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Clonal Hematopoiesis: Somatic Mutations in Blood Cells and Atherosclerosis

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Abstract

The most important prognostic factor for atherosclerotic cardiovascular disease (ASCVD) is age, independent of all other recognized risk factors. Recently, exome sequence analyses showed that somatic mutations in blood cells, a process termed clonal hematopoiesis, is common and increases in prevalence with age, with at least 1 in 10 adults older than 70 years affected. Carriers of clonal hematopoiesis have been shown to be not only at heightened risk for hematologic malignancy but also at increased risk for ASCVD. Here, we review the prior literature of clonal selection and expansion of hematopoietic stem cells and the evidence supporting its causal association with ASCVD.

Keywords

coronary artery disease; genetics, human; genetics, rat/mouse; blood cell; clonal hematopoiesis

Journal Subject Terms

Inflammation; Aging; Cardiovascular Disease; Genetics; Atherosclerosis

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) remains a leading cause of death in the United States and worldwide.^{1, 2} In fact, in a recent clinical trial where historically low low-density lipoprotein cholesterol (LDL-C) concentrations were achieved, 10% still had recurrent ASCVD events over the subsequent two years.³ As such, the identification of new

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risk pathways orthogonal to traditional risk factors continues to be an important research goal.

Age is the dominant risk factor for ASCVD but the mechanistic bases for why age predisposes to ASCVD is not completely understood.^{4, 5} While age is associated with the acquisition of and cumulative exposure of conventional risk factors, such factors do not appear to fully explain the association of age and ASCVD.⁵⁻⁸ Previously described plausible mechanisms accompanying aging include flow-mediated epigenetic alterations of endothelial cells⁹, diminished regenerative capabilities of bone marrow-derived vascular progenitor cells^{10, 11}, alteration of vascular smooth muscle cell function¹² and proliferation from epigenetic changes¹³, plaque instability from senescent foam macrophage cells¹⁴, with additional contributions from telomere ablation¹⁵ and oxidative stress¹⁶. Key challenges are disentangling whether proposed factors are causal for atherosclerosis or simply correlated with age.

The effect of hematopoietic cells on atherosclerosis pathogenesis has long been recognized.¹⁷⁻²² The acquisition of genetic mutations in cells, including hematopoietic stem cells, is a feature of aging.²³ We recently observed such somatic mutations leading to clonal expansion in the absence of other hematologic abnormalities, or “clonal hematopoiesis of indeterminate potential” (CHIP), not only predisposed to risk for hematologic malignancy but, surprisingly, predisposed to risk for ASCVD.²⁴⁻³¹ Here, we provide an overview of clonal hematopoiesis and the evidence linking it with ASCVD.

THE AGING HEMATOPOIETIC SYSTEM

The hematopoietic system relies on balancing regeneration, differentiation, and senescence. Notably, much of what is known about this balance is derived from murine studies and much less is known about human aging. In adult mammals, the former two processes occur within the bone marrow, with hematopoietic stem cells differentiating into lymphoid, myeloid, erythroid, and platelet cells. A combination of intrinsic and extrinsic cellular factors influencing cytokines and related receptors determine hematopoietic lineage commitment and potential amplification. Aging can influence multiple factors influencing lineage (Table 1).³²

The accumulation of genomic DNA damage is a well-known feature of cellular aging.²³ Like other stem cells, hematopoietic stem cells acquire mutations during their lifetimes but most have no significant consequences or result in cell death.³³ Most hematopoietic stem cells are maintained in a quiescent state to minimize the stresses of cellular metabolism and DNA replication. However, quiescence also promotes nonhomologous end joining-mediated DNA repair further contributing to mutagenesis.³⁴ Old hematopoietic stem cells in mice show greater signs of stress from cellular replication, which is tied to a decreased expression of all subunits of the mini-chromosome maintenance helicase.³⁵ Acquired mutations, age-related changes in DNA repair, and reduced regenerative potential with aging³⁶, provide an ideal opportunity for clonal selection and expansion.

INITIAL EVIDENCE FOR AGE-RELATED CLONAL HEMATOPOEISIS IN ASYMPTOMATIC INDIVIDUALS

Hematopoiesis is considered to be polyclonal with largely equipotent hematopoietic stem cells. Age-related changes in hematopoietic stem cell clonal contribution was first described approximately 20 years ago through analyses of chromosome X-inactivation patterns derived from peripheral leukocytes in women.^{37–39} It was observed that age strongly correlated with nonrandom patterns of chromosome X-inactivation indicating allelic skewing. These data demonstrated that clonality was present in blood cells and that age was a determinant.^{37–39}

Most mutations observed in hematopoietic stem cells are either benign or deleterious to the cell; it estimated that hematopoietic stem cells acquire approximately one exonic mutation per decade.²³ More recently, sequencing of peripheral leukocytes and buccal epithelial cells of older women with chromosome X-inactivation skewing identified recurrent somatic mutations in *TET2* (encoding tet methylcytosine dioxygenase 2) in leukocytes but not buccal cells in 10 of 182 individuals.⁴⁰ Additionally, among non-leukemic hematopoietic stem cells from individuals with acute myeloid leukemia, a high frequency of *DNMT3A* (encoding DNA (cytosine-5-)-methyltransferase 3 α) and *TET2* mutations were observed.^{41, 42} These data indicate the presence of “driver” mutations shared by clonal hematopoiesis and hematologic malignancy, in support of a proposed sequential model of leukemogenesis (Figure 1).⁴³

CLONAL HEMATOPOEISIS AND RISK OF HEMATOLOGICAL MALIGNANCY

Longitudinal analyses in large numbers of apparently healthy individuals are required to better understand the prevalence and clinical consequences of clonal hematopoiesis. Such analyses became feasible over the last decade through whole exome sequencing.⁴⁴ To perform exome sequencing for germline variant discovery, DNA is typically extracted from blood peripheral leukocytes. Discovery of somatic mutations in these leukocytes can be performed using separate bioinformatics approaches. Raw sequence data can be processed with conventional germline tools with increased sensitivity parameters, or dedicated somatic mutation detection software, while filtering likely germline calls.^{45–47} Next, rare disruptive mutations in hematologic malignancy-predisposing genes and mutations recurrently observed in hematological malignancy⁴⁸ may be annotated. In 2014, three studies ascertained data from thousands of blood DNA exomes to characterize prevalence and malignancy potential (Figure 2).^{24, 25, 49}

Xie M et al analyzed blood-derived exomes from 2,278 participants of The Cancer Genome Atlas (TCGA) with one of eleven non-hematological malignancies and no prior chemotherapy or radiation from age 10 to 90 years.⁴⁹ Given the unique presence of matched blood DNA and non-blood (i.e., tumor) DNA within the same individual, investigators were able to reliably discriminate acquired mutations to demonstrate hematopoietic clonal mosaicism. 77 blood-specific mutations in 58 individuals were identified. >80% of observed mutations were in 19 genes previously linked to hematologic malignancy, including: *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *SF3B1*, *BCORL1*, *ASXL2*, and *SH2B3*. The prevalence of these mutations increased with age: 0.9% for cases in their 40s to 6.1% for cases in their

80s. As studied participants already had cancer, an initial concern was whether these estimates of prevalence were generalizable.

Genovese G et al analyzed blood-derived exomes from 12,380 Swedish individuals, comprising of 4,970 with schizophrenia, 1,165 with bipolar disorder, and 6,245 controls, from age 19 to 93 years.²⁴ Health records were ascertained with 2 to 7 years of follow up data available for 11,164 participants. Genes linked to hematologic malignancy accounted for most of the observed somatic mutations at appreciable allele fractions, particularly *DNMT3A*, *ASXL1*, *TET2*, *JAK2*, and *PPM1D*. Observed mutations in these genes were also more likely to be protein-disruptive. Additionally, the pattern of mutations (largely C-to-T) were similar to those observed across diverse cancer types.⁵⁰ 1 in ~150 individuals younger than 50 years and 1 in ~17 older than 65 years had detectable clonal hematopoiesis. Individuals with clonal hematopoiesis had a nearly 13-fold increased risk of hematological malignancy and 1.4-fold increased risk of death.

We analyzed blood-derived exomes from 17,182 individuals from 22 population-based cohorts, which were largely case-control studies for type 2 diabetes (T2D), from age 19 to 108 years.²⁵ 805 candidate somatic mutations in 73 genes in 746 individuals were identified with similar increasing prevalence with age, with 1 in 10 older than 70 years having clonal hematopoiesis. The most commonly mutated genes were previously linked to hematologic malignancy, including *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2*, and *SF3B1*. 93% of individuals with mutations in a hematologic malignancy-associated gene had only one such mutation consistent with the hypothesis of clonal hematopoiesis representing an initiating state. Across a median follow-up of 7.9 years among 3,342 individuals, clonal hematopoiesis was associated with a 11-fold increased risk of hematologic malignancy. A higher variant allele fraction (>0.10) was linked to a much higher risk of hematologic malignancy – nearly 50-fold. Analyses of conventional complete blood count indices showed that clonal hematopoiesis was not associated with cytopenias. Carrier status was also linked to a 1.4-fold increased risk of death among 5,132 individuals with median follow-up of 96 months.

Cancer genes are classically binned as oncogenes and tumor suppressors. The two most frequently mutated genes linked to clonal hematopoiesis, *TET2*, and *DNMT3A*, regulate DNA methylation, an epigenetic mark that is thought to influence transcription. The mutations seen in *TET2* and *DNMT3A* are loss-of-function, consistent with these being tumor suppressor genes. The third most commonly mutated gene is *ASXL1*, which is involved in regulating polycomb mediated transcriptional repression, hence it is also an epigenetic regulator. However, the mutations seen in *ASXL1*, while truncating, are exclusively in exons 11 and 12, suggesting that they might lead to gain-of-function, or altered function (Table 2).

While competitive advantage in stem cells has been observed in *Dnmt3a* and *Tet2* loss-of-function mice, the mechanisms predisposing to this clonal advantage are not fully understood.^{51, 52} Large-scale changes in epigenetic regulation may enhance self-renewal but may also limit differentiation capabilities given complex coordination required for fate determination.⁴³ Nevertheless, despite similarities with acute myelogenous leukemia, the rate of leukemic transformation remains relatively low. Whether this reflects stochastic

variation in epigenetic heterogeneity from initiating mutations or the acquisition of additional cooperating events is not understood.

Additionally, McKerrell et al resequenced 15 mutation hotspots in blood DNA from 4,219 individuals observing a largely linear association of *DNMT3A* mutations with age but an exponential increase in *SF3B1* and *SRSF2* mutations only after the age of 70 years.⁵³ These data, suggest that spliceosome defects may preferentially select and expand clones within the aging hematopoietic stem cell niche. Splicing mutations may also yield analogous transcriptional heterogeneity, like broad epigenetic changes from *DNMT3A* and *TET2* mutations, which can promote selection or deficiencies in differentiation.⁵⁴

CLONAL HEMATOPOESIS OF INDETERMINATE POTENTIAL VERSUS MYELODYSPLASTIC SYNDROME

Clonal hematopoiesis broadly refers to the selective expansion of hematopoietic stem cells with somatic mutations. The term “clonal hematopoiesis of indeterminate potential (CHIP)” has been proposed to define individuals carrying clonal somatic mutations in genes linked to hematologic malignancy with a VAF of at least 2%, but without a known hematologic malignancy or other clonal disorder (Table 3).^{27, 29} Of note, sensitive deep sequencing strategies increase the apparent prevalence of clonal hematopoiesis with improved detection of somatic mutations in leukemogenic genes at lower VAF thresholds.^{55–57} Furthermore, using ultra-sensitive methods and a VAF threshold of at least 0.03%, almost all (95%) of healthy 50-60 year-olds are identified as having clonal hematopoiesis.^{27, 58} Since all hematologic malignancies in our prior study occurred among individuals with VAF > 10%, the presence of clonal hematopoiesis at such very low VAFs are unlikely to carry significant clinical relevance.

Of note, myelodysplastic syndrome (MDS) is a well-recognized low-grade clonal neoplasm with an increased risk of leukemic transformation. CHIP largely presents without cytopenia or dysplasia²⁵ which are defining features of MDS. In addition, CHIP often has only 1 driver mutation with a low VAF, as opposed to MDS which may have several driver events at high VAF. As a result, CHIP carries a more favorable prognosis than MDS. The rate of progression to hematologic malignancy is 0.5-1% per year for CHIP^{24, 25}, similar to rates observed for other pre-malignant clonal conditions, such as monoclonal gammopathy of undetermined significance (precursor for multiple myeloma) and monoclonal B-cell lymphocytosis (precursor for chronic lymphocytic leukemia and other B-cell lymphomas).²⁹

CLONAL HEMATOPOESIS AND RISK OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

To better understand the observed association of clonal hematopoiesis with all-cause mortality, we performed exploratory analyses testing CHIP status with cause-specific mortality.²⁵ Only one individual with clonal hematopoiesis had died from hematologic malignancy. Cause-specific analyses of 5,132 individuals confirmed a lack of association with cancer death but there was an association with cardiovascular death, particularly for

individuals with a variant allele fraction of at least 10% (HR 1.9). Secondary analyses suggested a potential association with coronary heart disease (CHD) and ischemic stroke despite adjusting for traditional cardiovascular risk factors, including age. A prior study showed that clonal large chromosomal rearrangements was associated with micro- and macrovascular complications among those with T2D (carriers 19/26 vs non-carriers 810/2,182).⁵⁹ It is unclear whether age was accounted for in this secondary analysis and whether there is a true association for atherosclerotic cardiovascular disease (i.e., macrovascular complications). Clonal mosaicism for large chromosomal rearrangements associates with age and increased risk for hematologic malignancy, but more commonly chronic lymphocytic and chronic myeloid leukemias.^{60, 61} *DNMT3A* and *TET2* mutations have not been typically observed with such large chromosomal rearrangements. Therefore, clonal large chromosomal rearrangements and CHIP may have distinct clinical consequences.

We subsequently tested the hypothesis that CHIP contributes to CHD risk.²⁶ We analyzed blood-derived exome data from four case-control studies together comprising 4,726 with CHD and 3,529 controls. We used a nested case-control approach from two prospective cohort studies: BioImage^{62, 63}, which was enriched for older individuals at higher cardiovascular disease risk, and Malmö Diet and Cancer (MDC)⁶⁴, which had a prolonged follow-up period. We selected individuals sustaining a first CHD event and controls matched by age, sex, T2D status, and smoking history. We also used data from two retrospective case-control studies: the Atherosclerosis, Thrombosis, and Vascular Biology (ATVB) Italian Study Group⁶⁵ and Pakistani Risk of Myocardial Infarction Study (PROMIS)^{66, 67}, with cases being individuals with early-onset (<50 years) myocardial infarction.

Similar to prior studies, somatic mutations were more commonly observed in *DNMT3A*, *TET2*, *ASXL1*, and *JAK2*. Clonal hematopoiesis was associated with a 1.9-fold increased risk of CHD in both BioImage (median age: 70 years, CHIP: 17% cases vs 10% controls) and MDC (median age: 60 years, CHIP: 7% cases vs 4% controls). Surprisingly, an even stronger association for early-onset myocardial infarction was observed, carrying a 4-fold increased risk, across both ATVB (median age: 41 years cases and 40 years controls, CHIP: 2.1% cases vs 0.4% controls) and PROMIS (median age: 45 years cases and 49 years controls, CHIP: 2.0% cases vs 0.9% controls).

Somatic mutations in blood cells might influence risk of atherosclerotic cardiovascular disease in two ways—by increasing the likelihood of thrombosis, or by increasing underlying atherosclerosis. We hypothesized that individuals with clonal hematopoiesis also harbored a greater burden of subclinical coronary atherosclerosis prior to clinical events. We examined the relationship between clonal hematopoiesis and subclinical coronary atherosclerosis in BioImage through measures of coronary artery calcification (CAC) measured by cardiac computed tomography.²⁶ Among controls, clonal hematopoiesis status was associated a 3.0-fold greater likelihood of having a CAC score greater than 615 Agatston units, a value described to indicate high CHD risk among older adults.^{26, 68} Similar to our observation that larger clones (variant allele fraction > 10%) were more strongly associated with hematologic malignancy²⁵, carrying a larger clone was associated with a 12-fold greater likelihood of having a CAC score greater than 615 Agatston units.

Concordantly, our data suggested that the presence of a larger clone was associated with a 2.2-fold greater risk of CHD compared to 1.4-fold greater risk for carriers of smaller clones.

BY WHAT MECHANISMS MIGHT CLONAL HEMATOPOIESIS LEAD TO ATHEROSCLEROSIS?

Despite independent associations of clonal hematopoiesis with clinical and subclinical atherosclerosis in statistical models accounting for age, confounding remains a potential concern.¹⁷ Causal inference from biomarker-disease correlation in observational studies is challenging.

In murine models, two groups have tested if and how clonal hematopoiesis leads to atherosclerosis (Figure 3).^{26, 69} *Tet2* is the second most commonly mutated gene observed in human clonal hematopoiesis and *Tet2* ablation in murine hematopoietic cells led to clonal selection and expansion.⁷⁰ We generated *Tet2*^{-/-} mice and their bone marrow (vs control bone marrow) was engrafted into irradiated, atherosclerosis-prone *Ldlr*^{-/-} mice. Compared with mice transplanted with wild-type bone marrow, chimeric mice with *Tet2* deficient bone marrow had nearly 3-fold larger descending aorta atherosclerotic plaques at 17 weeks despite similar blood counts and similar serum cholesterol concentrations. The effect size was nearly identical for *Ldlr*^{-/-} for bone marrow from heterozygous *Tet2*-deficient (*Tet2*^{+/-}) mice.

Fuster et al also evaluated *Tet2* deficiency in hematopoietic cells in mice and its effect on atherosclerosis.⁶⁹ Fuster et al created *Ldlr*^{-/-} mice engrafted with 10% *Tet2*^{-/-} bone marrow (tagged by CD45.2) and 90% *Tet2*^{+/+} bone marrow (tagged by CD45.1) to mimic observed circulating variant allele fractions in humans; control bone marrow consisted of *Tet2*^{+/+} labeled 10% CD45.2 and 90% CD45.1. At 13 weeks after engraftment, despite having a similar total number of hematopoietic stem cells, 69% of hematopoietic stem cells in the bone marrow were CD45.2 cells in those receiving 10% *Tet2*^{-/-} bone marrow. A slight predisposition toward myeloid differentiation was observed. Despite similar weight, insulin sensitivity, and plasma cholesterol levels, *Ldlr*^{-/-} mice engrafted with 10% *Tet2*^{-/-}/90% *Tet2*^{+/+} bone marrow had larger aortic root plaques with a proportional increase in macrophage content when compared with *Ldlr*^{-/-} mice with 100% *Tet2*^{+/+} bone marrow. Furthermore, engraftment 10% *Tet2*^{-/+}/90% *Tet2*^{+/+} bone marrow to *Ldlr*^{-/-} mice similarly promoted atherosclerosis compared to *Ldlr*^{-/-} mice with 100% *Tet2*^{+/+} bone marrow. These data demonstrate independent consistent observations between the two groups.

We tested the hypothesis that *Tet2* disruption was particularly influencing myeloid cells. *Ldlr*^{-/-} mice were engrafted with bone marrow from mice where *Tet2* had been selectively disrupted in myeloid cells; compared with mice transplanted with wild-type bone marrow, these mice had larger aortic root atheroma at 10 weeks. Fuster et al observed that CD45.2 cells preferentially differentiated to macrophages within atherosclerotic vascular walls. The authors similarly hypothesized a central role of clonally expanded macrophages in atherosclerosis. They also generated *Ldlr*^{-/-} mice selectively deficient of *Tet2* in myeloid cells and reported similar results as us.

Notably, current murine bone marrow transplant models of CHIP may not faithfully replicate the kinetics clonal expansion in human CHIP. Of note, we observed among 13 individuals (17 mutations in total) with CHIP at baseline and blood-cell DNA collection 4-8 years later, 10 VAFs did not increase and 7 did increase and none developed hematologic cancer over this time frame.²⁵ Additionally, Young et al observed a similar pattern of VAF stability in 20 individuals over ~10 years.⁵⁸

In our study, *Tet2*^{-/-} bone marrow-derived macrophages *in vitro* were exposed to native low-density lipoprotein (LDL), resulting in increased expression of several cytokines and chemokines, including *Cxcl1*, *Cxcl2*, *Cxcl3*, *Pf4*, *Il1b*, and *Il6*, compared to control macrophages. This was consistent with prior observations linking *Tet2* deficient bone marrow-derived macrophages with increased inflammatory gene expression.^{71, 72} Notably, CXC chemokines were also elevated in serum from *Ldlr*^{-/-} mice transplanted with *Tet2*-deficient bone marrow. In human plasma, IL8, a CXC chemokine analogue that mice lack, was approximately double among those with clonal hematopoiesis compared to those without.

In complementary studies, Fuster et al exposed *Tet2*^{-/-} peritoneal macrophages to lipopolysaccharide and interferon- γ *in vitro* and showed higher gene expression for several cytokines and chemokines, including *Ccl3*, *Ccl4*, *Cxcl1*, *Cxcl2*, *Cxcl3*, *Cxcl5*, *Cxcl13*, *C4b*, *Dcn*, *Il12b*, *Il1a*, *Il1b*, *Il6*, and *Osm*. Elevated IL-6 levels were also detected in culture supernatant. *Il1b* upregulation was particularly notable when exposed to a combination of oxidized LDL, tumor necrosis factor, and interferon- γ . Concordantly, unlike other chemokines, *Il1b* gene expression and IL-1 β concentration was increased in the aortic arches of *Ldlr*^{-/-} mice engrafted with 10% *Tet2*^{-/-}/90% *Tet2*^{+/+} bone marrow when compared with *Ldlr*^{-/-} mice with 100% *Tet2*^{+/+} bone marrow. Further, stimulation of *Tet2*^{-/-} macrophages *in vitro* led to increased IL-1 β which was mitigated by a NLRP3 inhibitor indicating that *Tet2* deficiency promotes NLRP3 inflammasome-mediated IL-1 β secretion. Similarly, aortic plaque size in chimeric *Ldlr*^{-/-} mice with 10% *Tet2*^{-/-} bone marrow treated with a NLRP3 inflammasome inhibitor was smaller compared to *Ldlr*^{-/-} mice with 10% *Tet2*^{+/+} bone marrow.

Both studies strikingly converge on the role of specific chemokines, particularly CXC family chemokines, IL-6, and IL-1 β , in promoting atherosclerosis in the setting of clonal hematopoiesis. CXC chemokines, such as IL-8, and their receptors have previously been shown to promote the adhesion of monocytes to vascular endothelium.⁷³⁻⁷⁵ Furthermore, macrophages in atheroma produce >8-fold greater IL-8 compared to circulating monocytes, with even greater production when exposed to oxidized LDL.^{76, 77} In the setting of oxidized LDL, IL-8 inhibits the production of specific tissue inhibitors of matrix metalloproteinases thereby contributing to local plaque instability. Additionally, CXC chemokines can recruit neutrophils, which can attach to atherosclerotic plaques via neutrophil extracellular traps (NETs) and separately promote atherothrombosis.⁷⁸⁻⁸³

In an exome-wide association analysis across >300,000 individuals, JAK2 p.V617F carriage detected by exome genotyping array was associated with lower (-0.32 total cholesterol and -0.30 LDL cholesterol).⁸⁴ Furthermore, *Ldlr*^{-/-} mice engrafted with Jak2 p.V617F bone

marrow had lower total cholesterol compared to *Ldlr*^{-/-} mice engrafted with wild-type bone marrow.⁸⁴ The apparent discordance between low LDL cholesterol and elevated CHD risk conferred by *JAK2* p.V617F requires further study. Notably, across CHIP broadly (which more frequently involves mutations in *DNMT3A* and *TET2*), there is no significant association with blood lipids.²⁶

GERMLINE GENETIC PREDISPOSITION TO CLONAL HEMATOPOIESIS

Prior efforts have evaluated the germline genetic predisposition to the occurrence of *JAK2* p.V617F at relatively small scale.⁸⁵⁻⁹⁰ *JAK2* p.V617F is the most frequently observed acquired mutation recurrently identified in myeloproliferative neoplasms (MPN)⁹¹, and is observed in CHIP. Kilpivaara O et al showed that, of 321 MPN cases compared to 3,000 convenience controls, germline rs10974944-G (MAF 25%) in a *JAK2* intron tagging the *JAK2* 46/1 haplotype was strongly associated with *JAK2*^{V617F}-positive MPN (OR = 4.0, p-value = 7.7×10^{-22}) and not *JAK2*^{V617F}-negative MPN (OR = 1.6, p-value = 0.06).⁸⁸ The *JAK2* 46/1 haplotype is estimated to account for 28% of the population attributable risk of MPN among Europeans.⁹²

More recently, Hinds D et al performed a genome-wide association analysis for both *JAK2*^{V617F}-positive MPN and *JAK2*^{V617F}-positive clonal hematopoiesis among 252,637 individuals ascertained from 23andme and 726 individuals with myeloproliferative neoplasms.⁹³ This specific mutation was chosen as it is a somatic mutation known to cause clonal hematopoiesis, occurs at appreciable frequency, and is present on most contemporary genome-wide genotyping arrays. Significant associations were observed at loci near the genes *JAK2* (including variants tagging the 46/1 haplotype), *TERT*, *SH2B3*, *TET2*, *CHEK2*, *ATM/PDGFD*, *PINT*, and *GFI1B*. The *TERT* association appears to be a more general effect on all MPN, regardless of *JAK2* p.V617F mutation status.^{86, 94}

Notably, the *SH2B3* and *ATM/PDGFD* loci were previously significantly associated with CHD in genome-wide association studies.⁹⁵ *SH2B3*, also known as *LNK*, is expressed in hematopoietic cells and is a negative regulator of cellular proliferation, including negatively regulating *JAK2* in stem cells.⁹⁶ Rare disruptive mutations in *SH2B3* predispose to MPN in humans, and targeted deletion in mice promotes multilineage expansion of hematopoietic stem cells.⁹⁶⁻⁹⁸ Wang W et al showed that both *Lnk*^{-/-} mice and *Ldlr*^{-/-} mice engrafted with *Lnk*^{-/-} bone marrow showed myelopoiesis, this was more prominent in the latter model.⁹⁸ They further observe a synergistic activation of platelets from *Lnk* deficiency and cholesterol loading.

While *JAK2* p.V617F is observed in the setting of CHIP, mutations in other genes (i.e., *DNMT3A*, *TET2*, *ASXL1*, et al) are more frequently observed in CHIP. Using targeted deep sequencing (>4,000-fold mean coverage), Buscarlet M et al observed leukemogenic mutations (93% in *DNMT3A* and *TET2*) in 13.7% of 2,530 apparently healthy adults.⁵⁶ Of 391 sibships within this cohort, recurrence risk of *TET2* mutations between siblings was ~2.5-fold and there was no recurrence risk observed for *DNMT3A* mutations between siblings. Additional work is required to understand the heritable basis of such mutations more frequently observed in CHIP.

CLONAL HEMATOPOIESIS WITH UNKNOWN DRIVER MUTATIONS

A hallmark of CHIP is the presence of initiating driver mutations.²⁹ However, Genovese et al observed the phenomenon of clonal hematopoiesis (detected by at least three putative somatic mutations at appreciable allele frequency) with unknown drivers (indicated by the lack of an initiating driver mutation in a previously implicated gene).²⁴ Prevalence also increases with age, with 0.9% carriers among individuals younger than 50 years and 10.4% in individuals older than 65 years. Among the 439 individuals with clonal hematopoiesis, 170 (39%) did not have a known driver mutation. The risk of hematologic malignancy was similar among individuals with clonal hematopoiesis with known drivers (HR 13.73) vs unknown drivers (HR 12.89).

Detection of somatic mutations outside of known driver genes may be enhanced via deep-coverage whole genome sequencing of blood-derived DNA given ability to detect mutations across the genome. This was recently assessed in 11,262 Icelanders participating in deCODE Genetics undergoing whole genome sequencing to average genome-wide coverage 36X.⁹⁹ Here, clonal hematopoiesis was defined as the presence of more than 20 singleton somatic mutations with variant allele fractions ranging 0.10-0.20 (99.5th percentile for subjects younger than 35 years). While 0.5% of participants younger than 35 years had clonal hematopoiesis, more than 50% older than 85 years had clonal hematopoiesis, approximately twice the prior yield from exome sequence analysis.^{24, 25} Additionally, only 1 in 8 individuals (177/1,403) with clonal hematopoiesis had a candidate driver mutation in at least 1 of 18 previously implicated genes. Deep resequencing at 54 myeloid malignancy-associated genes of a subset to variant allele fraction >0.01, identified clonal driver mutations in 40.7% still demonstrating that the majority have no detectable candidate driver mutation. However, the clinical relevance of clones with driver mutations at lower variant allele fractions (<0.10) appear to be less significant.²⁵ As before, risk of hematologic malignancy was similar for clonal hematopoiesis with or without a candidate driver mutation.

FUTURE RESEARCH DIRECTIONS

Given that the association of clonal hematopoiesis with ASCVD has just recently been discovered, many outstanding questions exist. Some of these research questions are discussed below.

First, is the relationship between clonal hematopoiesis and atherosclerosis homogenous across implicated genes? Most known driver mutations occur in *DNMT3A* and *TET2*. In current sample sizes, effect estimates appear different for early-onset myocardial infarction – 1.4 increased odds for *DNMT3A* mutations and 8.3 for *TET2* mutations.²⁶ Nevertheless, risk for CHD appears similar for older individuals is similar – 1.7-fold for *DNMT3A* mutations and 1.9-fold for *TET2* mutations. To better understand risks conferred from individual genes, larger sample sizes are required.

Second, why do some individuals develop clonal hematopoiesis without known driver mutations and do they also have elevated risk of ASCVD? Recent whole genome sequence

analyses suggest clonal hematopoiesis without known driver mutations is highly prevalent in older adults.⁹⁹ However, whether this phenomenon also increases risk for ASCVD is currently unknown.

Third, what are the determinants of clinical consequences among individuals with clonal hematopoiesis? Most individuals with clonal hematopoiesis followed in recent studies do not develop hematologic malignancy or atherosclerotic cardiovascular disease.^{24–26} Some differences may be explained by different genes. However, disruption of many such genes likely result in broad, varied epigenetic changes so specific epigenetic disruption patterns may yield differential clinical consequences. Transcriptional and epigenetic profiling in humans and model systems may provide new insights. Furthermore, smoking and diabetes have been associated with clonal hematopoiesis; the presence of such risk factors and others may influence risk of ASCVD in additive or multiplicative manners. Since CHIP promotes atherosclerosis in humans and hypercholesterolemia-prone mice, alteration of cholesterol-related pathways within hematopoietic stem cells related to dyslipidemia may differentially influence ASCVD in the setting of CHIP.

Fourth, can interventions reduce the risk for ASCVD associated with CHIP? Induction of the NLRP3 inflammasome is required for proteolytic cleavage to activate IL-1 β .¹⁰⁰ Individuals with rare Mendelian autoinflammatory conditions due to mutations activating the NLRP3 inflammasome suffer from symptoms related to overproduction of IL-1 β . Cholesterol crystals can stimulate the NLRP3 inflammasome within atheromata yielding IL-1 β production.^{101, 102} IL-1 β has been shown to promote hematopoietic stem cell proliferation as well as induce endothelial cell expression of monocyte adhesion and recruitment factors.^{103–105} IL-1 also promotes proliferation of vascular smooth muscle cells.^{106, 107} IL-1 in turn further promotes the secretion of IL-6 from both endothelial cells and vascular smooth muscle cells.^{108, 109} Mendelian randomization studies have causally associated the IL-6 receptor with CHD.^{110, 111} IL-6 stimulates the production of C-reactive protein, a circulating biomarker associated with incident CHD risk.¹¹²

Recently, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial tested the efficacy and safety of IL-1 β inhibition with canakinumab to prevent recurrent atherosclerotic cardiovascular disease events among individuals with elevated high-sensitivity C-reactive protein (hsCRP) levels (> 2 mg/L).¹¹³ Individuals receiving canakinumab 150 mg every three months had a 15% reduction in recurrent events at 4 years. Individuals who attained hsCRP < 2 mg/L on treatment appeared to have a greater efficacy (HR 0.75) versus those with persistent hsCRP > 2 mg/L (HR 0.90).¹¹⁴ A surprising secondary observation was that individuals treated with canakinumab had a lower risk of developing lung cancer (HR 0.61).¹¹⁵ Given that loss of *Tet2* leads to increased *Il1b* expression in mice and that atherosclerosis can be reduced in these mice with a NLRP3 inflammasome inhibitor,⁶⁹ it is possible that downstream blockade of IL-1 β will reduce the risk of atherosclerotic events among humans with *TET2*-mutated clonal hematopoiesis. Additionally, current laboratory testing does not capture individuals with clonal hematopoiesis well. Analyses of complete blood counts, suggests that red cell distribution width (RDW) is associated but this is not adequately sensitive or specific.²⁵ It is not known whether clonal hematopoiesis is associated with high sensitivity C-reactive protein.

CONCLUSIONS

Clonal hematopoiesis represents a new risk factor for ASCVD with an effect size estimate at least as large as conventional risk factors. Efforts to better characterize fundamental pathophysiology, identify determinants of initiation and progression, further improve diagnostic yield, and develop novel preventive and therapeutic strategies may have significant public health benefit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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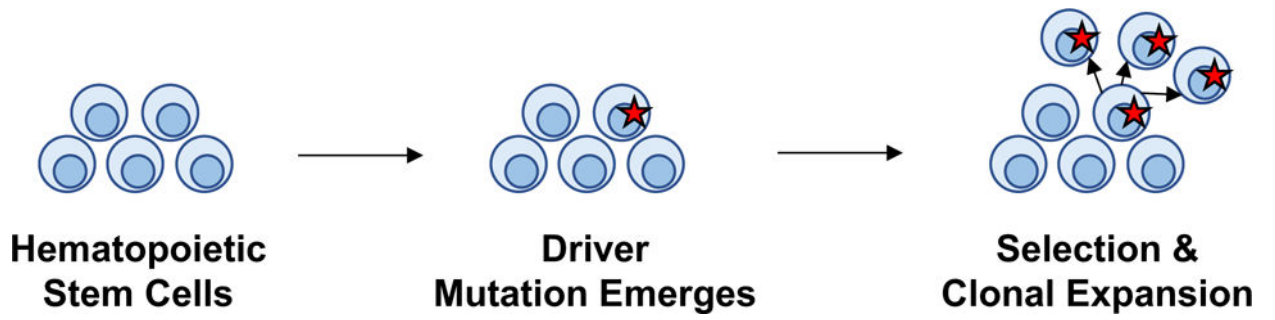


Figure 1. Sequential model of clonal hematopoiesis

A sequential model of progression from normal hematopoiesis to clonal hematopoiesis.

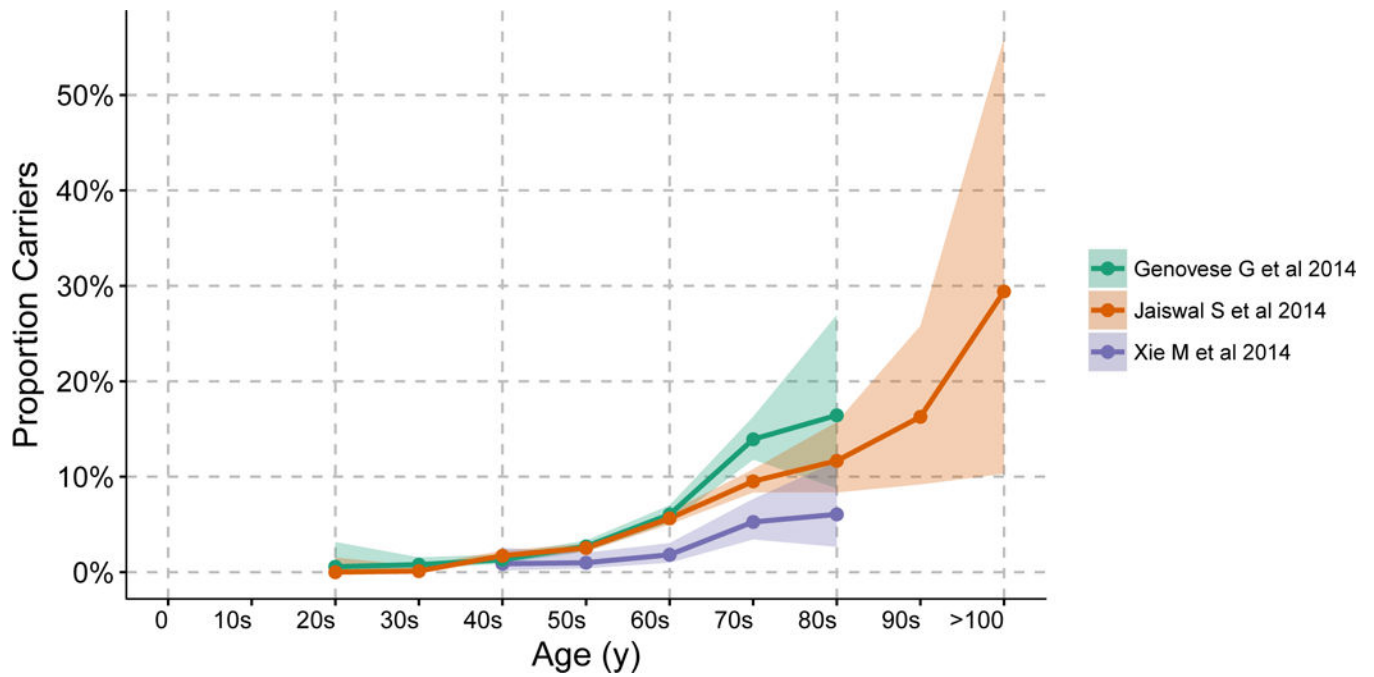


Figure 2. Prevalence of clonal hematopoiesis of indeterminate potential by age
 Clonal hematopoiesis of indeterminate potential prevalence by age detected by exome sequencing of blood-derived DNA in three studies of individuals without hematologic malignancy.^{24, 25, 49} Shaded areas represent 95% confidence intervals. Adapted from Jan M et al.⁴³

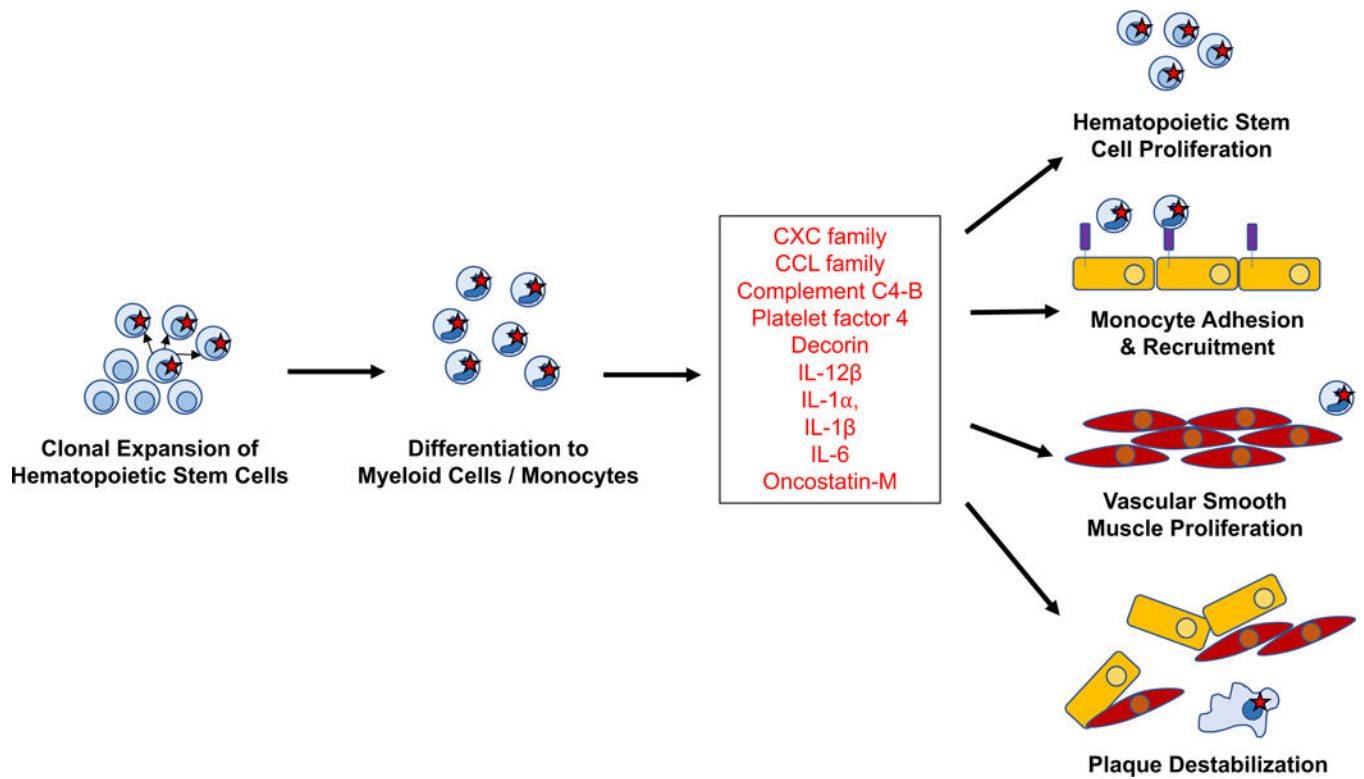


Figure 3. Inflammatory mediators of clonal hematopoiesis influencing atherosclerosis
 Summary of inflammatory factors observed to be upregulated in Jaiswal et al²⁶ and Fuster et al⁶⁹ and proposed influences on atherosclerosis plaque formation and destabilization.

Table 1

Distinctive features of aging hematopoietic stem cells

Features
Increased total count
Reduced adhesion to bone marrow stroma
Lineage skew favoring myeloid progenitors
Polarity shift
mTOR pathway perturbation
Altered DNA damage response
Increased reactive oxygen species
Global epigenetic shift

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Table 2

Genes with most frequent recurrent somatic mutations in blood cells.

Gene	Function of Gene Product	References
<i>DNMT3A</i>	DNA methyltransferase performing <i>de novo</i> genome-wide methylation	116–118
<i>TET2</i>	Methylcytosine dioxygenase catalyzing conversion of methylcytosine to 5-hydroxymethylcytosine, including downstream DNA demethylation	26, 51, 69, 119, 120
<i>ASXL1</i>	Chromatin-binding protein regulating transcription through nuclear hormone receptors, such as retinoic acid receptors and peroxisome proliferator-activated receptor gamma	121–123
<i>TP53</i>	Transcription factor regulating cell cycle arrest, apoptosis, senescence, DNA repair, and metabolism changes in response to diverse cellular stresses.	124–126
<i>JAK2</i>	Protein tyrosine kinase associated with the prolactin receptor, thrombopoietic receptor, and interferon gamma, involved in cell growth, development, differentiation, and histone modification.	127–130
<i>SF3B1</i>	Component of U2 snRNP which binds to pre-mRNA and is involved in RNA splicing regulation.	131, 132
<i>GNB1</i>	A G protein beta subunit involved in transmembrane signal transduction and modulation.	133, 134
<i>CBL</i>	E3 ubiquitin-protein ligase promoting proteosomal degradation.	135–137
<i>SRSF2</i>	Interacts with pre-mRNA and spliceosomal components to promote RNA splicing.	138, 139
<i>GNAS</i>	Stimulatory G-protein alpha subunit involved in transmembrane signal transduction and modulation.	140
<i>PPM1D</i>	PP2C family member induced by p53 and negatively regulates p38 MAPK.	141
<i>BCORL1</i>	Transcriptional corepressor interacting with histone deacetylases to repress transcription.	142

The genes with the largest burden of recurrent somatic mutations in blood cells detected by exome sequencing in three studies of individuals without hematologic malignancy are listed.^{24, 25, 49}

Table 3

Diagnostic features of clonal hematopoiesis of indeterminate potential

Features
Absence of definitive morphological evidence of a hematological neoplasm
Absence of cytopenia or dysplasia
Does not meet diagnostic criteria for PNH, MGUS, or MBL
Presence of a somatic mutation associated with hematological neoplasia at a variant allele frequency \geq 2% in peripheral blood

Adapted from Steensma et al²⁹ and Bowman et al²⁷.

MBL = monoclonal B-cell lymphocytosis; MGUS = monoclonal gammopathy of uncertain significance; PNH = paroxysmal nocturnal hemoglobinuria

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