Comment

Clonal hematopoiesis, multi-omics and coronary artery disease

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Studies of the genetic architecture of cardiovascular disease once focused on heritable germline factors. Newer work has shed light on the role of somatic mutations in blood cells. These mechanistic and multi-omics studies, along with phenotypic analyses, offer the prospect of new precision cardiovascular medicine paradigms.

Despite advances, cardiovascular disease (CVD) remains the leading cause of death worldwide. Although chronological age is the overwhelmingly dominant risk factor for CVD, the mechanisms by which aging promotes CVD risk are poorly understood.

Research from approximately 50 years ago demonstrated the enrichment of monoclonality in atherosclerotic plaques[1]. Although the drivers of clonality at the time were thought to be chronic infections, previous investigations indicated greater mutational burden in peripheral blood lymphocytes among patients with greater atherosclerosis burden. Indeed, aging hematopoietic stem cells have decreased capacity to prevent and repair DNA replication errors[2]. Although the acquisition, retention and clonal expansion of mutations altering a stem cell's fitness is a hallmark of carcinogenesis, its role in cardiovascular disease is not immediately obvious in light of the sparse antecedent scientific literature.

With the advent of population-based genetic analysis of blood DNA, investigators have empirically demonstrated the commonality of expanded mutations indicative of clonal hematopoiesis. Expanded single-nucleotide polymorphisms and short insertions or deletions recurrently observed in blood cancers among individuals without other cytological abnormalities, termed 'clonal hematopoiesis of indeterminate potential' (CHIP), are surprisingly common in the general population[3]. Mutations are typically in *DNMT3A*, *TET2*, *ASXL1*, *JAK2* and *TP53*. Furthermore, analyses of haplotype imbalance can also detect the presence of larger structural variants, such as large genomic insertions or deletions or copy number neutral loss of heterozygosity, but not always with a concomitant cancer driver mutation[4]. Therefore, these variants are termed 'mosaic chromosomal alterations' (mCAs). Both phenomena are associated with high mortality as well as blood cancer, with myeloid malignancies more common for CHIP and lymphoid malignancies for mCAs, probably reflecting differences in the underlying cellular populations implicated[3,5].

CHIP and mCA share major risk factors, including age, male gender and smoking, though they slightly differ in ancestry prevalence: Hispanic individuals have a lower prevalence of CHIP than other races, whereas individuals of African descent have a high risk of mosaic loss of Y chromosome (mLOY, a subtype of mCA). Importantly, CHIP is

associated with coronary artery disease[6-8]. On the other hand, mCAs are not associated with CAD in the general population[4,5]. However, a 2022 preprint reported that mCA is associated with increased CAD death among individuals with previous diagnoses of solid cancers. In the general population, mCAs have been linked to increased risks for severe infectious diseases, including severe COVID-19, and the risk is particularly heightened among individuals with previous diagnoses of solid cancers[5]. Interestingly, a 2022 study confirmed that CHIP is associated with cardiovascular mortality and that mCAs are not, but showed that concomitant mCAs among individuals with CHIP further increased cardiovascular mortality[9]. These individuals generally also had large clone sizes defined by the variant allele frequency, such as >10%, and were more likely to have cytopenias and were at greater future risk for hematological malignancy compared to those with small clone sizes.

Several experimental designs have been used to probe for mechanistic insights into CHIP-associated CAD and are beginning to uncover both common and distinct pathways depending on the gene implicated. Atherosclerotic plaque size increases in hypercholesterolemic mice transplanted with *Tet2*$^{-/-}$, *Tp53*$^{-/-}$ and *Jak2*^{V617F} (*Jak2*^{VF}) bone marrow compared with mice transplanted with wild-type bone marrow. Macrophages with CHIP-related mutations tend to exacerbate the production of inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and IL-18, via NLRP3 and AIM2 inflammasome activation in *Tet2* and *Jak2*^{VF} CHIP[7,8,10], respectively (Fig. 1). In addition, individuals with *DNMT3A* CHIP have greater *NLRP3* mRNA expression in monocytes and higher circulating IL-6 than those without[11]. These findings were further corroborated in a macrophage cell line with CRISPR-mediated disruption of *Dnmt3a*, which had increased *Il6* mRNA expression compared with cells without the disruption. Concordant elevations of each circulating cytokine in human cohorts are observed[11], such as elevated plasma IL-1β and IL-6 in *TET2* CHIP carriers and IL-6 in *DNMT3A* CHIP, which supports the inflammasome–cytokine production pathway (that is, NLRP3–IL-1β–IL-6) being implicated in the path from CHIP to CAD. Furthermore, individuals with CHIP who harbour *IL6R* p.Asp358Ala, a well-established germline allele associated with modest CAD reduction in the general population, have a substantial reduction of the increased risk of CAD seen in CHIP carriers[6]. The CANTOS trial demonstrated the efficacy of an IL-1β inhibitor (canakinumab) for secondary prevention of major adverse cardiovascular events in those with high C-reactive protein[12]. In an exploratory post hoc analysis of the CANTOS trial, canakinumab showed substantially greater cardiovascular risk reduction among *TET2* CHIP carriers than in the rest of the population, providing additional support for an inflammatory link between CHIP and CAD.

Besides atherosclerotic plaque formation, thrombosis is another major cause of CAD. Indeed, venous thromboembolism is associated with CHIP, especially JAK2 V617F (*JAK2*^{VF}) CHIP. Mechanistically, hematopoietic *Jak2*^{VF} in mice leads to increased thrombosis owing to increased neutrophil extracellular trap (NET) formation,
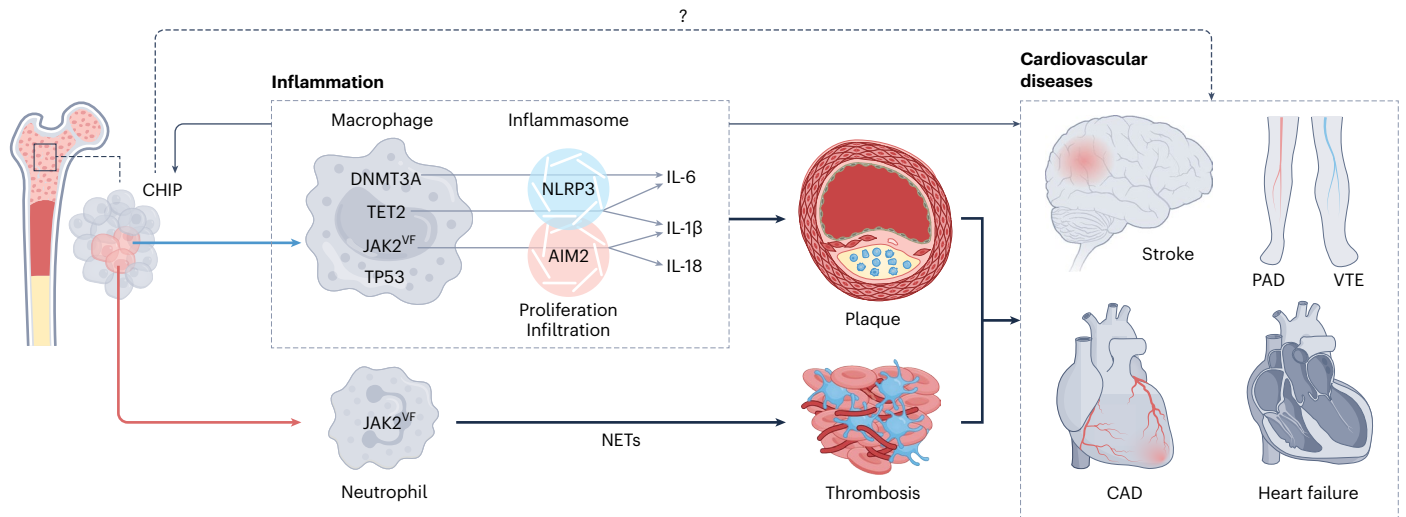
Fig. 1 | Proposed mechanisms of association between clonal hematopoiesis of intermediate potential and cardiovascular diseases. Clonal expansion of hematopoietic stem cells with leukaemogenic somatic driver mutations in bone marrow cells (known as clonal hematopoiesis, CH) results in clones of peripheral hematological cells, including macrophages and neutrophils, with those mutations. Clonal hematopoiesis of indeterminate potential (CHIP) refers to CH without overt hematological abnormality. Monocyte/macrophages with CHIP-related mutations in *DNMT3A*, *TET2* or *JAK2* V617F (*JAK2*^{VF}) promote

inflammatory cytokine production via inflammasome activation, which might in turn favourably influence the fitness of these mutations. *JAK2*^{VF} promotes neutrophil extracellular trap (NET) formation to increase the risk of thrombosis through inflammation, plaque formation, thrombosis and potentially unknown mechanisms. CHIP is associated with an increased risk for atherothrombotic diseases: stroke, coronary artery disease (CAD), peripheral artery disease (PAD) and venous thromboembolism (VTE). Those mechanisms are also associated with risk of heart failure in CHIP carriers.

consistent with the increased NET formation by neutrophils from patients with myeloproliferative neoplasms harboring *JAK2*^{VF}, which is abrogated by the JAK2 inhibitor. These data indicate that *JAK2*^{VF} CHIP is associated with CAD via increased thrombosis. Consequently, atherothrombotic diseases in other vascular beds, including ischemic stroke and peripheral artery disease, are also associated with CHIP.

Beyond atherothrombotic CVDs, there is robust evidence that CHIP is associated with an increased risk of heart failure both in murine models and in multiple human epidemiological cohorts in both ischemic and non-ischemic contexts. In addition to *TET2*, *ASXL1* and *JAK2* CHIP, a 2022 study showed that mLOY was linked to heart failure in a murine model, with support in one human cohort.

Intersections exist between CH and other traits that might highlight essential insights for both CH biology and CAD association. For example, individuals with premature natural menopause, which is linked to heightened risk of multiple cardiovascular diseases, also have an elevated likelihood of CHIP, especially *DNMT3A* CHIP. Given that only natural premature menopause, but not surgical menopause, is associated with greater CHIP prevalence, hormonal deficiency alone does not explain the mechanisms of this association. Considering that DNA damage repair pathways account for two-thirds of identified genetic determinants of age at natural menopause, natural premature menopause might reflect a latent predisposition to the accumulation of somatic mutations, which leads to CHIP acquisition.

Few known modifiable factors for CH exist. Although smoking is associated with CHIP and mCA, there is heterogeneity by subtypes. *ASXL1* CHIP is most significantly associated with smoking among CHIP subtypes, and mLOY is the most significant among mCA subtypes. Healthy diet behavior, defined by self-reported consumption of healthy

(fruits and vegetables) and unhealthy (red meat, processed food and added salt) foods, is associated with a lower prevalence of CHIP.

Genome-wide association studies (GWAS) to discover the germline genetic basis for somatic CH may yield important insights into disease pathogenesis[11,13]. The most robust susceptibility locus for CHIP includes the *TERT* locus, which is also the lead locus influencing leukocyte telomere length (LTL). However, Mendelian randomization studies have indicated a complex relationship, supporting a bidirectional and inverse causal relationship. Longer LTL increases the chance of acquiring CHIP, and, in turn, CHIP shortens LTL once acquired[14]. These observations pose limitations to conventional Mendelian randomization analyses of CHIP and highlight the potential presence of complementary CAD risk pathways. Other GWAS loci for CHIP harbour genes responsible for DNA damage repair, hematopoietic proliferation and DNA methylation.

The top two most frequently mutated genes in CHIP, *DNMT3A* and *TET2*, both encode epigenetic regulators, with DNMT3A generally leading to hypermethylation and TET2 to hypomethylation. CHIP is associated with accelerated methylation aging clocks, which are validated methylation scores predictive of mortality and cardiovascular disease, independent of chronological age. Interestingly, individuals with CHIP have greater cardiovascular risk stratification with accelerated methylation aging clocks compared to individuals without. Consistent with murine studies, epigenome-wide association studies show that *TET2* CHIP is associated with increased CpG methylation, whereas *DNMT3A* CHIP is associated with decreased CpG methylation in blood cells[15]. Mendelian randomization analyses indicate that some of the CpG changes may promote CHIP-associated CAD risk.

Beyond the acquisition of mutated clones, we are keen to understand better how the clones expand, as CHIP mutations with larger

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clone sizes are more likely to lead to adverse clinical outcomes. Longitudinal observation in small cohorts does not always show the expected clonal growth over time, and many even shrink. Deep targeted sequencing reveals that most middle-aged individuals have quiescent CHIP mutations, indicating that many CHIP mutations occurred many years before, often very early in life. Therefore, understanding the various features influencing mutational fitness in longitudinal studies will yield improved opportunities for risk stratification as well as early treatment.

In summary, new analyses of CH leveraging somatic mutation intrinsic features and burden, germline genetics, telomere length and epigenomics with experimental models have all led to new insights in understanding the path from CHIP and CAD. Nevertheless, several outstanding questions remain, such as what mechanisms drive clonal expansion and how to manage the risk of comorbidities, which necessitate longitudinal analyses with complementary deep molecular profiling to better elucidate this new opportunity for CAD precision medicine.

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