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REVIEW

Clonal Hematopoiesis

Somatic Mutations in Blood Cells and Atherosclerosis

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ABSTRACT: The most important prognostic factor for atherosclerotic cardiovascular disease 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, are common and increase 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 atherosclerotic cardiovascular disease. Here, we review the prior literature of clonal selection and expansion of hematopoietic stem cells and the evidence supporting its causal association with atherosclerotic cardiovascular disease.

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 LDL-C (low-density lipoprotein cholesterol) concentrations were achieved, 10% still had recurrent ASCVD events over the subsequent 2 years.[3] As such, the identification of new 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 are not completely understood.[4,5] Although age is associated with the acquisition of and cumulative exposure of conventional risk factors, such factors do not seem to fully explain the association of age and ASCVD.[5–8] Previously described plausible mechanisms accompanying aging include flow-mediated epigenetic alterations of endothelial cells,[9] diminished regenerative capabilities of bone marrow–derived vascular progenitor cells,[10,11] alteration of vascular smooth muscle cell function[12] and proliferation from epigenetic changes,[13] plaque instability from senescent foam macrophage cells,[14] with additional contributions from telomere ablation[15] and oxidative stress.[16] 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.

Key Words: blood cell ■ clonal evolution ■ coronary artery disease ■ genetics ■ hematologic neoplasms ■ hematopoiesis ■ human

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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 2 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 aging[36] provide an ideal opportunity for clonal selection and expansion.

INITIAL EVIDENCE FOR AGE-RELATED CLONAL HEMATOPOIESIS IN ASYMPTOMATIC INDIVIDUALS

Hematopoiesis is considered to be polyclonal with largely equipotent hematopoietic stem cells. Age-related changes in hematopoietic stem cell clonal contribution were first described ≈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 ≈1 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] In addition, among nonleukemic hematopoietic stem cells from individuals with acute myeloid leukemia, a high frequency of *DNMT3A* (encoding DNA (cytosine-5-)-methyltransferase 3α) 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]

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

mTOR indicates mammalian target of rapamycin.

CLONAL HEMATOPOIESIS AND RISK OF HEMATOLOGIC 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 cells to identify somatic mutations.[45–47] Next, rare disruptive mutations in hematologic malignancy-predisposing genes and mutations recurrently observed in hematologic malignancy[48] may be annotated. In 2014, 3 studies ascertained data from thousands of blood DNA exomes to characterize prevalence and malignancy potential (Figure 2).[24,25,49]

Xie et al[49] analyzed blood-derived exomes from 2278 participants of The Cancer Genome Atlas with 1 of 11 nonhematologic malignancies and no prior chemotherapy or radiation from age 10 to 90 years. Given the unique presence of matched blood DNA and nonblood (ie, tumor) DNA within the same individual, investigators were able to reliably discriminate acquired mutations to demonstrate hematopoietic clonal mosaicism. Seventy-seven blood-specific mutations in 58 individuals were identified. More than 80% of observed mutations were in 19 genes previously linked to hematologic malignancy, including *DNMT3A*,

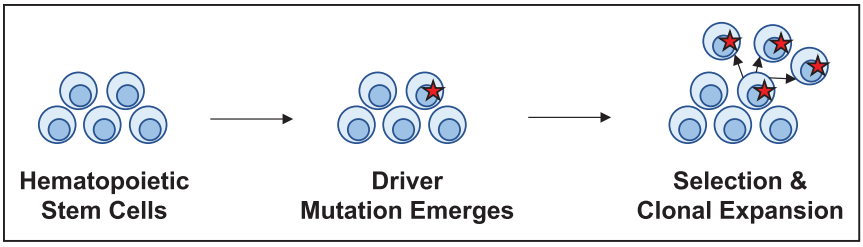

Figure 1. Sequential model of clonal hematopoiesis.
A sequential model of progression from normal hematopoiesis to clonal hematopoiesis.

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 et al[24] analyzed blood-derived exomes from 12 380 Swedish individuals, comprising of 4970 with schizophrenia, 1165 with bipolar disorder, and 6245 controls, from age 19 to 93 years. 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* (encoding janus kinase 2), and *PPM1D*. Observed mutations in these genes were also more likely to be protein disruptive. In addition, the pattern of mutations (largely C-to-T) was similar to those observed across diverse cancer types.[50] One 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 hematologic 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 mellitus, from age 19 to 108 years.[25] Eight hundred five 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*. Ninety-three percent 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 3342 individuals, clonal hematopoiesis was associated with an 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 5132 individuals with median follow-up of 96 months.

Cancer genes are classically binned as oncogenes and tumor suppressors. The 2 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).

Although 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.[54,80] Large-scale changes in epigenetic regulation may enhance self-renewal but

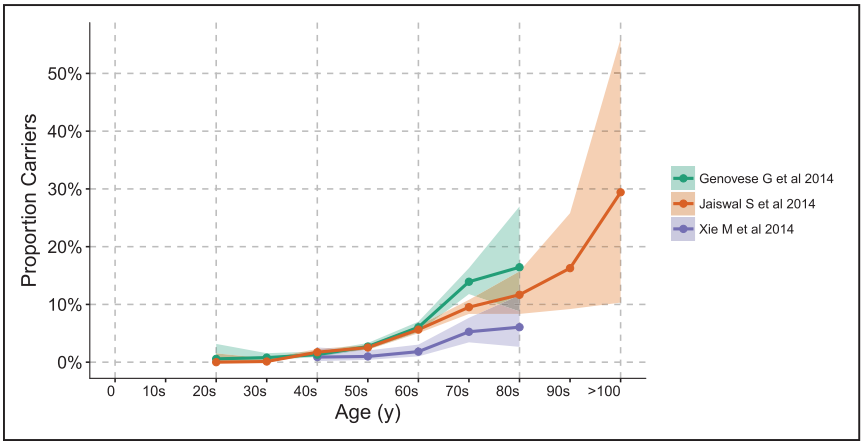

Figure 2. Prevalence of clonal hematopoiesis of indeterminate potential by age.
Clonal hematopoiesis of indeterminate potential prevalence by age detected by exome sequencing of blood-derived DNA in 3 studies of individuals without hematologic malignancy.[24,25,49] Shaded areas represent 95% confidence intervals. Adapted from Jan et al[43] with permission. Copyright ©2017, Wolters Kluwer Health, LWW.

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Table 2. Genes With Most Frequent Recurrent Somatic Mutations in Blood Cells

Gene	Function of Gene Product	References
DNMT3A	DNA methyltransferase performing de novo genome-wide methylation.	51–53
TET2	Methylcytosine dioxygenase catalyzing conversion of methylcytosine to 5-hydroxymethylcytosine, including downstream DNA demethylation.	26,54–57
ASXL1	Chromatin-binding protein regulating transcription through nuclear hormone receptors, such as retinoic acid receptors and peroxisome proliferator–activated receptor γ.	58–60
TP53	Transcription factor regulating cell cycle arrest, apoptosis, senescence, DNA repair, and metabolism changes in response to diverse cellular stresses.	61–63
JAK2	Protein tyrosine kinase associated with the prolactin receptor, thrombopoietic receptor, and interferon-γ involved in cell growth, development, differentiation, and histone modification.	64–67
SF3B1	Component of U2 snRNP which binds to pre-mRNA and is involved in RNA splicing regulation.	68,69
GNB1	A G protein β subunit involved in transmembrane signal transduction and modulation.	70,71
CBL	E3 ubiquitin-protein ligase promoting proteosomal degradation.	72–74
SRSF2	Interacts with pre-mRNA and spliceosomal components to promote RNA splicing.	75,76
GNAS	Stimulatory G-protein α subunit involved in transmembrane signal transduction and modulation.	77
PPM1D	PP2C family member induced by p53 and negatively regulates p38 MAPK.	78
BCORL1	Transcriptional corepressor interacting with histone deacetylases to repress transcription.	79

The genes with the largest burden of recurrent somatic mutations in blood cells detected by exome sequencing in 3 studies of individuals without hematologic malignancy are listed.[24,25,49] MAPK indicates Mitogen-activated protein kinase; PP2C, Protein phosphatase 2C; and U2 snRNP, U2 small nuclear ibonucleoprotein.

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.

In addition, McKerrell et al[81] resequenced 15 mutation hotspots in blood DNA from 4219 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. 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.[82]

CHIP VERSUS MYELODYSPLASTIC SYNDROME

Clonal hematopoiesis broadly refers to the selective expansion of hematopoietic stem cells with somatic mutations. The term CHIP has been proposed to define individuals carrying clonal somatic mutations in genes linked to hematologic malignancy with a variant allele fraction (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.[83–85] Furthermore, using ultrasensitive methods and a VAF threshold of at least 0.03%, almost all (95%) of healthy 50- to 60-year-olds are identified as having clonal hematopoiesis.[27,86] Because all hematologic malignancies in our prior study occurred among individuals with VAF >10%, the presence of clonal hematopoiesis at such very low VAFs is unlikely to carry significant clinical relevance.

Of note, myelodysplastic syndrome is a well-recognized low-grade clonal neoplasm with an increased risk of leukemic transformation. CHIP largely presents without cytopenia or dysplasia[25] which are defining features of myelodysplastic syndrome. In addition, CHIP often has only 1 driver mutation with a low VAF, as opposed to myelodysplastic syndrome which may have several driver events at high VAF. As a result, CHIP carries a more favorable prognosis than myelodysplastic syndrome. The rate of progression to hematologic malignancy is 0.5% to 1% per year for CHIP,[24,25] similar to rates observed for other premalignant 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 HEMATOPOIESIS AND RISK OF ASCVD

To better understand the observed association of clonal hematopoiesis with all-cause mortality, we performed

Table 3. Diagnostic Features of Clonal Hematopoiesis of Indeterminate Potential

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

MBL indicates monoclonal B-cell lymphocytosis; MGUS, monoclonal gammopathy of uncertain significance; and PNH, paroxysmal nocturnal hemoglobinuria.

Adapted from Steensma et al.[29]

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exploratory analyses testing CHIP status with cause-specific mortality.[25] Only 1 individual with clonal hematopoiesis had died from hematologic malignancy. Cause-specific analyses of 5132 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% (hazard ratio [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 were associated with micro- and macrovascular complications among those with type 2 diabetes mellitus (carriers 19/26 versus noncarriers 810/2182).[87] It is unclear whether age was accounted for in this secondary analysis and whether there is a true association for ASCVD (ie, 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.[88,89] *DNMT3A* and *TET2* mutations have not be 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 4 case-control studies together comprising 4726 with CHD and 3529 controls. We used a nested case-control approach from 2 prospective cohort studies: BioImage,[90,91] which was enriched for older individuals at higher cardiovascular disease risk, and Malmö Diet and Cancer,[92] which had a prolonged follow-up period. We selected individuals sustaining a first CHD event and controls matched by age, sex, type 2 diabetes mellitus status, and smoking history. We also used data from two retrospective case-control studies: the ATVB (Atherosclerosis, Thrombosis, and Vascular Biology) Italian Study Group[93] and PROMIS (Pakistani Risk of Myocardial Infarction Study),[94,95] 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 versus 10% controls) and Malmö Diet and Cancer (median age: 60 years, CHIP: 7% cases versus 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 versus 0.4% controls) and PROMIS (median age: 45 years cases and 49 years controls, CHIP: 2.0% cases versus 0.9% controls).

Somatic mutations in blood cells might influence risk of ASCVD in 2 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 before clinical events. We examined the relationship between clonal hematopoiesis and subclinical coronary atherosclerosis in BioImage through measures of coronary artery calcification measured by cardiac computed tomography.[26] Among controls, clonal hematopoiesis status was associated a 3.0-fold greater likelihood of having a coronary artery calcification score >615 Agatston units, a value described to indicate high CHD risk among older adults.[26,96] Similar to our observation that larger clones (variant allele fraction ≥10%) were more strongly associated with hematologic malignancy,[25] carrying a larger clone was associated with a 12-fold greater likelihood of having a coronary artery calcification score >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 with 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, 2 groups have tested if and how clonal hematopoiesis leads to atherosclerosis (Figure 3).[26,55] *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.[97] We generated *Tet2*$^{-/-}$ mice and their bone marrow (versus 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[55] also evaluated *Tet2* deficiency in hematopoietic cells in mice and its effect on atherosclerosis. Fuster et al[55] created *Ldlr*$^{-/-}$ mice engrafted with 10% *Tet2*$^{-/-}$ bone marrow (tagged by CD45.2) and 90% *Tet2*$^{+/+}$ bone marrow (tagged by CD45.1) to mimic

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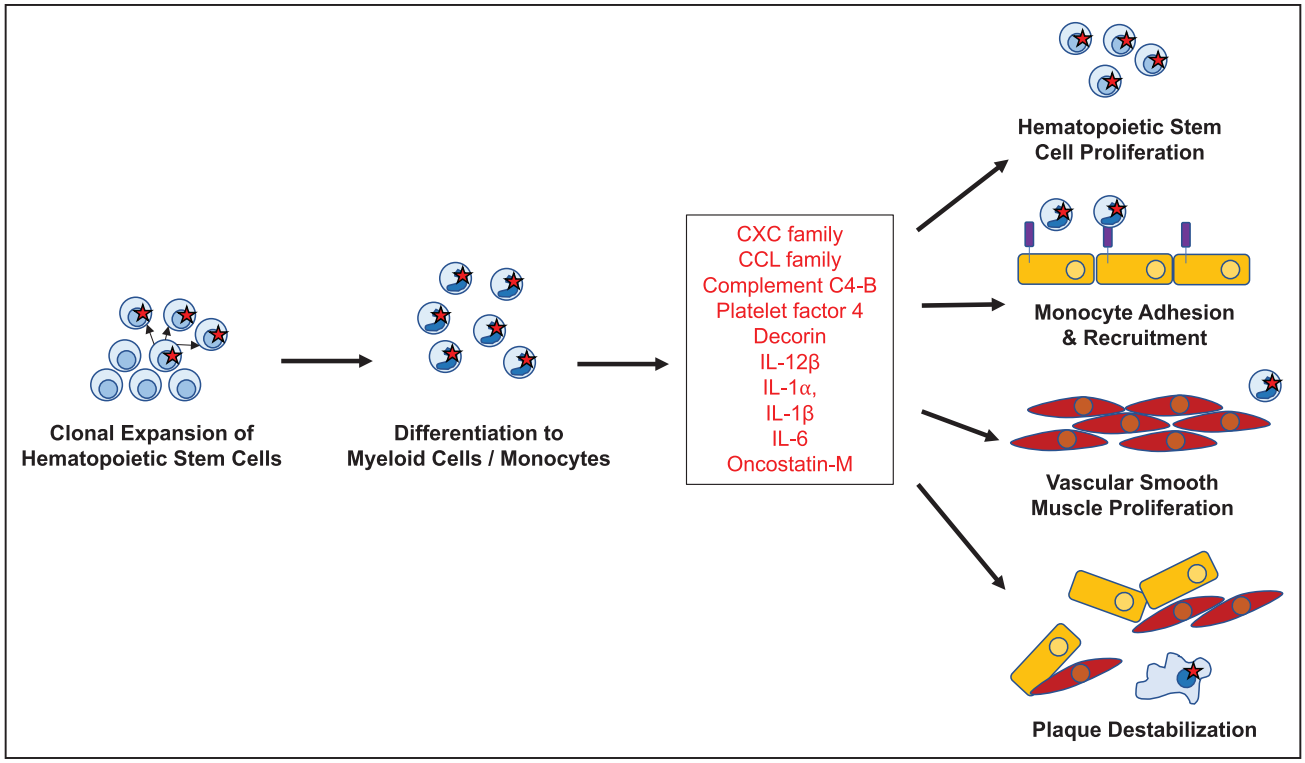

Figure 3. Inflammatory mediators of clonal hematopoiesis influencing atherosclerosis.
Summary of inflammatory factors observed to be upregulated in Jaiswal et al[26] and Fuster et al[55] and proposed influences on atherosclerosis plaque formation and destabilization.

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 with *Ldlr*$^{-/-}$ mice with 100% *Tet2*$^{+/+}$ bone marrow. These data demonstrate independent consistent observations between the 2 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[55] 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 to 8 years later, 10 VAFs did not increase, and 7 did increase and none developed hematologic cancer over this time frame.[25] In addition, Young et al[86] observed a similar pattern of VAF stability in 20 individuals over ≈10 years.

In our study, *Tet2*$^{-/-}$ bone marrow–derived macrophages in vitro were exposed to native LDL (low-density lipoprotein), resulting in increased expression of several cytokines and chemokines, including *Cxcl1, Cxcl2, Cxcl3*, *Pf4*, *Il1b*, and *Il6*, compared with control macrophages. This was consistent with prior observations linking *Tet2*-deficient bone marrow–derived macrophages with increased inflammatory gene expression.[98,99] Notably, cysteine-X-cysteine (CXC) chemokines were also elevated in serum from *Ldlr*$^{-/-}$ mice transplated with *Tet2*-deficient bone marrow. In human plasma, IL (interleukin) 8, a CXC chemokine analogue that mice lack, was approximately double among those with clonal hematopoiesis compared with those without.

In complementary studies, Fuster et al[55] exposed *Tet2*$^{-/-}$ peritoneal macrophages to lipopolysaccharide and interferon-γ in vitro and showed higher gene

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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-1β 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-1β which was mitigated by an nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain containing 3 (NLRP3) inhibitor indicating that *Tet2* deficiency promotes NLRP3 inflammasome-mediated IL-1β secretion. Similarly, aortic plaque size in chimeric *Ldlr*$^{-/-}$ mice with 10% *Tet2*$^{-/-}$ bone marrow treated with an NLRP3 inflammasome inhibitor was smaller compared with *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-1β, 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.[100–102] Furthermore, macrophages in atheroma produce >8-fold greater IL-8 compared with circulating monocytes, with even greater production when exposed to oxidized LDL.[103,104] In the setting of oxidized LDL, IL-8 inhibits the production of specific tissue inhibitors of matrix metalloproteinases thereby contributing to local plaque instability. In addition, CXC chemokines can recruit neutrophils, which can attach to atherosclerotic plaques via neutrophil extracellular traps and separately promote atherothrombosis.[105–110]

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-C).[111] Furthermore, *Ldlr*$^{-/-}$ mice engrafted with *Jak2* p.V617F bone marrow had lower total cholesterol compared with *Ldlr*$^{-/-}$ mice engrafted with wild-type bone marrow.[111] The apparent discordance between low LDL-C 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.[112–117] *JAK2* p.V617F is the most frequently observed acquired mutation recurrently identified in myeloproliferative neoplasms (MPNs)[118] and is observed in CHIP. Kilpivaara et al[115] showed that, of 321 MPN cases compared with 3000 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 (odds ratio, 4.0; P=7.7×10^{-22}) and not *JAK2*^{V617F}-negative MPN (odds ratio, 1.6; P=0.06). The JAK2 46/1 haplotype is estimated to account for 28% of the population attributable risk of MPN among Europeans.[119]

More recently, Hinds et al[120] 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 MPNs. 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 seems to be a more general effect on all MPN, regardless of *JAK2* p.V617F mutation status.[113,121]

Notably, the *SH2B3* and *ATM/PDGFD* loci were previously significantly associated with CHD in genome-wide association studies.[122] SH2B3, also known as LNK (encoding lymphocyte adapter protein), is expressed in hematopoietic cells and is a negative regulator of cellular proliferation, including negatively regulating JAK2 in stem cells.[123] Rare disruptive mutations in *SH2B3* predispose to MPN in humans, and targeted deletion in mice promotes multilineage expansion of hematopoietic stem cells.[123–125] Wang et al[125] showed that both *Lnk*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice engrafted with *Lnk*$^{-/-}$ bone marrow showed myelopoiesis, this was more prominent in the latter model. They further observe a synergistic activation of platelets from Lnk deficiency and cholesterol loading.

Although *JAK2* p.V617F is observed in the setting of CHIP, mutations in other genes (ie, *DNMT3A*, *TET2*, *ASXL1*) are more frequently observed in CHIP. Using targeted deep sequencing (>4000-fold mean coverage), Buscarlet et al[84] observed leukemogenic mutations (93% in *DNMT3A* and *TET2*) in 13.7% of 2530 apparently healthy adults. 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[24] observed the phenomenon of clonal hematopoiesis (detected by at least 3 putative somatic mutations at appreciable allele frequency) with unknown drivers (indicated by the lack of an initiating driver mutation in a previously implicated gene). 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) versus 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.[126] Here, clonal hematopoiesis was defined as the presence of >20 singleton somatic mutations with variant allele fractions ranging 0.10 to 0.20 (99.5th percentile for subjects younger than 35 years). Although 0.5% of participants younger than 35 years had clonal hematopoiesis, >50% older than 85 years had clonal hematopoiesis, approximately twice the prior yield from exome sequence analysis.[24,25] In addition, only 1 in 8 individuals (177/1403) 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) seems 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 seem 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.[126] 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 ASCVD.[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 mellitus have been associated with clonal hematopoiesis; the presence of such risk factors and others may influence risk of ASCVD in additive or multiplicative manners. Because 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-1β.[127] Individuals with rare Mendelian autoinflammatory conditions due to mutations activating the NLRP3 inflammasome have symptoms related to overproduction of IL-1β. Cholesterol crystals can stimulate the NLRP3 inflammasome within atheromata yielding IL-1β production.[128,129] IL-1β has been shown to promote hematopoietic stem cell proliferation as well as induce endothelial cell expression of monocyte adhesion and recruitment factors.[130–132] IL-1 also promotes proliferation of vascular smooth muscle cells.[133,134] IL-1 in turn further promotes the secretion of IL-6 from both endothelial cells and vascular smooth muscle cells.[135,136] Mendelian randomization studies have causally associated the IL-6 receptor with CHD.[137,138] IL-6 stimulates the production of C-reactive protein, a circulating biomarker associated with incident CHD risk.[139]

Recently, the CANTOS trial (Canakinumab Antiinflammatory Thrombosis Outcome Study) tested the efficacy and safety of IL-1β inhibition with canakinumab to prevent recurrent ASCVD events among individuals with elevated hsCRP (high-sensitivity C-reactive protein) levels (>2 mg/L).[140] Individuals receiving canakinumab 150 mg every 3 months had a 15% reduction in recurrent events

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at 4 years. Individuals who attained hsCRP <2 mg/L on treatment seemed to have a greater efficacy (HR, 0.75) versus those with persistent hsCRP >2 mg/L (HR, 0.90).[141] A surprising secondary observation was that individuals treated with canakinumab had a lower risk of developing lung cancer (HR, 0.61).[142] Given that loss of *Tet2* leads to increased *Il1b* expression in mice and that atherosclerosis can be reduced in these mice with an NLRP3 inflammasome inhibitor,[55] it is possible that downstream blockade of IL-1β will reduce the risk of atherosclerotic events among humans with *TET2*-mutated clonal hematopoiesis. In addition, current laboratory testing does not capture individuals with clonal hematopoiesis well. Analyses of complete blood counts suggest that red cell distribution width is associated, but this is not adequately sensitive or specific.[25] It is not known whether clonal hematopoiesis is associated with hsCRP.

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.

ARTICLE INFORMATION

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REFERENCES

1. Benjamin EJ, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation*. 2017;135:e146–e603. doi: 10.1161/CIR.0000000000000485.
2. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390:1151–1210. doi: 10.1016/S0140-6736(17)32152-9.
3. Sabatine MS, et al. Evolocumab in patients with cardiovascular disease. *N Engl J Med*. 2017;377:787–788. doi: 10.1056/NEJMc1708587.
4. Karmali KN, et al. A systematic examination of the 2013 ACC/AHA pooled cohort risk assessment tool for atherosclerotic cardiovascular disease. *J Am Coll Cardiol*. 2014;64:959–968. doi: 10.1016/j.jacc.2014.06.1186.
5. Wang JC, et al. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res*. 2012;111:245–259. doi: 10.1161/CIRCRESAHA.111.261388.
6. Jousilahti P, et al. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. *Circulation*. 1999;99:1165–1172.
7. Castelli WP. Epidemiology of coronary heart disease: the Framingham study. *Am J Med*. 1984;76(2A):4–12.
8. Tunstall-Pedoe H, et al. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;90:583–612.
9. Dunn J, et al. Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. *J Clin Invest*. 2014;124:3187–3199. doi: 10.1172/JCI74792.
10. Rauscher FM, et al. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation*. 2003;108:457–463. doi: 10.1161/01.CIR.0000082924.75945.48.
11. Karra R, et al. Molecular evidence for arterial repair in atherosclerosis. *Proc Natl Acad Sci USA*. 2005;102:16789–16794.
12. Olive M, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. *Arterioscler Thromb Vasc Biol*. 2010;30:2301–2309. doi: 10.1161/ATVBAHA.110.209460.
13. Ying AK, et al. Methylation of the estrogen receptor-alpha gene promoter is selectively increased in proliferating human aortic smooth muscle cells. *Cardiovasc Res*. 2000;46:172–179.
14. Childs BG, et al. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science*. 2016;354:472–477. doi: 10.1126/science.aaf6659.
15. Edo MD, et al. Aging, telomeres, and atherosclerosis. *Cardiovasc Res*. 2005;66:213–221. doi: 10.1016/j.cardiores.2004.09.007.
16. Finkel T, et al. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000;408:239–247. doi: 10.1038/35041687.
17. Libby P, et al. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473:317–325. doi: 10.1038/nature10146.
18. Swirski FK, et al. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science*. 2013;339:161–166. doi: 10.1126/science.1230719.
19. Woollard KJ, et al. Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol*. 2010;7:77–86. doi: 10.1038/nrcardio.2009.228.
20. Gerrity RG. The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions. *Am J Pathol*. 1981;103:181–190.
21. Gerrity RG. The role of the monocyte in atherogenesis: II. Migration of foam cells from atherosclerotic lesions. *Am J Pathol*. 1981;103:191–200.
22. Gerrity RG, et al. Ultrastructural identification of monocyte-derived foam cells in fatty streak lesions. *Artery*. 1980;8:208–214.
23. Welch JS, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150:264–278. doi: 10.1016/j.cell.2012.06.023.
24. Genovese G, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371:2477–2487. doi: 10.1056/NEJMoa1409405.
25. Jaiswal S, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371:2488–2498. doi: 10.1056/NEJMoa1408617.
26. Jaiswal S, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–121. doi: 10.1056/NEJMoa1701719.
27. Bowman RL, et al. Clonal hematopoiesis and evolution to hematopoietic malignancies. *Cell Stem Cell*. 2018;22:157–170. doi: 10.1016/j.stem.2018.01.011.
28. Fuster JJ, et al. Somatic mutations and clonal hematopoiesis: unexpected potential new drivers of age-related cardiovascular disease. *Circ Res*. 2018;122:523–532. doi: 10.1161/CIRCRESAHA.117.312115.
29. Steensma DP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126:9–16. doi: 10.1182/blood-2015-03-631747.
30. Tall AR, et al. Cardiovascular disease: commonality with cancer. *Nature*. 2017;543:45–47. doi: 10.1038/nature21505.
31. Keaney JF Jr. CHIP-ping away at atherosclerosis. *N Engl J Med*. 2017;377:184–185. doi: 10.1056/NEJMe1706173.
32. Elias HK, et al. Molecular mechanisms underlying lineage bias in aging hematopoiesis. *Semin Hematol*. 2017;54:4–11. doi: 10.1053/j.seminhematol.2016.11.002.

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33. Mardis ER, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med*. 2009;361:1058–1066. doi: 10.1056/NEJMoa0903840.
34. Mohrin M, et al. Hematopoietic stem cell quiescence promotes error-prone DNA repair and mutagenesis. *Cell Stem Cell*. 2010;7:174–185. doi: 10.1016/j.stem.2010.06.014.
35. Flach J, et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature*. 2014;512:198–202. doi: 10.1038/nature13619.
36. Wagner W, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. *PLoS One*. 2009;4:e5846. doi: 10.1371/journal.pone.0005846.
37. Busque L, et al. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. *Blood*. 1996;88:59–65.
38. Gale RE, et al. Acquired skewing of X-chromosome inactivation patterns in myeloid cells of the elderly suggests stochastic clonal loss with age. *Br J Haematol*. 1997;98:512–519.
39. Fey MF, et al. Clonality and X-inactivation patterns in hematopoietic cell populations detected by the highly informative M27 beta DNA probe. *Blood*. 1994;83:931–938.
40. Busque L, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012;44:1179–1181. doi: 10.1038/ng.2413.
41. Jan M, et al. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Sci Transl Med*. 2012;4:149ra118. doi: 10.1126/scitranslmed.3004315.
42. Shlush LI, et al; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506:328–333. doi: 10.1038/nature13038.
43. Jan M, et al. Clonal hematopoiesis. *Semin Hematol*. 2017;54:43–50. doi: 10.1053/j.seminhematol.2016.10.002.
44. Manolio TA, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–753. doi: 10.1038/nature08494.
45. Van der Auwera GA, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics*. 2013;11:11 10 11–11 10 33.
46. Cibulskis K, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*. 2013;31:213–219. doi: 10.1038/nbt.2514.
47. Costello M, et al. Discovery and characterization of artifactual mutations in deep coverage targeted capture sequencing data due to oxidative DNA damage during sample preparation. *Nucleic Acids Res*. 2013;41:e67. doi: 10.1093/nar/gks1443.
48. Forbes SA, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res*. 2017;45(D1):D777–D783. doi: 10.1093/nar/gkw1121.
49. Xie M, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014;20:1472–1478. doi: 10.1038/nm.3733.
50. Alexandrov LB, et al; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415–421. doi: 10.1038/nature12477.
51. Kaneda M, et al. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature*. 2004;429:900–903. doi: 10.1038/nature02633.
52. Okano M, et al. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet*. 1998;19:219–220. doi: 10.1038/890.
53. Yang L, et al. DNMT3A in haematological malignancies. *Nat Rev Cancer*. 2015;15:152–165. doi: 10.1038/nrc3895.
54. Ko M, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010;468:839–843. doi: 10.1038/nature09586.
55. Fuster JJ, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355:842–847. doi: 10.1126/science.aag1381.
56. Delhommeau F, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009;360:2289–2301. doi: 10.1056/NEJMoa0810069.
57. Langemeijer SM, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet*. 2009;41:838–842. doi: 10.1038/ng.391.
58. Boultwood J, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. *Leukemia*. 2010;24:1062–1065. doi: 10.1038/leu.2010.20.
59. Cho YS, et al. Additional sex comb-like 1 (ASXL1), in cooperation with SRC-1, acts as a ligand-dependent coactivator for retinoic acid receptor. *J Biol Chem*. 2006;281:17588–17598. doi: 10.1074/jbc.M512616200.
60. Fisher CL, et al. A human homolog of Additional sex combs, ADDITIONAL SEX COMBS-LIKE 1, maps to chromosome 20q11. *Gene*. 2003;306:115–126.
61. Kern SE, et al. Identification of p53 as a sequence-specific DNA-binding protein. *Science*. 1991;252:1708–1711.
62. Matlashewski G, et al. Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. *EMBO J*. 1984;3:3257–3262.
63. McBride OW, et al. The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13). *Proc Natl Acad Sci USA*. 1986;83:130–134.
64. Dawson MA, et al. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature*. 2009;461:819–822. doi: 10.1038/nature08448.
65. Sakatsume M, et al. The Jak kinases differentially associate with the alpha and beta (accessory factor) chains of the interferon gamma receptor to form a functional receptor unit capable of activating STAT transcription factors. *J Biol Chem*. 1995;270:17528–17534.
66. Saltzman A, et al. Cloning and characterization of human Jak-2 kinase: high mRNA expression in immune cells and muscle tissue. *Biochem Biophys Res Commun*. 1998;246:627–633. doi: 10.1006/bbrc.1998.8685.
67. Jäkel H, et al. Phosphorylation of p27Kip1 by JAK2 directly links cytokine receptor signaling to cell cycle control. *Oncogene*. 2011;30:3502–3512. doi: 10.1038/onc.2011.68.
68. Golas MM, et al. Molecular architecture of the multiprotein splicing factor SF3b. *Science*. 2003;300:980–984. doi: 10.1126/science.1084155.
69. Tanuma N, et al. Nuclear inhibitor of protein phosphatase-1 (NIPP1) directs protein phosphatase-1 (PP1) to dephosphorylate the U2 small nuclear ribonucleoprotein particle (snRNP) component, spliceosome-associated protein 155 (Sap155). *J Biol Chem*. 2008;283:35805–35814. doi: 10.1074/jbc.M805468200.
70. Ray K, et al. Isolation of cDNA clones encoding eight different human G protein gamma subunits, including three novel forms designated the gamma 4, gamma 10, and gamma 11 subunits. *J Biol Chem*. 1995;270:21765–21771.
71. Yan K, et al. Differential ability to form the G protein betagamma complex among members of the beta and gamma subunit families. *J Biol Chem*. 1996;271:7141–7146.
72. Kirsch KH, et al. The adapter type protein CMS/CD2AP binds to the proto-oncogenic protein c-Cbl through a tyrosine phosphorylation-regulated Src homology 3 domain interaction. *J Biol Chem*. 2001;276:4957–4963. doi: 10.1074/jbc.M005784200.
73. Petrelli A, et al. The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature*. 2002;416:187–190. doi: 10.1038/416187a.
74. Schmidt MH, et al. The Cbl interactome and its functions. *Nat Rev Mol Cell Biol*. 2005;6:907–918. doi: 10.1038/nrm1762.
75. Wu JY, et al. Specific interactions between proteins implicated in splice site selection and regulated alternative splicing. *Cell*. 1993;75:1061–1070.
76. Zahler AM, et al. SR proteins: a conserved family of pre-mRNA splicing factors. *Genes Dev*. 1992;6:837–847.
77. Abramowitz J, et al. XLalphas, the extra-long form of the alpha-subunit of the Gs G protein, is significantly longer than suspected, and so is its companion Alex. *Proc Natl Acad Sci USA*. 2004;101:8366–8371.
78. Fiscella M, et al. Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. *Proc Natl Acad Sci USA*. 1997;94:6048–6053.
79. Pagan JK, et al. A novel corepressor, BCoR-L1, represses transcription through an interaction with CtBP. *J Biol Chem*. 2007;282:15248–15257. doi: 10.1074/jbc.M700246200.
80. Challen GA, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet*. 2011;44:23–31. doi: 10.1038/ng.1009.
81. McKerrell T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep*. 2015;10:1239–1245.
82. Landau DA, et al. Locally disordered methylation forms the basis of intratumor methylome variation in chronic lymphocytic leukemia. *Cancer Cell*. 2014;26:813–825. doi: 10.1016/j.ccell.2014.10.012.
83. Arends CM, et al. Hematopoietic lineage distribution and evolutionary dynamics of clonal hematopoiesis [published online ahead of print March 1, 2018]. *Leukemia*. doi: 10.1038/s41375-018-0047-7.
84. Buscarlet M, et al. DNMT3A and TET2 dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood*. 2017;130:753–762. doi: 10.1182/blood-2017-04-777029.
85. Acuna-Hidalgo R, et al. Ultra-sensitive sequencing identifies high prevalence of clonal hematopoiesis-associated mutations throughout adult life. *Am J Hum Genet*. 2017;101:50–64. doi: 10.1016/j.ajhg.2017.05.013.

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86. Young AL, et al. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016;7:12484. doi: 10.1038/ncomms12484.
87. Bonnefond A, et al. Association between large detectable clonal mosaicism and type 2 diabetes with vascular complications. *Nat Genet*. 2013;45:1040–1043. doi: 10.1038/ng.2700.
88. Jacobs KB, et al. Detectable clonal mosaicism and its relationship to aging and cancer. *Nat Genet*. 2012;44:651–658. doi: 10.1038/ng.2270.
89. Laurie CC, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat Genet*. 2012;44:642–650. doi: 10.1038/ng.2271.
90. Baber U, et al. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BioImage study. *J Am Coll Cardiol*. 2015;65:1065–1074. doi: 10.1016/j.jacc.2015.01.017.
91. Muntendam P, et al; High-Risk Plaque Initiative. The BioImage Study: novel approaches to risk assessment in the primary prevention of atherosclerotic cardiovascular disease–study design and objectives. *Am Heart J*. 2010;160:49.e1–57.e1. doi: 10.1016/j.ahj.2010.02.021.
92. Berglund G, et al. The Malmo Diet and Cancer Study. Design and feasibility. *J Intern Med*. 1993;233:45–51.
93. Atherosclerosis T, et al. No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. *Circulation*. 2003;107:1117–1122.
94. Saleheen D, et al. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature*. 2017;544:235–239. doi: 10.1038/nature22034.
95. Saleheen D, et al. The Pakistan Risk of Myocardial Infarction Study: a resource for the study of genetic, lifestyle and other determinants of myocardial infarction in South Asia. *Eur J Epidemiol*. 2009;24:329–338. doi: 10.1007/s10654-009-9334-y.
96. Elias-Smale SE, et al. Coronary calcium score improves classification of coronary heart disease risk in the elderly: the Rotterdam study. *J Am Coll Cardiol*. 2010;56:1407–1414. doi: 10.1016/j.jacc.2010.06.029.
97. Moran-Crusio K, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell*. 2011;20:11–24. doi: 10.1016/j.ccr.2011.06.001.
98. Cull AH, et al. Tet2 restrains inflammatory gene expression in macrophages. *Exp Hematol*. 2017;55:56.e13–70.e13. doi: 10.1016/j.exphem.2017.08.001.
99. Zhang Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature*. 2015;525:389–393. doi: 10.1038/nature15252.
100. Boisvert WA, et al. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest*. 1998;101:353–363. doi: 10.1172/JCI1195.
101. Gerszten RE, et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*. 1999;398:718–723. doi: 10.1038/19546.
102. Schwartz D, et al. Role of the GRO family of chemokines in monocyte adhesion to MM-LDL-stimulated endothelium. *J Clin Invest*. 1994;94:1968–1973. doi: 10.1172/JCI117548.
103. Apostolopoulos J, et al. Interleukin-8 production by macrophages from atheromatous plaques. *Arterioscler Thromb Vasc Biol*. 1996;16:1007–1012.
104. Liu Y, et al. Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols may have a regulatory function for IL-8 production. *Arterioscler Thromb Vasc Biol*. 1997;17:317–323.
105. Warnatsch A, et al. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science*. 2015;349:316–320. doi: 10.1126/science.aaa8064.
106. Eash KJ, et al. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest*. 2010;120:2423–2431. doi: 10.1172/JCI41649.
107. Suratt BT, et al. Role of the CXCR4/SDF-1 chemokine axis in circulating neutrophil homeostasis. *Blood*. 2004;104:565–571. doi: 10.1182/blood-2003-10-3638.
108. Döring Y, et al. Lack of neutrophil-derived CRAMP reduces atherosclerosis in mice. *Circ Res*. 2012;110:1052–1056. doi: 10.1161/CIRCRESAHA.112.265868.
109. Döring Y, et al. Neutrophil extracellular traps in atherosclerosis and atherothrombosis. *Circ Res*. 2017;120:736–743. doi: 10.1161/CIRCRESAHA.116.309692.
110. Wantha S, et al. Neutrophil-derived cathelicidin promotes adhesion of classical monocytes. *Circ Res*. 2013;112:792–801. doi: 10.1161/CIRCRESAHA.112.300666.
111. Liu DJ, et al; Charge Diabetes Working Group; EPIC-InterAct Consortium; EPIC-CVD Consortium; GOLD Consortium; VA Million Veteran Program. Exome-wide association study of plasma lipids in >300,000 individuals. *Nat Genet*. 2017;49:1758–1766. doi: 10.1038/ng.3977.
112. Tapper W, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. *Nat Commun*. 2015;6:6691. doi: 10.1038/ncomms7691.
113. Trifa AP, et al. TERT rs2736100 A>C SNP and JAK2 46/1 haplotype significantly contribute to the occurrence of JAK2 V617F and CALR mutated myeloproliferative neoplasms - a multicentric study on 529 patients. *Br J Haematol*. 2016;174:218–226. doi: 10.1111/bjh.14041.
114. Trifa AP, et al. MECOM, HBS1L-MYB, THRB-RARB, JAK2, and TERT polymorphisms defining the genetic predisposition to myeloproliferative neoplasms: a study on 939 patients. *Am J Hematol*. 2018;93:100–106. doi: 10.1002/ajh.24946.
115. Kilpivaara O, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet*. 2009;41:455–459. doi: 10.1038/ng.342.
116. Koh SP, et al. Genetic association between germline JAK2 polymorphisms and myeloproliferative neoplasms in Hong Kong Chinese population: a case-control study. *BMC Genet*. 2014;15:147. doi: 10.1186/s12863-014-0147-y.
117. Pardanani A, et al. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. *Blood*. 2008;111:2785–2789. doi: 10.1182/blood-2007-06-095703.
118. Levine RL, et al. X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. *Blood*. 2006;107:4139–4141. doi: 10.1182/blood-2005-09-3900.
119. Jones AV, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet*. 2009;41:446–449. doi: 10.1038/ng.334.
120. Hinds DA, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. *Blood*. 2016;128:1121–1128. doi: 10.1182/blood-2015-06-652941.
121. Oddsson A, et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. *Leukemia*. 2014;28:1371–1374. doi: 10.1038/leu.2014.48.
122. Nikpay M, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47:1121–1130.
123. Bersenev A, et al. Lnk controls mouse hematopoietic stem cell self-renewal and quiescence through direct interactions with JAK2. *J Clin Invest*. 2008;118:2832–2844. doi: 10.1172/JCI35808.
124. McMullin MF, et al. A nonsynonymous LNK polymorphism associated with idiopathic erythrocytosis. *Am J Hematol*. 2011;86:962–964. doi: 10.1002/ajh.22154.
125. Wang W, et al. LNK/SH2B3 loss of function promotes atherosclerosis and thrombosis. *Circ Res*. 2016;119:e91–e103. doi: 10.1161/CIRCRESAHA.116.308955.
126. Zink F, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood*. 2017;130:742–752. doi: 10.1182/blood-2017-02-769869.
127. Latz E, et al. Activation and regulation of the inflammasomes. *Nat Rev Immunol*. 2013;13:397–411. doi: 10.1038/nri3452.
128. Duewell P, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938.
129. Paramel Varghese G, et al. NLRP3 inflammasome expression and activation in human atherosclerosis. *J Am Heart Assoc*. 2016;5:e003031. doi: 10.1161/JAHA.115.003031.
130. Sager HB, et al. Targeting interleukin-1β reduces leukocyte production after acute myocardial infarction. *Circulation*. 2015;132:1880–1890. doi: 10.1161/CIRCULATIONAHA.115.016160.
131. Shemesh S, et al. Interleukin-1 receptor type-1 in non-hematopoietic cells is the target for the pro-atherogenic effects of interleukin-1 in apoE-deficient mice. *Atherosclerosis*. 2012;222:329–336. doi: 10.1016/j.atherosclerosis.2011.12.010.
132. Kirii H, et al. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2003;23:656–660. doi: 10.1161/01.ATV.0000064374.15232.C3.

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133. Libby P, et al. Interleukin 1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids. *J Clin Invest*. 1988;81:487–498. doi: 10.1172/JCI113346.
134. Alexander MR, et al. Interleukin-1β modulates smooth muscle cell phenotype to a distinct inflammatory state relative to PDGF-DD via NF-κB-dependent mechanisms. *Physiol Genomics*. 2012;44:417–429. doi: 10.1152/physiolgenomics.00160.2011.
135. Loppnow H, et al. Proliferating or interleukin 1-activated human vascular smooth muscle cells secrete copious interleukin 6. *J Clin Invest*. 1990;85:731–738. doi: 10.1172/JCI114498.
136. Sironi M, et al. IL-1 stimulates IL-6 production in endothelial cells. *J Immunol*. 1989;142:549–553.
137. Swerdlow DI, et al; Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium. The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. *Lancet*. 2012;379:1214–1224. doi: 10.1016/S0140-6736(12)60110-X.
138. Sarwar N, et al; IL6R Genetics Consortium Emerging Risk Factors Collaboration. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*. 2012;379:1205–1213. doi: 10.1016/S0140-6736(11)61931-4.
139. Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. *Circ Res*. 2016;118:145–156. doi: 10.1161/CIRCRESAHA.115.306656.
140. Ridker PM, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914.
141. Ridker PM, et al; CANTOS Trial Group. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet*. 2018;391:319–328. doi: 10.1016/S0140-6736(17)32814-3.
142. Ridker PM, et al; CANTOS Trial Group. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet*. 2017;390:1833–1842. doi: 10.1016/S0140-6736(17)32247-X.

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