Thrombosis and Haemostasis

CHIP and atherothrombotic risk

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DOI: 10.1055/a-1830-2147

Please cite this article as: Murphy AJ, Dragoljevic D, Natarajan P et al. CHIP and atherothrombotic risk. Thromb Haemost 2022. doi: 10.1055/a-1830-2147

Conflict of Interest: P.N. reports grants from Amgen, Apple, AstraZeneca, Boston Scientific, and Novartis, personal fees from Apple, AstraZeneca, Blackstone Life Sciences, Foresite Labs, Genentech / Roche, Novartis, and TenSixteen Bio, equity in geneXwell, TenSixteen Bio, and Zizi, and spousal employment at Vertex. P.N. is a co-founder of TenSixteen Bio, a company focusing on somatic mutations in blood cells to reduce risks for blood cancer and atherosclerotic cardiovascular disease; his interests were reviewed and are managed by Massachusetts General Hospital and Mass General Brigham in accordance with their conflict of interest policies.

This study was supported by 
Jack Brockhoff Foundation (http://dx.doi.org/10.13039/100012698), JBF 4867-2021, National Health and Medical Research Council (http://dx.doi.org/10.13039/501100009255), APP1142938,APP1194329, National Heart, Lung, and Blood Institute (http://dx.doi.org/10.13039/100000050), R01HL118567, R01HL127564, R01HL135242, R01HL142711, R01HL148050, R01HL148071, R01HL148565, R01HL151152, R01HL151283, National Institute of Diabetes and Digestive and Kidney Diseases (http://dx.doi.org/10.13039/100000062), R01DK125782, Fondation Leducq (http://dx.doi.org/10.13039/1000001674), TNE-18CVD04, CSL Behring (http://dx.doi.org/10.13039/100008322), Centenary Award

Abstract:
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CHIP and Atherothrombotic Risk

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Abstract

Haematopoiesis is the process of blood production, essential for the continued supply of immune cells and red blood cells. However, the proliferative nature of haematopoietic stem cell (HSCs) renders them susceptible to developing somatic mutations. HSCs carrying a mutation can gain a selective advantage compared to normal HSCs and result in haematological disorders. One such disorder is termed clonal haematopoiesis of indeterminate potential (CHIP), a pre-malignant state associated with ageing, where the mutant HSCs are responsible for producing a small portion of the mature immune cells in the circulation and subsequently in tissues. People with CHIP have been shown to have an increased risk of mortality due to cardiovascular disease (CVD). Why this occurs is under rigorous investigation, but the majority of the studies to date suggest increased atherosclerosis is due to heightened inflammatory cytokine release from mutant lesional macrophages. However, given CHIP is driven by several mutations, other haematopoietic lineages can be altered to promote CVD. In this review we explore the relationship between mutations in genes causing CHIP and atherothrombotic disorders, along with potential mechanisms of enhanced clonal outgrowth and potential therapies and strategies to slow CHIP progression.

Haematopoiesis is the tightly regulated and hierarchical process of blood cell production. This process originates from haematopoietic stem cells (HSCs) through to lineage committed progenitors and mature leukocytes, red blood cells and platelets. The development of specific lineages is regulated through intrinsic factors including precise combinations of transcription factors and epigenetic modifications, along with extrinsic cues such as cytokines and growth factors, resulting in the expression or repression of gene signatures to shape the morphological and functional capabilities of the mature cell. However, these processes can be altered with aging causing fundamental alterations to the haematopoietic system. One of the irreversible changes that occurs in all somatic cells is the acquisition and persistence of mutations due to inefficiencies in DNA repair mechanisms. In HSCs, somatic mutations can accumulate with each division, with most having no overt functional effect and many resulting in diminished HSC function initiating programmed cell death and clearance of the mutant cell. However, some mutations in HSCs evade clearance and can result in a competitive advantage, characterized by increased self-renewal, proliferation, survival, and biased lineage output. To put this into numbers, HSCs are thought to acquire 1-2 mutations per division, which when extrapolated, equates to ~10 mutations/year and once we reach adulthood, modelling suggest that most of our HSCs will have 2 coding mutations and as many as 200,000 non-coding mutations. This suggests that by the time we
are adults each HSC is unique and keeps acquiring mutations. These mutations can result in a process termed clonal haematopoiesis of indeterminate potential (CHIP), which is a form of clonal haematopoiesis, but is not driven by other mechanisms such as clonal mosaicism. Individuals with CHIP have an increased risk of mortality, which is now linked to cardiovascular disease (CVD). In this review, we will focus on CHIP mutations associated with thrombotic disease, the mechanisms contributing to this pathology and potential therapies.

**Clonal haematopoiesis of indeterminate potential**

Somatic mutations can result in several haematological disorders. CHIP occurs when HSCs acquire a somatic mutation providing them with a competitive growth advantage over normal HSCs. This results in a relative increased number of mutated haematopoietic cells in the bone marrow and blood. While the overall abundance of white blood cells (WBCs) is only modestly affected, over time the proportion of mutated cells in the blood grows at the expense of normal WBCs. CHIP is an ageing phenomenon, because a key feature is insufficient repair of damaged DNA which may then be differentially propagated depending on mutational fitness. CHIP is defined as a variant allele frequency (VAF) of >2% in circulating WBCs (i.e. >4% of WBCs carry the mutation in one allele). Largely based on whole exome sequence studies of blood DNA in various datasets, it is estimated that ~5% of individuals aged under 60 years display CHIP, which increase to ~10% aged over 60 years and is continued to increase with age. Deep targeted sequencing indicates that haematological somatic leukemogenic mutations at very low VAF (i.e., median 0.2%) are almost ubiquitous in middle age healthy adults. This finding infers that we are all at some point in our lives at risk of developing CHIP and other haematological disorders.

Mutations indicative of CHIP are most commonly observed in the genes DNMT3A, TET2, ASXL1, JAK2, and TP53. Why mutations in the epigenetic modifiers DNMT3A and TET2 are such prevalent drivers of CHIP is not known. However, the relatively open chromatin structure of HSCs suggest gene expression is largely governed by methylation status and may reveal why mutations in DNMT3A (methylation) and TET2 (hydroxymethylation) cause such dominant changes in HSCs to promote their outgrowth. Certainly, studies exploring the deletion or loss of function of these genes demonstrate the competitive advantage these mutant HSCs acquire. The loss in DNMT3A and thus reduced DNA methylation results in the increased expression of genes involved in HSC proliferation and self-renewal, while the prevention of hydroxymethylation when TET2 is non-functional destabilises key HSC maintenance genes which promotes both hyperproliferation and myeloid skewing.

**CHIP & CVD**

CHIP driving mutations are known to increase the risk of hematologic malignancy and carriers have 10 times the risk of hematologic cancer as those without such mutations do. Initial analysis has found an association of CHIP with increased all-cause mortality, but the increased risk of haematological malignancies of 0.5-1% per year is not nearly enough to account for the 40% increase in mortality. Further analyses identified a strong association of CHIP with a higher risk of CVD independent of age and other traditional risk factors. The direct evidence for causality was first provided by animal studies.
Haematopoietic Tet2$^{-/-}$ or Tet2$^{+/+}$ markedly increased atherosclerosis in hypercholesterolemic Ldlr$^{-/-}$ mice$^{21,23}$. Mechanistically, Tet2 deficient macrophages showed increased NLRP3 inflammasome activation and elevated IL-1β production (Figure 1A)$^{21,23}$. Concentrations of related biomarkers are also increased among individuals with CHIP$^{24,25}$. An NLRP3 inhibitor selectively reversed the increased atherosclerosis in the Tet2$^{-/-}$ CHIP model$^{23}$.

JAK2V617F (JAK2$^{VF}$) is less common than the mutations of epigenetic modifiers such as TET2, DNMT3A or ASXL1$^{13,21}$. Nevertheless, a recent study, with innovative deep targeted sequencing that had a screening sensitivity as low as VAF 0.01%, found the JAK2$^{VF}$ mutation is detectable in almost 4% of a general European population$^{26}$. Among the JAK2$^{VF}$ individuals in this population, approximately 60% had a VAF of >0.1% but most of whom did not have features of myeloproliferative neoplasm (MPN). CHIP-associated JAK2$^{VF}$ occurs at a younger age than the other CHIP variants$^{27}$ and dramatically increases risk of myocardial infarction by as much as 12-fold in younger people$^{21}$. We found that JAK2$^{VF}$ increases atherosclerotic disease despite lowering LDL cholesterol in both mice and humans$^{28,29}$. Interestingly, it has been shown that mouse models of JAK2$^{VF}$ resembling a MPN (i.e. 100% JAK2$^{VF}$ bone marrow transplant; BMT), or CHIP (20% Jak2$^{VF}$:80% WT BMT) both increase atherosclerosis$^{29,30}$. JAK2$^{VF}$ causes altered functionality of multilineage blood cells$^{29}$. Selective expression of JAK2$^{VF}$ in monocyte/macrophage increases atherosclerosis in association with increased generation of IL-1β and IL-18, the product of inflammasome activation$^{30}$. Unlike Tet2 deficiency, knockout of NLRP3 has little effect while deletion of AIM2, the essential component of AIM2 inflammasome, reduces the increased atherosclerosis in JAK2$^{VF}$ models (Figure 1B). Inflammasome activation can lead to programmed cell death that is mediated by the pyroptosis executioner gasdermin D (Gsdmd), leading to release of inflammasome activation product such as IL-1β. Gsdmd$^{-/-}$ reduces atherosclerosis in JAK2$^{VF}$ mice$^{30}$.

Chronic inflammation associated with atherosclerosis has long been thought to mediate atherosclerosis progression$^{31}$. The recent Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) largely validated this notion$^{32}$. IL-1β inhibition by canakinumab reduces incident CVD events in individuals with prevalent CVD and elevated high-sensitivity C-reactive protein (hsCRP), a marker of inflammation$^{32}$. However, canakinumab therapy was associated with only a moderate clinical benefit and an increase in infections. Consequently, canakinumab has not been marketed for cardiovascular indications. Similarly, colchicine, which inhibits the microtubule-dependent assembly of the NLRP3 inflammasome and IL-1β secretion,$^{33,34}$ appeared to benefit CVD but also increased pneumonia$^{35,36}$.

A more precise approach to identify patients who may benefit most from anti-inflammatory therapy, as well as identification of upstream therapeutic targets, could lead to more effective, safer anti-inflammatory treatments for CVD. Enhanced inflammation in NLRP3 or AIM2 inflammasome/IL-1β axis in TET2 mutant or JAK2$^{VF}$ atherosclerosis models suggests individuals with TET2 mutant or JAK2$^{VF}$ CHIP and increased risk of CVD could benefit more from anti-inflammation therapy. In the preclinical JAK2VF CHIP model, administration of an anti-IL-1β antibody improved readouts of plaque stability, but did not change overall lesion size and had no apparent effect in control mice$^{30}$. Consistently, in an exploratory preliminary analysis, patients with a TET2 mutant have an improved response to canakinumab (HR=0.36) relative to the response to overall response to canakinumab (HR=0.85) in the CANTOS trial$^{37}$. In addition to the pharmacological evidence, human genetic studies provide
more support for a causal role of inflammation in CVD. IL-6 can be a downstream product of IL-1β signaling and increased IL-6 production and activity are considered to be a common mediator in chronic inflammation associated with CVD. A disruptive IL-6R missense variant is associated with 5% reduced CVD risk in a general population.\textsuperscript{38,39} A more recent study indicates that this IL-6R variant attenuates CVD risk in individuals with CHIP by 54%, supporting the notion that individuals with CHIP may benefit most from IL-6 inhibition to reduce the risk of CHIP-associated CVD.\textsuperscript{11}

**CHIP and Thrombosis**

Several somatic mutations have been linked to quantitative or qualitative abnormalities in platelets (reviewed by Veninga et al\textsuperscript{40}). Among the common CHIP driving mutations that effect platelets, the evidence for increased risk of atherothrombosis primarily comes from studies of JAK2\textsuperscript{VF} associated MPNs and CHIP. Patients with MPNs have increased risk of arterial and venous thrombosis and thrombotic complications\textsuperscript{41-42}. The studies of thrombotic risk conferred by JAK2\textsuperscript{VF} CHIP without apparent MPNs was significantly advanced relatively recently. In a population study of over 10,000 individuals without a known myeloid disorder, JAK2\textsuperscript{VF} CHIP was associated with an increased incidence of thrombosis\textsuperscript{44} (Figure 1). Polycythemia vera (PV), in which more than 90% of the patients are JAK2\textsuperscript{VF} positive,\textsuperscript{45} has increased haematocrit in association with increased blood viscosity,\textsuperscript{46} a major risk factor of thrombosis in PV patients. Phlebotomy has been successfully used to maintain the haematocrit and reduce the risk of thrombosis in PV patients\textsuperscript{43}. Thrombotic risk also is increased in JAK2\textsuperscript{VF} MPN patients without apparently increased haematocrit. JAK2\textsuperscript{VF} increases myeloid cell production, causing leukocytosis. Increased WBC count has been associated with an increased risk of thrombosis in MPNs. However, many with JAK2\textsuperscript{VF} CHIP do not show signs of abnormal blood cell counts yet they still have increased risk of thrombosis.\textsuperscript{44} JAK2\textsuperscript{VF} is generally thought to only affect platelets, but platelets can interact with many cell types. In the circulation, neutrophils often form aggregates with platelets which can activate these cells. A consequence of neutrophil activation is the formation of neutrophil extracellular traps (NETs), where neutrophils release their contents leading to the formation of web-like structures (termed NETosis) made of DNA, myeloperoxidase, citrullinated histones and proteases that entrap and kill bacteria.\textsuperscript{47} While NETs may help to suppress infections, the formation of NETs in blood vessels can promote atherosclerosis and thrombosis\textsuperscript{48,49} (Figure 1B). NETs may serve as biomarkers predicting the risk of thrombosis\textsuperscript{49}. Neutrophils from patients with MPNs display some features of enhanced activation\textsuperscript{50,51}. Subsequent studies showed that neutrophils from MPN patients are primed for NETosis\textsuperscript{44}. However, this isn’t always observed\textsuperscript{52}. JAK2\textsuperscript{VF} modelled mice showed increased NET formation and venous thrombosis, which were reduced by DNase treatment or hematopoietic deficiency of peptidyl-arginine deiminase 4 (PAD4), the enzyme essential for citrullination of histones in NET formation\textsuperscript{44}.

Various mouse models have been generated to assess the impact of JAK2\textsuperscript{VF} on arterial thrombosis, including mouse JAK2\textsuperscript{VF} knockin, human JAK2\textsuperscript{VF} transgenic and knockin of human JAK2\textsuperscript{VF} cDNA into the mouse Jak2 allele, with constitutive or tamoxifen induced expression in hematopoietic and endothelial cells.\textsuperscript{53-55} These models have different levels of mouse or human JAK2\textsuperscript{VF} expression in different types of cells and generate various
thrombotic and haemostasis phenotypes, varying from increased platelet activation, reduced tail vein bleeding time and increased thrombus formation in a flow chamber assay to decreased platelet activation in response to convulxin or TRAP4, increased tail vein bleeding and accelerated arterial thrombosis with unstable thrombi in vivo. A common feature of these studies is modelling of MPNs with high JAK2VF burden but not CHIP. The impact of JAK2VF CHIP with lower burden on platelet activity and arterial thrombosis in mice is not known (Figure 1B). We have some evidence that modelling CHIP in mice with a small fraction (2-20%) of hematopoietic cells expressing JAK2VF shows increased platelet activation to thrombin and accelerated arterial thrombosis, without increased bleeding (unpublished observations).

SH2B3/LNK encodes an adaptor protein that acts as a negative regulator of JAK2 mediated hematopoietic cell proliferation. Lnk deficiency promotes multilineage expansion of HSCs in mice. LDLR−/− mice with hematopoietic LNK deficiency display increased atherogenesis and accelerated atherothrombosis. Lnk deficiency and hypercholesterolemia act synergistically promoting platelet activation and myelopoesis. More recently, we show that the increased arterial thrombosis in hematopoietic Lnk deficient mice is due to NETosis in the thrombi and accelerated thrombosis is completely reversed by neutrophil depletion or PAD4 deficiency. Mechanistic studies have identified oxidized phospholipids (OxPL) released and presented by activated platelets mediate neutrophil activation and NETosis (Figure 1C). Lnk−/− mice show increased plasma OxPL levels and transgenic expression of E06-scFv, that specifically binds and neutralizes OxPL activity, selectively and completely reverses NETosis in thrombi and accelerated thrombosis in Lnk deficiency. A common SH2B3 polymorphism (p.R262W, c.784T>C) is a loss of function LNK variant in association with increased platelet and neutrophil counts and the risk of CVD. Consistent with this, we observed increased NETosis in co-cultures of human induced pluripotent stem cell (iPSC)-derived neutrophils and activated platelets carrying isogenic LNK(TT) relative to LNK(CC). Interestingly, the LNK(R262W, T allele) predisposes to both JAK2VF MPN and CHIP. We also confirmed this with data from UK Biobank, showing that individuals with the JAK2VF mutation only displayed increased CAD when also carrying the LNK(TT) allele.

Accelerators of CHIP

Clone size, as estimated by VAF, is strongly associated with the prognosis of CHIP, whether it be cancer, subclinical atherosclerosis, atherosclerotic CVD, or heart failure. Thus, identifying CHIP early will provide a window of opportunity to slow clonal growth and avoid CV complications. However, there is a scarcity of longitudinal sampling to confidently explore and define drivers of clonal outgrowth over time, with much of our knowledge coming from pre-clinical models. Thus, a major outstanding question is what drives clonal outgrowth and how can this be halted? Given that CHIP is driven by somatic mutations providing a competitive advantage, one strategy could be to define driver genes and target their expression or protein function to slow proliferative rates or kill the mutant HSCs. This is a complicated option as the major genes mutated in CHIP are epigenetic modifiers and kinases which have a significant impact on a network of genes involved in stem cell maintenance and proliferation. We suggest that understanding the interactions between the mutations and environmental drivers of CHIP is key in delaying clonal outgrowth and may reduce the risk of CVD in the context of CHIP.
An alternative approach to exploring the gene regulatory networks that are altered by somatic mutations is to identify the extrinsic drives of clonal outgrowth. It is slowly emerging that clonal outgrowth is linked with extrinsic factors including co-morbidities, diets, smoking along with inflammatory status and infections\textsuperscript{13,21,25,63-65} (Figure 2). In the initial studies linking CHIP to mortality due to CVD, an over representation of individuals with metabolic disorders, namely diabetes, was noted\textsuperscript{13}. Indeed, diabetes has been linked with leukaemia\textsuperscript{66,67} and in murine models diabetes has been shown to cooperate with Tet2 heterozygosity to cause leukaemia\textsuperscript{68}. TET2 deficiency can also aggravate insulin resistance in mice\textsuperscript{69}. Metabolic stressors such as unhealthy diets have now been linked to a higher prevalence of CHIP\textsuperscript{70,71}. Additional evidence to support the hypothesis that altered lipid and glucose metabolism often seen in individuals consuming unhealthy diets or with diabetes is linked with clonal outgrowth was seen in the Swedish Obese Subjects (SOS) study\textsuperscript{65}. In this pre-print manuscript, the authors report that over a 20 year follow up period, growing clones in the obese individuals were found to correlate with low HDL levels insulin levels and HOMA-index as a read out of insulin resistance (Figure 2). However, this data was based off a small sample size of <40 individuals and requires a larger follow study. Nonetheless, the mechanism(s) responsible for this are unknown, but could relate to low grade chronic inflammation, which is a known consequence of obesity, providing cytokines that would enhance the proliferation of the HSCs harbouring mutations. This hypothesis was also illustrated by the Naxerova group, revealing that disorders underpinned by enhanced haematopoietic activity (i.e. atherosclerosis and sleep fragmentation) accelerated clonal outgrowth\textsuperscript{64}. However, it is important to note in the SOS study, hsCRP was not associated with clonal outgrowth\textsuperscript{65}. We hypothesize that clonal outgrowth in metabolic disorders is due to an alternative mechanism\textsuperscript{72}. Firstly, we have previously shown that obesity and diabetes promotes increased haematopoiesis at the level of the CMP and GMP, while HSCs are largely unaffected in respects to abundance\textsuperscript{73,74}. This suggests that enhanced haematopoiesis at the level of the HSCs due to extrinsic signalling is unlikely to be responsible. Instead, high energy environments such as obesity and diabetes could reduce the activity of epigenetic modifiers particularly TET2, which is reliant on AMPK activity\textsuperscript{75}. Through this mechanism, further loss of TET2 function in TET2 mutant cells (i.e. 1 mutant allele), or combination with mutations in other genes may synergize to cause myeloid skewing and increase clonal outgrowth. This is consistent with haematopoietic TET2 heterozygote mice that display transition to leukaemia in a model of hyperglycaemia\textsuperscript{68}. If this is true, treating individuals with agents such as metformin or novel AMPK activators along with life-style interventions may be effective in slowing the expansion of mutant cells in these individuals. However, the impact on extrinsic stimuli in promoting clonal outgrowth is largely limited to mice and required large longitudinal clinical studies to address our hypothesis more accurately.

**Interventions for individuals with CHIP**

There is no approved treatment for CHIP related CVD risk. CANTOS\textsuperscript{32} and genetic evidence\textsuperscript{11} suggest that anti-IL-1β and anti-IL-6 could be particularly effective and potentially broadly beneficial to CHIP carriers. Targeting NLRP3 or AIM2 inflammasome may need to be tailored to the specific genetic factors responsible for CHIP. While ruxolitinib (RUX), a JAK1/2 inhibitor, has been approved for JAK2\textsuperscript{67} MPNs, it shows no benefit in atherogenesis in the
JAK2\textsuperscript{VF} modelled mice.\textsuperscript{30} Fedratinib, a selective JAK2 inhibitor, is newly approved for MPN associated myelofibrosis\textsuperscript{76}. We showed that Fedratinib reduced atherogenesis in Apoe\textsuperscript{-/-} mice at least partly by reducing aberrant myelopoiesis\textsuperscript{77} but its impact on CHIP-driven atherogenesis is not known. The ASPREE trial indicates that aspirin use in the healthy elderly does not provide benefit against CVD but increases the risk of major hemorrhage,\textsuperscript{78} suggesting the need for more targeted therapy. Interestingly, evidence exists suggesting that individuals with JAK2\textsuperscript{VF} MPNs benefit more from aspirin relative to MPN patients carrying no JAK2\textsuperscript{VF} \textsuperscript{79,80}. Potentially, aspirin could be more effective in JAK2\textsuperscript{VF} CHIP carriers to reduce thrombosis risk. OxPL has long been considered as a risk factor for CVD\textsuperscript{81}. Anti-OxPL therapy could potentially be particularly beneficial for LNK mutant carriers or individuals with LNK risk polymorphism who have increased atherothrombotic risk.

Another important point to consider is surveillance for and managing clonal expansion. It has been shown that individuals with larger clones are at great risk of mortality and that people with small stable clones generally live healthy lives. As discussed above there is growing knowledge surrounding what lifestyle factors and co-morbidities drive clonal outgrowth\textsuperscript{18,34,64,73}. We suggest another approach to avoid small clones becoming problematic could be to effectively treat co-morbidities, alter lifestyles (i.e. diets, cessation of smoking) or activate pathways that might slow the proliferation of the mutated cells, which will likely be dependent on the mutated gene.

Nonetheless, since discovering the link between CHIP and CVD, experimental interventions targeting inflammation may find an indication in individuals with CHIP. With the movement towards precision medicine in the cardiovascular field, it may be important to define the genetic drives of CHIP to treat these individuals effectively and significantly reduce their risk of CVD.

Funding
A.J.M is supported by project (APP1142938) and investigator (APP1194329) grants from the National Health and Medical Research Council and a CSL Centenary Award; D.D is supported by a grant from the Jack Brockhoff foundation (JBF 4867-2021) and Diabetes Australia. P.N. is supported by grants from the National Heart, Lung, and Blood Institute (R01HL142711, R01HL148050, R01HL151283, R01HL127564, R01HL148565, R01HL135242, R01HL151152), National Institute of Diabetes and Digestive and Kidney Diseases (R01DK125782), and Massachusetts General Hospital (Paul and Phyllis Fireman Endowed Chair in Vascular Medicine) and by a grant from Fondation Leducq (TNE-18CVD04). N.W. is supported by grants from the National Heart, Lung, and Blood Institute (R01HL118567, R01HL148071).

Disclosures
P.N. reports grants from Amgen, Apple, AstraZeneca, Boston Scientific, and Novartis, personal fees from Apple, AstraZeneca, Blackstone Life Sciences, Foresite Labs, Genentech / Roche, Novartis, and TenSixteen Bio, equity in geneXwell, TenSixteen Bio, and Zizi, and spousal employment at Vertex. P.N. is a co-founder of TenSixteen Bio, a company focusing on somatic mutations in blood cells to reduce risks for blood cancer and atherosclerotic cardiovascular disease; his interests were reviewed and are managed by Massachusetts
General Hospital and Mass General Brigham in accordance with their conflict of interest policies.

Conflict of Interest
None declared.

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Figure 1. Mechanistic features of haematopoietic mutations resulting in increased risk of atherosclerosis and atherothrombotic disease. A. Somatic mutations in TET2 have been shown to accelerate atherosclerosis through inflammatory macrophage signaling driven by the NLRP3/IL-1β axis, which when inhibited reduces atherosclerosis. B. Somatic mutations in JAK2 can result in myeloproliferative neoplasms which influence atherothrombotic disorders, namely venous thrombosis. However, when JAK2 mutations result in CHIP enhanced activation of the AIM2 inflammasome occurs to release inflammatory cytokines through gasdermin D pores. Whether this causes atherothrombosis is not yet known. C. Germline mutations in SH2B3 increases the risk of atherothrombotic disease. Inflammatory interactions between neutrophils and platelets occur, particularly driven by platelet release oxidised phospholipids (oxPL), causing NETosis. Interestingly, JAK2 mutations are linked with SH2B3 mutations, where the LNK(R262W, T allele) predisposes individuals to JAK2 vulgar MPN and CHIP, along with coronary artery disease.

Figure 2. Accelerators of clonal haematopoiesis. Somatic mutations are ubiquitous in people of middle age. However, what promotes the proliferation of these mutated cells and development of CHIP is relatively unknown. To date some metabolic disorders/stressors, sleep fragmentation and chronic inflammation and viral infections have been associated with CHIP. The extrinsic environment could aid in the increase clonal outgrowth of mutated haematopoietic stem cells (HSCs), influencing VAF and contributing to atherosclerosis.
A. TET2 mutation
- ↑ myeloid skewing (e.g. monocytes)
- ↑ Lesional Macrophages
- ↑ Lesional Macrophages
- ↑ NLRP3 Inflammasome activation (i.e. IL-18)

B. VAK2<sup>VF</sup> mutation
- → Leukocytosis and ↑ Polycythaemia Vera
- ↑ Platelets and platelet Activation
- ↑ NETosis
- ↑ NLRP3/AIM2 Inflammasome activation (i.e. IL-16, IL-18)

C. SH2B3/LNK mutation
- ↑ Neutrophils
- ↑ Platelets, Platelet Activation and oxPL
- ↑ NETosis

Bone Marrow

- ↑ Atherosclerosis
- ↑ Arterial/Venous thrombosis
- ↑ Arterial thrombosis

↑ CVD

Extrinsic factors

- ↑ VAF
- (Mutant clonal outgrowth)

- ↑ Atherosclerosis

Unhealthy Diet

Chronic Inflammation

Insulin Resistance (high HOMA-index)

Low HDL

Infection

Smoking

Fragmented Sleep

Aging

Legend

CHIP+ HSC
CHIP+ Monocyte
CHIP+ Neutrophil
CHIP+ Macrophage

CHIP- HSC
Normal HSC
Normal Monocyte
Normal Neutrophil
Platelet

Diabetes

Obesity

Accepted Manuscript

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