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## Clonal hematopoiesis in cardiovascular disease and therapeutic implications

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### Abstract

Clonal hematopoiesis arises from somatic mutations that provide a fitness advantage to hematopoietic stem cells and the outgrowth of clones of blood cells. Clonal hematopoiesis commonly involves mutations in genes that are involved in epigenetic modifications, signaling and DNA damage repair. Clonal hematopoiesis has emerged as a major independent risk factor in atherosclerotic cardiovascular disease, thrombosis and heart failure. Studies in mouse models of clonal hematopoiesis have shown an increase in atherosclerosis, thrombosis and heart failure, involving increased myeloid cell inflammatory responses and inflammasome activation. Although increased inflammatory responses have emerged as a common underlying principle, some recent studies indicate mutation-specific effects. The discovery of the association of clonal hematopoiesis with cardiovascular disease and the recent demonstration of benefit of anti-inflammatory treatments in human cardiovascular disease converge to suggest that anti-inflammatory treatments should be directed to individuals with clonal hematopoiesis. Such treatments could target specific inflammasomes, common downstream mediators such as IL-1 $\beta$  and IL-6, or mutations linked to clonal hematopoiesis.

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Clonal hematopoiesis (CH) occurs when somatic mutations in leukemogenic genes provide a selective advantage to haematopoietic stem and progenitor cells (HSPCs), leading to the expansion of mutant blood cells<sup>1</sup>. CH commonly involves mutations in genes mediating epigenetic modifications (*TET2*, *DNMT3A* and *ASXL1*), hematopoietic cytokine signaling (*JAK2*), DNA damage repair (*PPM1D* and *TP53*) or messenger RNA splicing (*SF3B1* and *SRSF2*). Clonal hematopoiesis of indeterminate potential (CHIP) is a term used to describe the presence of a driver gene mutation with a variant allele frequency (VAF) of at least 2% in peripheral blood, in the absence of clinical criteria of hematological malignancy<sup>2</sup>.

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CHIP increases in frequency with aging and increases the risk of developing atherosclerotic cardiovascular disease (CVD) as well as hematological malignancies<sup>3</sup>. CHIP has also been associated with adverse outcomes in heart failure (HF), an increasing problem in cardiology practice<sup>4–6</sup>, and also following transcatheter aortic valve replacement of stenotic aortic valves, a common procedure in older people<sup>7</sup>.

Many excellent reviews on CHIP have been published, and CHIP has been associated with several other chronic diseases, including cirrhosis and chronic obstructive pulmonary disease<sup>1,8–11</sup>. This Review will focus on potential mechanisms that link CHIP to CVD and on how mechanistic and clinical studies may inform future potential therapies for CHIP-associated CVD. We will critically evaluate a recent proposal that the relationship between CHIP and atherosclerotic CVD may involve reverse causation<sup>12</sup>. Although there may be some shared general mechanisms for CHIP-associated CVD, such as macrophage inflammatory responses, a more granular view indicates that several mechanisms linking CHIP to CVD are mutation specific. Consequently, future therapies may target either common downstream pathways or mutation-specific mechanisms.

## CHIP and atherosclerosis

Atherosclerosis develops as a result of the accumulation of cholesterol-rich low-density lipoprotein (LDL) particles in the artery wall, provoking an inflammatory response that eventually leads to the rupture or erosion of atherosclerotic plaques and blockage of arteries by thrombus<sup>13</sup>. Jaiswal et al.<sup>3</sup> studied the relationship between CHIP mutations and the development of hematological neoplasms. Expectedly, CHIP increased the risk of developing hematological malignancy; unexpectedly, CHIP also predisposed to an increase in total mortality and to an increased risk of coronary artery disease (CAD) and thrombotic stroke, independently of traditional cardiovascular risk factors. In follow-up studies, Jaiswal et al. used whole-exome sequencing and a case–control design to assess the relationship between CHIP mutations and CVD<sup>3,14</sup>. People carrying genetic variants in *TET2*, *DNMT3A*, *ASXL1* or *JAK2* had a risk of CAD that was 1.9 times higher than that of non-carriers. In two retrospective case–control studies, participants with CHIP had a fourfold-higher risk of early-onset myocardial infarction. CHIP carriers also had increased coronary artery calcification, a marker of coronary atherosclerosis. These CHIP variants, as well as the DNA damage response (DDR) variants in *TP53* and *PPM1D*, predict incident atherosclerotic disease in multiple vascular beds, including coronary, peripheral and mesenteric arteries<sup>15</sup>. CHIP has thus emerged as a major independent CVD risk factor, with comparable impact to traditional CVD risk factors, such as smoking or high LDL (Fig. 1). The causal relationship between CHIP and atherosclerosis has been established in hyperlipidemic mouse models transplanted with mixtures of wild-type (WT) and *Tet2*<sup>-/-</sup>, *Jak2*<sup>V617F</sup> or *Tp53*<sup>-/-</sup> bone marrow (BM) (Table 1).

CHIP increases with aging and occurs in more than 10% of people over age 70. More sensitive DNA-sequencing methods can detect even higher frequencies of CH mutations<sup>16</sup>, but the clinical significance of very-low-burden CH mutations is poorly understood. Aging is also associated with altered methylation at a subset of CpGs<sup>17</sup>. Measurements of these modifications in epidemiological studies have been used to accurately predict chronological

age in healthy individuals. Accelerated aging is inferred when measured methylation age is greater than chronological age. Epigenetic aging is associated with increased risk of CVD and all-cause mortality<sup>18</sup>. CHIP is strongly associated with epigenetic age acceleration<sup>19,20</sup> and only the 40% of people with CHIP who had increased aging biomarkers also had increased CVD risk, suggesting that epigenetic aging could be used to identify people with CHIP who have greatest need for treatment<sup>20</sup>.

## TET2.

Levine and colleagues showed that older people with clonal expansion of blood cells in the absence of concomitant hematological malignancy commonly had mutations in a potential driver gene, *TET2* (ref.<sup>21</sup>). TET2 catalyzes the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, leading to widespread epigenetic modifications. TET2 deficiency increases HSPC self-renewal, leading to expansion of the HSPC compartment and increased production of blood cells, especially myeloid cells<sup>22,23</sup>. Loss of *Tet2* resulted in the upregulation of several inflammatory mediators in macrophages, including IL-6, during the late-phase response to lipopolysaccharide challenge<sup>24</sup>. *Tet2*-deficient mice were more susceptible to endotoxin shock and dextran-sulfate-induced colitis, displaying a more severe inflammatory phenotype and increased IL-6 production compared with that of WT mice. The inflammatory repression mechanism involved TET2 recruitment of HDAC2 and repression of *Il6* via histone deacetylation, independent of DNA methylation and hydroxymethylation<sup>24</sup>.

Fuster et al.<sup>25</sup> showed that transplantation of *Tet2*<sup>-/-</sup> BM in a 10:90 ratio with WT BM resulted in an increased size of early atherosclerotic lesions in 9-week-old *Ldlr*<sup>-/-</sup> mice fed a Western-type diet compared with mice transplanted with 100% WT BM. *Tet2*-deficient cells expanded in the BM, spleen and blood but were not increased within atherosclerotic plaques relative to the bloodstream, and blood cell counts were unchanged. Although all *Tet2*-deficient blood cells were expanded, myeloid deficiency of *Tet2* produced a similar increase in the size of atherosclerotic lesions, indicating a central role of macrophages or neutrophils in accelerated atherosclerosis. *Tet2*<sup>+/-</sup> BM cells modeling human *TET2* CHIP also accelerated atherosclerosis. Early atherosclerotic lesions showed an increase in macrophage content, but there was no change in macrophage proliferation or apoptosis within plaques. Atherosclerotic plaques from *Tet2*<sup>-/-</sup> CH mice displayed prominently increased *Il1b* mRNA and increased IL-1 $\beta$  protein levels. The NLRP3 inhibitor MCC950 reduced atherosclerosis in *Tet2*<sup>-/-</sup> CH mice but not in controls, indicating a major role of the NLRP3 inflammasome in the accelerated progression of atherosclerosis in TET2-mutant CH. Inflammasome activation leads to the secretion of active IL-1 $\beta$ , which has the potential to increase recruitment of leukocytes into plaques. Accordingly, aortic endothelial P-selectin expression and monocyte recruitment to lesions were increased in *Tet2*<sup>-/-</sup> CH mice, with reversal of increased P-selectin by MCC950 treatment. On a mechanistic level, *Tet2* deficiency resulted in increased *Il1b* and *Il6* expression in response to inflammatory stimuli, which appeared to be independent of TET2 enzymatic activity on the basis of experiments with cultured macrophages expressing a catalytically inactive mutant TET2. This was associated with increased histone acetylation over the *Il1b* promoter and reversal of the difference by HDAC inhibitors, implicating increased histone acetylation. *Tet2*-

deficient macrophages exhibited a widespread increase in the expression of inflammatory genes, including increased mRNA for *I1b* and *Nlrp3*, indicating increased inflammasome priming, and secreted increased IL-1 $\beta$  in response to lipopolysaccharide and ATP, indicating increased inflammasome activation. These studies provide strong evidence for increased *I1b* transcription and NLRP3 inflammasome priming in *Tet2*-deficient macrophages and a central role of the NLRP3 inflammasome in *Tet2*<sup>-/-</sup> CH-accelerated atherosclerosis (Fig. 2a).

### **JAK2<sup>VF</sup>.**

*JAK2*<sup>V617F</sup> is a gain-of-function mutation that is associated with myeloproliferative neoplasms (MPNs) and an increased risk of atherothrombotic disease. In exome-wide sequencing studies, *JAK2*<sup>VF</sup> was associated with increased CAD despite lower levels of LDL<sup>26</sup>. Among the more frequent CHIP variants that associate with CVD, the *JAK2* mutation (*JAK2*(V617F)) is less common but occurs at a younger age and has the greatest effect on CVD risk<sup>27</sup>. In a meta-analysis of four studies, *JAK2*<sup>VF</sup> endowed a 12-fold increase in risk of CAD compared with about a 2-fold increase for the other variants<sup>14</sup>. CHIP, especially that involving *JAK2*<sup>VF</sup>, has also been associated with increased risk of venous thrombo-embolic disease. In a general European population, *JAK2*<sup>VF</sup> was detected by droplet digital PCR in 3.1% of adults not selected for age<sup>28</sup>. *JAK2*<sup>VF</sup> increased thrombotic risk at allele frequency 1% or more<sup>28</sup>. In a study from Japan, *JAK2*<sup>VF</sup> allele frequencies of less than 2% were associated with increased CVD<sup>29</sup>. Reconstruction of clonal histories using whole-genome sequencing of single-cell hematopoietic stem cell (HSC)-derived colonies from people with MPNs has shown that *Jak2*<sup>VF</sup> mutations can be acquired several decades earlier than the appearance of MPN<sup>30</sup>, emphasizing the disease-potentiating effect of *JAK2*<sup>VF</sup> from the early years of life.

Mice with a conditional knock-in of *Jak2*<sup>VF</sup> and pan-hematopoietic expression of *Jak2*<sup>VF</sup> displayed accelerated atherosclerosis despite lower LDL-cholesterol levels<sup>31</sup>. Mice with macrophage-specific expression of *Jak2*<sup>VF</sup> and *Jak2*<sup>VF</sup> chimeric mice modeling CHIP displayed increased lesion area, increased proliferation of macrophages and larger necrotic cores<sup>32</sup>. In contrast to *Tet2*<sup>-/-</sup> CH mice<sup>25</sup>, there was little or no expansion of *Jak2*<sup>VF</sup> cells in blood. However, atherosclerotic lesions showed an enrichment of *Jak2*<sup>VF</sup> cells relative to the bloodstream, reflecting both increased recruitment of *Jak2*<sup>VF</sup> monocytes likely owing to increased surface expression of integrins<sup>33</sup>, as well as increased cell-autonomous proliferation of *Jak2*<sup>VF</sup> macrophages in lesions. Deletion of *Casp1* and *Casp11*, which encode essential inflammasome components, in *Jak2*<sup>VF</sup> mice abrogated increased proliferation of macrophages in lesions; moreover, features of plaque stability, including macrophage accumulation, cap thickness, and necrosis, were markedly improved. Inflammasome activation in *Jak2*<sup>VF</sup> macrophages increased secretion of IL-1 $\beta$ , which increased AKT and ERK signaling and thereby led to both increased proliferation and pyroptotic cell death (Fig. 2b). Proliferating *Jak2*<sup>VF</sup> macrophages displayed increased glycolysis and mitochondrial respiration, as well as increased mitochondrial reactive oxygen species (ROS) and oxidized DNA. Increased mitochondrial ROS can lead to oxidation of DNA and activation of NLRP3 inflammasomes<sup>34</sup> but can also promote damage of nuclear DNA, formation of double-stranded DNA breaks and activation of AIM2

inflammasomes<sup>35</sup>. Immunofluorescence staining of lesions from mice with *Jak2<sup>VF</sup>* CH showed accumulation of oxidized DNA and pγH2AX-positive macrophages, which suggests ROS-mediated oxidative DNA damage and replication stress. *Jak2<sup>VF</sup>* bone-marrow-derived macrophages (BMDMs) and human induced-pluripotent-stem-cell-derived macrophages showed increases in both polydAdT-mediated AIM2 activation and ATP-mediated NLRP3 inflammasome activation. However, AIM2 protein was increased, whereas NLRP3 was decreased. IFNγ-neutralizing antibodies reversed the increase in *Aim2* expression in *Jak2<sup>VF</sup>* BMDMs, indicating a direct link between JAK2 gain-of-function, IFNγ signaling and *Aim2* expression. Whereas *Nlrp3* deficiency had no significant effect on lesion or necrotic core areas, *Aim2* deficiency markedly reduced both. Consistent with cell culture studies, AIM2 levels were increased in *Jak2<sup>VF</sup>* lesions. Single-cell RNA sequencing (scRNA-seq) studies showed an increase in inflammatory, proliferative myeloid populations in *Jak2<sup>VF</sup>* lesions that were reduced by *Gsdmd* deficiency, but also revealed complex changes, with a reduced population of noninflammatory *Trem2<sup>hi</sup>* macrophages and a potentially adverse increase in monocytes and pyroptotic ‘zombie’ macrophages in *Gsdmd<sup>-/-</sup>* mice. *Jak2<sup>VF</sup>* chimeric mice treated with antibodies against IL-1β showed enhanced features of plaque stability, including decreased macrophage density, decreased necrotic cores and increased fibrous-cap thickness. These reciprocal changes did not result in an overall change in plaque size. Even though mice do not reliably rupture atherosclerotic plaques, such changes in macrophage density, necrotic core size and fibrous-cap thickness have been implicated in plaque stabilization in humans<sup>36</sup>.

### DNMT3A.

The most common genetic variants in CH involve *DNMT3A*, which encodes a methyltransferase enzyme that catalyzes DNA methylation at CpG sites and is a critical epigenetic regulator of gene expression<sup>37</sup>. The majority of pathogenic mutations in *DNMT3A* are loss-of-function, including missense mutations in regulatory and catalytic domains as well as nonsense and dominant negative mutations. *DNMT3A* pathogenic variants enhance HSPC self-renewal and reduce differentiation potential. Paradoxically, DNMT3A and TET2 have opposing enzymatic activities with regard to DNA methylation while their reduced function in CHIP has parallel effects on atherosclerosis<sup>38</sup> and heart failure<sup>4,14,39</sup>. Both *Tet2<sup>-/-</sup>* and *Dnmt3a<sup>-/-</sup>* CH increased atherosclerosis and promoted accumulation of a distinctive population of resident-like, inflammatory macrophages by scRNA-seq<sup>38</sup>. *TET2* and *DNMT3A* CHIP both associate with epigenetic aging and have largely similar associations with reduced methylation of CpG islands<sup>20</sup>. However, there seems to be some differences in the mechanisms linking *Tet2* and *Dnmt3a* CHIP to CVD. Monoallelic *DNMT3A* inactivation in macrophage-like cells (derived from embryonic stem cells) causes manifold complex alterations in immunomodulatory gene expression, with increased expression of many chemokine genes and *IL6*, but decreased expression of TLR4-responsive genes, including *IL1B*<sup>40</sup>. CHIP mutations have recently been associated with increased osteoporosis in the UK Biobank and in mouse models<sup>41</sup>. In *Dnmt3a<sup>-/-</sup>* CHIP mice, increased osteoporosis reflected increased osteoclastogenesis as a result of increased inflammatory gene expression and increased IL-20 secretion by *Dnmt3a<sup>-/-</sup>* macrophages. While ELISA assays from BMDM and RAW264.7 macrophage cultured medium showed increased IL-20, IL-1β levels were not increased in *Dnmt3a<sup>-/-</sup>* relative to the level in WT

cultures. Together, these studies suggest distinctive effects of *Tet2* and *Dnmt3a* mutations on macrophage inflammatory activities. Further studies are needed to resolve whether TET2-mutant CHIP and DNMT3A-mutant CHIP are impacting CVD by distinctive or convergent pathways.

### PPM1D and TP53.

These DDR CHIP variants are increased in individuals who have been exposed to chemotherapy or radiation and are associated with increased risk of incident coronary and peripheral artery disease<sup>15</sup>. In mice, competitive bone marrow transplantation (BMT) of 20% *Trp53*<sup>-/-</sup> with 80% WT BM leads to an increase in early atherosclerotic lesions in the proximal aorta<sup>15</sup>. p53-deficient cells are moderately expanded in the BM and blood and incrementally increased in plaques relative to the bloodstream, likely as a result of increased p53-deficient macrophage proliferation within plaques. However, in contrast to findings with *Tet2*<sup>-/-</sup> and *Jak2*<sup>VF</sup> CHIP models, TP53-mutant CHIP plaques did not show evidence of increased *Il1b* or *Il6* mRNA, providing further evidence for diverse mechanisms that increase atherosclerosis in murine CHIP models.

### CHIP and thrombotic disease

It has long been known that *JAK2*<sup>VF</sup> individuals with myeloproliferative neoplasms have an increased risk of venous and arterial thrombosis, leading to thrombo-embolism and ischemic heart disease<sup>42</sup>. In mice, haematopoietic *Jak2*<sup>VF</sup> increased venous thrombosis in association with increased formation of neutrophil extracellular traps (NETs) within thrombi, whereas neutrophils from people with myeloproliferative disorders showed increased NETosis<sup>43</sup>. Genetic deficiency of PAD4, an essential enzyme in NET formation, appeared to decrease venous thrombosis. Another study identified pleckstrin-2 (PLEK2) as a downstream target of the JAK2–STAT5 pathway in erythroid and myeloid cells and showed that it is upregulated in a *Jak2*<sup>VF</sup> mouse model and in people with myeloproliferative disorders<sup>44</sup>. Loss of PLEF2 ameliorated *JAK2*<sup>VF</sup>-induced myeloproliferative phenotypes, thereby reverting the widespread vascular occlusions and lethality in *Jak2*<sup>VF</sup> knock-in mice. A reduction in red blood cell mass was the main contributing factor in the reversion of vascular occlusions. Together, these studies suggest that erythrocytosis, leukocytosis and intrinsic red blood cell and white blood cell abnormalities increase thrombotic risk in *Jak2*<sup>VF</sup> myeloproliferative disorders and could potentially also contribute to the increased thrombotic risk in *JAK2*<sup>VF</sup> CH.

LNK (also known as SH2B3) inhibits JAK–STAT signaling by hematopoietic cytokine receptors. Genome-wide association studies have shown the association of a common single nucleotide polymorphism in *LNK* (R262W, T allele) with neutrophilia, thrombocytosis and CAD<sup>45</sup>. *LNK(TT)* reduces LNK function and thus increases signaling downstream of *JAK2(V617F)* (ref.<sup>46</sup>). *Lnk*-deficient mice show accelerated atherosclerosis and arterial thrombosis. Increased arterial thrombosis reflected increased NET formation in thrombi<sup>47</sup>. NETosis was dependent on oxidized phospholipid and p-selectin released by activated platelets; consequently, accelerated thrombosis and atherosclerosis were reversed by an antibody targeting oxidized phospholipids. In the UK Biobank, individuals with the *JAK2*<sup>VF</sup>

mutation only showed increased CAD when also carrying the LNK(R262W) allele<sup>47</sup>. Modeling CHIP in mice with all hematopoietic cells being *Lnk*<sup>+/-</sup> and with *Jak2*<sup>VF</sup> induced in a subset of hematopoietic cells showed accelerated thrombosis, but not atherosclerosis. This suggests that the increased CAD in JAK2<sup>VF</sup> individuals with LNK(R262W) might primarily reflect increased thrombotic risk.

## CHIP and heart failure

HF arises when the heart cannot pump enough blood to meet the body's needs. HF is a heterogeneous clinical syndrome that can be driven by a variety of etiologies, including hypertension and CAD, and it may involve infiltration of the myocardium with inflammatory cells and fibrotic remodeling. Recent epidemiological studies have demonstrated a robust link between CH and HF. In a meta-analysis of >56,000 participants in 5 cohorts who were free of HF at baseline, carriers of a CHIP mutation exhibited a 25% increased risk of incident HF, independent of traditional risk factors<sup>4</sup>. Single-gene-specific analyses suggested a differential effect of mutations in different genes in this context. Mutations in *TET2*, *JAK2* and *ASXL1* were separately associated with risk of incident HF that was increased by 1.5- to 2.5-fold, whereas the more prevalent mutations in *DNMT3A* were not associated with HF risk. CH has also been investigated in cohorts of people with established HF, in whom it is highly prevalent, being typically detected in approximately one-third of individuals with reduced left ventricular ejection fraction<sup>5,48,49</sup>. In this setting, carriers of CHIP-linked mutations exhibit a substantially higher risk of adverse clinical progression, defined as higher risk of death or composites of all-cause or HF-specific death and HF-related hospitalizations<sup>5,6,48,50,51</sup>. Both *DNMT3A* and *TET2* mutations have been associated with worse outcomes in individuals with HF<sup>5,6,50</sup>, and emerging evidence suggests that less frequent CH variants in other genes may also have predictive value<sup>48,51</sup>. The association between CH and HF is similar in individuals with and without coronary heart disease<sup>4,5</sup>, which suggests a direct connection between CH and HF pathophysiology and argues against the possibility that these associations merely reflect the strong association between CH and atherosclerosis or effects of myocardial ischemia on the hematopoietic system. The observations in these epidemiological studies are also consistent with the idea that the clinical significance of CH may depend on the specific mutated gene and, in some cases, the specific context (that is, onset versus progression of HF).

Altered inflammatory responses contribute to the pathogenesis and progression of HF<sup>52</sup>, and CH has been shown to correlate with heightened systemic inflammation in people with HF. Circulating IL-6 levels are increased in individuals with HF with reduced ejection fraction and CH mutations in either *DNMT3A* or *TET2*<sup>5</sup>. In addition, scRNA-seq studies have shown that blood monocytes from a small sample of individuals with HF carrying *DNMT3A* mutations exhibit increased expression of *IL6*, *IL1B*, *IL8* and *NLRP3*, several chemokines and their receptors, and cell surface receptors involved in monocyte-T cell interactions<sup>53</sup>. An important question is whether the association between CH and HF differs between HF with reduced left ventricular ejection fraction and HF with preserved ejection fraction.

**TET2.**

TET2 is the CH driver gene most extensively studied in the context of HF. Sano et al. found that competitive transplantation of *Tet2*<sup>-/-</sup> BM cells in a 10:90 ratio with WT BM leads to greater left ventricular dysfunction after surgical ligation of the left anterior descending (LAD) artery<sup>54</sup>. This impaired cardiac function was paralleled by substantial increases in myocardial fibrosis, myocyte hypertrophy and macrophage content within the cardiac tissue. *Tet2*-deficient CH also worsened cardiac function and remodeling in an experimental model of non-ischemic pressure-overload-induced cardiac hypertrophy, achieved by transverse aortic constriction (TAC). In both experimental models, myeloid-restricted ablation of *Tet2* was sufficient to aggravate cardiac dysfunction. Mechanistic studies indicated that overproduction of IL-1 $\beta$  by TET2-deficient macrophages and the ensuing exacerbation of cardiac inflammation has a central role in these aggravated HF phenotypes. Accordingly, the detrimental cardiac effects of hematopoietic TET2 deficiency, including increased fibrosis, were suppressed by the NLRP3 inhibitor MCC950. A study based on lentiviral-vector-mediated CRISPR-Cas9 editing of *Tet2* corroborated the damaging effects of loss of function of TET2 in an experimental model of hypertension and cardiac dysfunction induced by angiotensin II infusion<sup>39</sup>. Furthermore, TET2-deficient CH has also been shown to accelerate age-related cardiac dysfunction and fibrotic remodeling in the absence of external insults in a model of CH achieved by non-conditioned BM transplantation<sup>55</sup>. Collectively, these studies provide strong evidence for a direct contribution of TET2-mutant CH to accelerated HF development and support a central role of NLRP3-IL-1 $\beta$ -driven inflammation, similar to the scenario in atherosclerosis.

**DNMT3A.**

The only report so far on the effects of DNMT3A-mutant CH on cardiac function showed that angiotensin II infusion induced greater cardiac dysfunction, fibrotic remodeling and immune cell infiltration into cardiac tissue in mice carrying *Dnmt3a*<sup>-/-</sup>-mutant cells than in controls<sup>39</sup>. However, these findings need to be interpreted with caution, as mutations were generated using a lentiviral-vector-mediated CRISPR-Cas9 approach, and the potential off-target effects of this intervention were not investigated. Furthermore, it remains unknown whether DNMT3A deficiency affects cardiac function in experimental conditions other than after chronic infusion of angiotensin II levels.

**JAK2<sup>VF</sup>.**

Similar to the strategy used in experimental atherosclerosis studies, myeloid-restricted JAK2<sup>VF</sup> expression has been used to model JAK2-mutant CH in the absence of major hematological abnormalities in experimental HF studies. Sano et al.<sup>56</sup> used an ex vivo lentivirus-mediated approach to overexpress the *JAK2*<sup>VF</sup> mutation specifically in myeloid cells under control of a combined synthetic SP146 promoter-gp91 enhancer combination. In chronic LAD ligation and TAC conditions, myeloid *JAK2*<sup>VF</sup>-expressing mice showed accelerated cardiac dysfunction and fibrosis, as well as heightened expression of proinflammatory cytokines in cardiac tissue, supporting the notion that the *JAK2*<sup>VF</sup> mutation can contribute to accelerated HF development.



### DDR gene mutations.

CHIP mutations affecting DDR genes are also being investigated in the context of cardiac disease, particularly as potential contributors to HF in cancer survivors, in whom these mutations are considerably more prevalent than in the general population owing to the selective pressure associated with genotoxic therapies<sup>57</sup>. Hematopoietic TP53 mutations have been reported to exacerbate doxorubicin-induced cardiac toxicity in mice, in parallel with increased neutrophil infiltration into the myocardium and elevated expression of *IL6*, *IL1B* and *TNF*<sup>58</sup>, which encode proinflammatory cytokines. CHIP mutations in the DDR-related phosphatase PPM1D have also been investigated in the context of experimental cardiac disease. These mutations typically result in a C-terminal-truncated protein that exhibits increased stability and activity, which has been investigated through an ex vivo lentivirus-mediated CRISPR–Cas9 approach coupled to BMT. Using this strategy, PPM1D-mutant-cell-carrying mice exhibit increased angiotensin-II-induced cardiac hypertrophy and fibrosis, as well as worsened cardiac function, compared with control mice<sup>59</sup>. Mechanistically, this cardiac phenotype has been linked to augmented expression of IL-1 $\beta$  and IL-18 expression in PPM1D-mutant myeloid cells.

### CHIP and reverse causation

While mouse models mimicking CHIP variants show worsened atherosclerosis and HF, indicating causation, a recent study has suggested that atherosclerosis could accelerate expansion of CH mutant clones by promoting HSPC proliferation, indicating reverse causation<sup>12</sup>. Mathematical modeling showed that the combination of a CH variant that endows an intrinsic advantage to HSPCs with extrinsic factors that increase the rate of HSPC proliferation will inevitably amplify the CH allele burden over time. For example, inflammatory cytokines associated with accelerated atherosclerosis could promote proliferation of HSPCs, resulting in accelerated expansion of mutant cells. Accordingly, in *Tet2*<sup>-/-</sup> CHIP mice the expansion of *Tet2*<sup>-/-</sup> HSPCs was increased by hyperlipidemia and atherosclerosis and by sleep-fragmentation-accelerated atherosclerosis. Infectious stimuli can also promote CH. Chronic myco-bacterial infection increased expansion of mutant HSPCs in a *Dnmt3a*<sup>-/-</sup> CHIP model<sup>60</sup>. This effect was mediated through IFN $\gamma$  and involved decreased differentiation and decreased apoptosis of *Dnmt3a*<sup>-/-</sup> HSPCs with widespread increases in gene methylation. Similarly, lipopolysaccharide treatment augmented the competitive advantage of TET2-deficient HSPCs in mice, owing to an overactivation of IL-6 signaling and improved TET2-deficient HSPC survival<sup>61</sup>. Consistent with bidirectional causation, a study in zebrafish suggests that enhanced fitness of ASXL1 mutant HSCs arises from increased expression of anti-inflammatory genes, which endows resistance to inflammatory signals arising from mutant mature cell progeny<sup>62</sup>.

Dyslipidemia involving elevated levels of atherogenic lipoproteins, decreased levels of HDL, or defects in cholesterol efflux genes *Abc1* and *Abcg1*, could also lead to both increased atherosclerosis and expansion of CHIP mutant HSPCs<sup>63</sup>. Dyslipidemia acts both at the level of cells in atherosclerotic plaques and in BM HSPCs to stimulate their proliferation. In *ApoE*<sup>-/-</sup> mice, HSPC proliferation and myeloid bias increase production of monocytes that enter the artery wall in increased amounts, promoting atherosclerosis<sup>64</sup>. Hyperlipidemia

promoted increased proliferation of HSPCs containing the *Jak2<sup>VF</sup>* mutation compared to chow-fed *JAK2<sup>VF</sup>* mice or WT controls<sup>31</sup>. In humans, a Mendelian randomization study showed that single nucleotide polymorphisms in genes associated with lower HDL levels are associated with higher WBC counts<sup>65</sup>, which in turn are associated with increased CVD in epidemiological studies<sup>66</sup>. CHIP variants are associated with type 2 diabetes<sup>14,67</sup>. *Tet2<sup>-/-</sup>* CHIP has been shown to foster insulin resistance in aging and obese mice with increased IL-1 $\beta$  in adipose tissue and reversal by the NLRP3 inhibitor MCC950 (ref.<sup>68</sup>). Together, these studies indicate the potential for a vicious cycle in which CHIP promotes insulin resistance and dyslipidemia that in turn induces expansion of *Tet2<sup>-/-</sup>* clones. Further prospective longitudinal studies in larger population samples are needed to ascertain the natural history of CH over time and to clarify the relationship between insulin resistance, dyslipidemia and emergence of CHIP.

Another potential example of reverse causation is the association of CHIP in later life with premature menopause and CVD<sup>69</sup>. In postmenopausal women, the presence of CHIP involving *DNMT3A* was associated with natural, but not surgical, premature menopause (age < 40). A genome-wide association study examining age of menopause revealed that the large majority of associations are for genes involved in DDR<sup>70</sup>. Causal inference analysis indicates that variants associated with early ovarian failure were associated with type 2 diabetes. This suggests a potential pathway in which genetic predisposition to early menopause promotes type 2 diabetes that in turn may facilitate the emergence of CH.

Although a vicious cycle connecting CHIP with atherosclerosis and other diseases is highly plausible<sup>71</sup>, the magnitude and consistency of this effect is uncertain<sup>72</sup>. In humans with atherosclerosis, the rate of HSPC proliferation over decades may not be as high as suggested by its measurement at a single time point in people of uncertain clinical status<sup>12</sup>. IL-6 is a key inflammatory factor in atherosclerosis that also increases HSPC proliferation<sup>73</sup>, but individuals with *TET2* or *DNMT3A* CHIP and reduced-function IL-6R variants had reduced CVD without a change in CHIP allele burden<sup>74</sup>, even though *TET2* and *DNMT3A* CHIP carriers have elevated IL-6 levels<sup>74</sup>. The finding that atherosclerosis promoted expansion of *Tet2<sup>-/-</sup>* BM progenitors<sup>12</sup> conflicts with earlier studies in which *Tet2<sup>-/-</sup>* BM progenitor and blood cells expanded similarly in mice fed chow diets (without atherosclerosis) or fed high-cholesterol diets (with early atherosclerosis); moreover, inhibition of NLRP3 reduced atherosclerosis without diminishing the expansion of *Tet2<sup>-/-</sup>* cells<sup>25</sup>. Also, *Jak2<sup>VF</sup>* allele burden in blood cells did not increase over time in CHIP atherosclerotic mice<sup>32</sup>. Nonetheless, the possibility of bidirectional causation, especially over longer time periods or with more advanced atherosclerosis, remains likely. This highlights the importance of mechanistic studies in mouse models in which the CH variant is clearly initiating atherosclerosis and reverse causation can be assessed by measurements of HSPC and myeloid progenitor populations in bone marrow and blood under atherosclerotic or non-atherosclerotic conditions. Further research is needed to disentangle forward and reverse causation and the underlying mechanisms in the association between CHIP and CVD. From a therapeutic perspective, inflammatory cytokines such as IL-1 $\beta$ , IL-6 or IFN $\gamma$ <sup>60</sup> or dyslipidemia could be driving both CHIP emergence and atherosclerosis and are thus attractive targets for therapeutic control of atherosclerosis and potentially CHIP emergence.

## Therapeutic approaches

Despite the success of LDL-lowering treatments, atherosclerotic CVD remains the major cause of morbidity and mortality in the United States and accounts for 28% of deaths<sup>75</sup>. Residual CVD remains substantial even in clinical trials with marked lowering of LDL cholesterol<sup>76–78</sup>, pointing to the need for development of new treatments. Recent clinical trials using anti-inflammatory therapies, notably IL-1 $\beta$  antibodies<sup>79</sup> or colchicine<sup>80,81</sup>, have shown a reduction in CVD. IL-1 $\beta$  antibodies inactivate a major product of all inflammasomes, while colchicine inhibits the microtubule-dependent assembly of the NLRP3 inflammasome and IL-1 $\beta$  secretion<sup>82,83</sup> and has other anti-inflammatory activities<sup>84</sup>. These studies have validated the importance of inflammation and inflammasomes in human atherothrombotic disease. However, IL-1 $\beta$  treatment caused an increase in fatal infections, whereas colchicine doubled the risk of pneumonia. IL-1 $\beta$  antibodies have not been marketed for CVD indications, and the therapeutic potential of colchicine may be limited<sup>85</sup>. This indicates that more precise targeting of anti-inflammatory treatments to individuals with higher levels of inflammatory CVD risk is essential to improve the benefit/risk ratio. The presence of CHIP mutations may help to identify such a population of people. CHIP carriers displayed increased aging biomarkers, suggesting that individuals with CHIP and increased aging biomarkers might represent a risk-enriched population that could be the target of anti-inflammatory therapies<sup>20</sup>. The identification of inherited variants that modulate CHIP-related CVD risk<sup>74</sup> may also help identify high-risk individuals in this context. The treatments to reduce CVD risk in CHIP carriers could involve targeting upstream inflammatory factors, such as NLRP3 or AIM2 inflammasomes, downstream common mediators, such as IL-1 $\beta$  or IL-6, or specific driver gene mutations, such as those involving *TET2* or *JAK2*.

### Inhibition of inflammasome components.

In mouse CHIP models, *Tet2* deficiency activated the NLRP3 inflammasome, while JAK2<sup>VF</sup> activated the AIM2 inflammasome. This suggests that specific inhibitors of NLRP3 or AIM2 inflammasomes may be required to treat CHIP-associated CVD according to CH status. A variety of NLRP3 inhibitors are in development including those that target the BRCC3/ABRO1 pathway that mediates specific deubiquitination and activation of NLRP3<sup>86</sup>. Clinical trials using the NLRP3 inhibitor CRID3 (the same molecule as MCC950 in a different formulation) to treat rheumatoid arthritis were stopped owing to hepatotoxicity<sup>87</sup>. Inhibitors of the AIM2 inflammasome have been reported to increase features of plaque stability in *ApoE*<sup>-/-</sup> mice<sup>88</sup>; however, the specificity of such oligonucleotide inhibitors is uncertain. Whether direct targeting of inflammasomes reduces CVD risk without increasing infections remains to be determined.

### Inhibition of pathways downstream of inflammasomes.

NLRP3 and AIM2 inflammasome activation that have been implicated in CHIP-promoted atherosclerosis leads to secretion of active IL-1 $\beta$  and IL-18. Plasma IL-1 $\beta$  is increased in TET2 CHIP, whereas IL-18 is increased in JAK2<sup>VF</sup> CHIP<sup>27</sup>. Given the positive outcome of CANTOS, and the results of preclinical studies cited above, IL-1 $\beta$  antagonism seems like a logical first choice for targeting TET2 and possibly JAK2<sup>VF</sup> CHIP-associated CVD.

IL-18 has a major role in the production of IFN $\gamma$  by T cells and natural killer cells<sup>89</sup> and increases atherosclerosis in mice via release of IFN $\gamma$  (ref.<sup>90</sup>) and other mechanisms<sup>91</sup>. IFN $\gamma$  is potentially atherogenic<sup>92</sup> and has a specific role in JAK2<sup>VF</sup>-promoted atherosclerosis via upregulation of AIM2 (ref.<sup>32</sup>). The therapeutic potential of IL-18 inhibition in CHIP-associated atherosclerosis deserves further exploration.

IL-6 may be increased downstream of inflammasome activation; IL-1 and IL-6 levels were moderately increased in the three commonest forms of CHIP that associate with CVD (TET2, DNMT3A and ASXL1)<sup>27</sup>. However, this was not associated with increases in CRP levels. The increased CVD risk associated with TET2- and DNMT3A-mutant CHIP was abrogated by the D358A variant of IL-6R, pointing to a key role of IL-6 in the development of CVD in people with CHIP<sup>74</sup>. In another study, this variant did not impact total mortality or CHD in overall CHIP; however, protection was seen in the subgroup of individuals with TET2 CHIP and aging biomarkers<sup>20</sup>. Direct signaling of IL-6 occurs when it binds to its receptor (IL-6R) in the liver and WBCs. This leads to cleavage of the IL6R  $\alpha$ -chain that forms a circulating complex with IL-6; the complex interacts with gp130 in a variety of cells to mediate *trans*-signaling<sup>93</sup>. The D358A variant is associated with reduced cell surface expression of IL-6R along with an increase in soluble IL6R receptor that has the potential to mediate *trans*-signaling<sup>94</sup>. Several studies have implicated IL-6 in the pathogenesis of atherosclerosis in mice<sup>95</sup>, especially the *trans*-signaling pathway<sup>96</sup>. However, knockout of the *Il6* gene in *Apoe*<sup>-/-</sup> mice exacerbated hyperlipidemia and promoted advanced atherosclerosis<sup>93,97</sup>. Despite this complexity and apparent inconsistency, it remains plausible that targeting IL-6 might improve the net therapeutic benefit in selected individuals with atherosclerotic CVD<sup>93</sup>. One complication of IL-6 antagonism with tocilizumab in people with rheumatoid arthritis has been elevated levels of triglycerides, non-HDL-cholesterol and APOB<sup>98</sup>. The IL-6 antagonist ziltivekimab is being developed for chronic-kidney-disease associated atherosclerotic disease and was found to reduce inflammatory biomarkers in a phase 2 trial; however, this appeared to be accompanied by an increase in APOB levels<sup>99</sup>. Further assessment of IL-6 antagonism combined with rigorous control of plasma lipoprotein levels as a potential treatment option for CHIP-associated CVD is warranted.

### CHIP-mutation-specific therapies.

Vitamin C metabolites activate *TET2* and can mimic restoration of *TET2* by enhancing 5-hydroxymethylcytosine formation in *TET2*-deficient mice, leading to reversal of aberrant HSC self-renewal; this could represent a potential preventive treatment for *TET2* CHIP carriers to prevent CVD<sup>100,101</sup>. JAK2<sup>VF</sup>, although less common than TET2 and DNMT3A variants, has important attributes that make it an attractive target for therapy to reduce CVD. JAK2<sup>VF</sup> increases venous and arterial thrombotic disease and atherosclerosis considerably more than the other CHIP variants, likely increases disease at lower allele burden than other variants, occurs at a younger age and is amenable to multiple approaches to inhibition that may prove beneficial for both MPN and CHIP. In JAK2<sup>VF</sup> CHIP, treatment with the JAK1/JAK2 inhibitor ruxolitinib reduced abnormal NET formation and deep vein thrombosis<sup>43</sup>. Ruxolitinib causes substantial increases in LDL cholesterol in JAK2<sup>VF</sup> mice and humans. In a JAK2<sup>VF</sup> mouse CHIP model, ruxolitinib treatment led to the development of slightly smaller atherosclerotic lesions with markedly increased

necrotic cores and thinner caps<sup>32</sup>, which are features of unstable plaques in humans. By contrast, JAK2 inhibition by fedratinib in *ApoE*<sup>-/-</sup> mice suppressed myelopoiesis and the development of atherosclerosis<sup>102</sup>. Ruxolitinib inhibits both JAK1 and JAK2 and thus could have broad effects on signaling pathways that are required for cell survival. Ruxolitinib also reduces oxidative metabolism of glucose in *Jak2*<sup>VF</sup> cells, leading to depletion of NADPH and glutathione with the potential to promote oxidative cell death<sup>103</sup>. While one should exercise caution in extrapolating from mouse models to humans, it is notable that the Food and Drug Administration recently issued a regulatory warning concerning potential increased thrombosis and CAD in people taking the related JAK inhibitors baricitinib, tofacitinib and upadacitinib for treatment of arthritis or ulcerative colitis. This could indicate the need for more specific targeting of JAK2 or JAK2<sup>VF</sup>. To reduce athero-thrombotic risk, people carrying JAK2<sup>VF</sup> might benefit from more effective anti-thrombotic therapy, such as multiple dosing with aspirin, to compensate for increased platelet production<sup>104</sup>, or specific JAK2 inhibitors. Since platelet-associated oxidized phospholipids promote NETosis and arterial thrombosis in Lnk-deficient mice, anti-OxPL antibodies that reduced both atherosclerosis and arterial thrombosis in JAK2<sup>VF</sup> CVD could be a novel therapeutic approach<sup>47</sup>. LNK inhibits both JAK2 and JAK2<sup>VF</sup> signaling<sup>105</sup>. LNK increases the association of JAK2 with the E-3 ligase CBL, promoting its ubiquitination and degradation<sup>106</sup>. Thus, increasing the activity of the E-3 ligase CBL on JAK2<sup>VF</sup>, for example, via proteolysis targeting chimeras (PROTACs), could lead to increased JAK2<sup>VF</sup> degradation and thus a reduction in MPN and CVD.

## Outlook

The convergence of recent evidence showing the efficacy of anti-inflammatory treatments in atherosclerotic CVD with the discovery that CHIP promotes atherosclerosis via increased plaque inflammation and inflammasome activation presents an important new therapeutic opportunity that could potentially lead to an acceptable risk/benefit ratio of anti-inflammatory therapy in individuals with CHIP. A variety of potential therapies may be considered, but those targeting common downstream factors, such as IL-1 $\beta$  or IL-6, and CHIP-mutation-specific therapies appear to be most promising. Future clinical trials enriched for individuals with CHIP and high atherosclerotic risk will be required to establish the efficacy and safety of such approaches.

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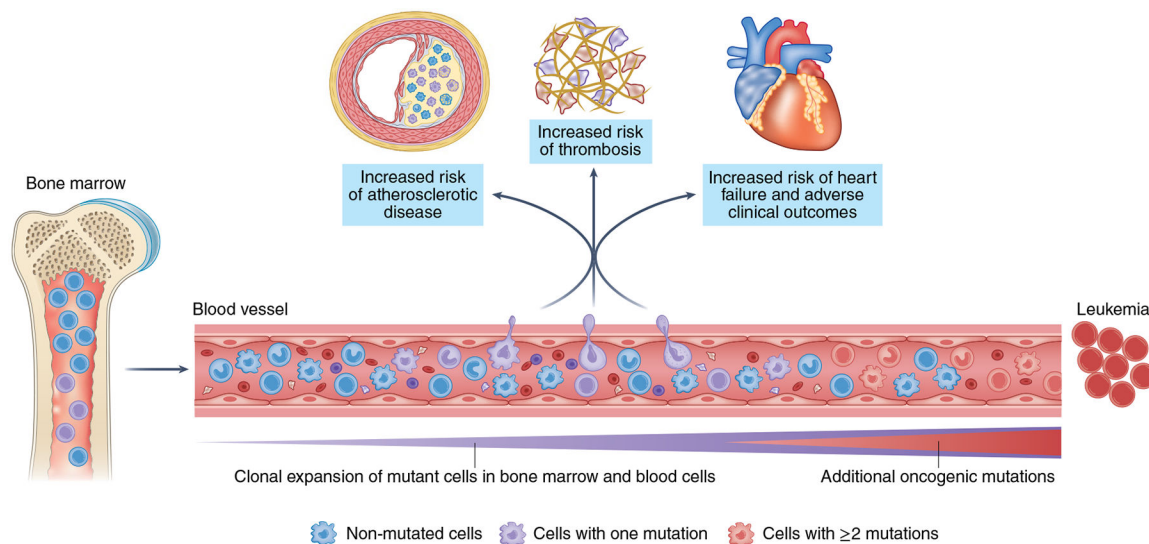
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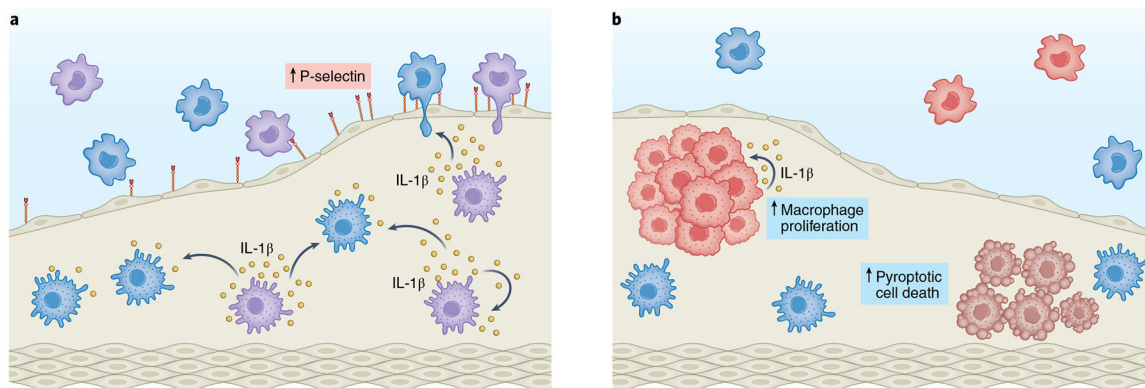
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**Fig. 1 | Somatic mutations and clonal hematopoiesis: at the crossroads of hematological malignancies and cardiovascular disease.**

Hematopoietic stem cells accumulate somatic mutations continuously with aging. Some of these mutations confer a competitive advantage to the mutant cell, leading to its clonal expansion in bone marrow and blood. This clonal hematopoiesis is most frequently driven by one single mutation. While clonal hematopoiesis is associated with a large increase in the relative risk of developing a hematologic neoplasia, transition to malignancy typically requires the acquisition of multiple mutations, which is infrequent, even in individuals with clonal hematopoiesis. The main cause of death in individuals exhibiting clonal hematopoiesis is cardiovascular disease. Epidemiological and experimental evidence suggest that some clonal-hematopoiesis-related mutations can contribute to the development and clinical progression of atherosclerosis, heart failure and thrombosis. Heightened inflammatory responses mediated by tissue-infiltrating mutant immune cells are emerging as a central link between clonal hematopoiesis and cardiovascular disease.



**Fig. 2 |. Mutation-specific mechanisms linking clonal hematopoiesis to atherosclerotic cardiovascular disease.**

Emerging evidence supports that different mutant genes promote inflammation and atherosclerosis through diverse mechanisms. **a**, TET2-deficient macrophages (purple cells) exhibit increased IL-1 $\beta$  transcript levels and higher NLRP3 inflammasome-mediated IL-1 $\beta$  secretion. Heightened IL-1 $\beta$  levels in the plaque promote P-selectin expression and endothelial cell activation, leading to increased recruitment of circulating leukocytes. IL-1 $\beta$  may further stimulate its own expression in both TET2-mutant cells and WT macrophages (light blue cells) through an autoregulatory autocrine–paracrine feedback loop. **b**, JAK2<sup>VF</sup> expression in macrophages leads to an overactivation of the AIM2 inflammasome, which increases JAK2<sup>VF</sup> mutant macrophage proliferation (red cells) in a manner dependent on intrinsic JAK2<sup>VF</sup> function and IL-1 $\beta$  signaling. Increased AIM2 inflammasome activity also leads to greater pyroptotic cell death of mutant macrophages (darker red cells), which contributes to the expansion of necrotic cores within the atherosclerotic plaque.

Summary of experimental studies in mouse models that reported the effects of the most frequent CH candidate driver mutations on cardiovascular disease

Table 1 |

CH candidate gene	Experimental atherosclerosis phenotype	Experimental cardiac phenotype
<i>DNMT3A</i>	Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice after competitive BMT with 10% <i>Dnmt3a</i> <sup>-/-</sup> BM cells <sup>38</sup>	Worse cardiac function and remodeling in angiotensin-II-infused mice bearing CRISPR-Cas9-edited <i>Dnmt3a</i> in hematopoietic cells <sup>39</sup>
<i>TEI2</i>	Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice after competitive BMT with 10% <i>Tet2</i> <sup>-/-</sup> or <i>Tet2</i> <sup>+/+</sup> BM cells <sup>25</sup> Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice with pan-hematopoietic ablation of <i>Tet2</i> (ref. <sup>14</sup> ) Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> with myeloid-restricted ablation of <i>Tet2</i> (refs. <sup>14,25</sup> )	Worse cardiac function and remodeling after LAD ligation or TAC in mice subjected to competitive BMT with 10% <i>Tet2</i> <sup>-/-</sup> or <i>Tet2</i> <sup>+/+</sup> BM cells <sup>34</sup> Worse cardiac function and remodeling after LAD ligation or TAC in mice with myeloid-restricted <i>Tet2</i> deficiency <sup>34</sup> Worse cardiac function and remodeling in angiotensin-II-infused mice bearing CRISPR-Cas9-edited <i>Tet2</i> in hematopoietic cells <sup>39</sup> Worse cardiac function and remodeling in aged mice after non-conditioned BM transfer of <i>Tet2</i> <sup>-/-</sup> cells <sup>55</sup>
<i>ASXL1</i>	Unknown	Unknown
<i>JAK2</i>	Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice with pan-hematopoietic expression of <i>Jak2</i> <sup>Δ617E</sup> (ref. <sup>31</sup> ) Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice with competitive BMT or macrophage-specific expression of <i>Jak2</i> <sup>Δ617E</sup> (ref. <sup>32</sup> )	Worse cardiac function and remodeling after LAD ligation or TAC in mice with lentivirus-mediated expression of <i>Jak2</i> <sup>Δ617E</sup> in myeloid cells <sup>36</sup>
<i>TP53</i>	Accelerated atherosclerosis in <i>Ldlr</i> <sup>-/-</sup> mice after competitive BMT with 20% <i>Tip53</i> <sup>-/-</sup> BM cells <sup>15</sup>	Greater doxorubicin-induced cardiac toxicity in mice after non-conditioned BM transfer of <i>Tip53</i> <sup>-/-</sup> cells <sup>38</sup>
<i>PPM1D</i>	Unknown	Worse cardiac function and remodeling in angiotensin-II-infused mice bearing hematopoietic cells expressing a CRISPR-Cas9-edited <i>Ppm1d</i> mutant <sup>59</sup>

LAD, left anterior descending artery; TAC, transverse aortic constriction