Published in final edited form as:

Curr Opin Cardiol. 2020 May ; 35(3): 219–225. doi:10.1097/HCO.0000000000000726.

Genetics of age-related clonal hematopoiesis and atherosclerotic cardiovascular disease

Changhee Jung^{a,b}, Megan A. Evans^a, Kenneth Walsh^a

^aHematovascular Biology Center, Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine, Charlottesville, Virginia, USA

^bDepartment of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

Abstract

Purpose of review—While advanced age is the major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD), we have a poor understanding of how aging promotes the progression of this disease. Recent evidence suggests that the age-dependent accumulation of somatic mutations in hematopoietic cells may represent a new causal risk factor for ASCVD.

Recent findings—A hallmark of aging is the accumulation of somatic DNA mutations in all tissues of the body. Accordingly, evidence shows that hematopoietic stem/progenitor cells accumulate somatic mutations as a function of age in nonsymptomatic individuals. When these mutations occur in driver genes that provide a selective advantage to the hematopoietic stem/progenitor cells, they undergo a clonal expansion and progressively give rise to blood leukocytes that harbor these mutations. This phenomenon, referred to as clonal hematopoiesis, has been associated with the increased risk of mortality, hematologic malignancy, ASCVD, and related diseases. Notably, many individuals exhibiting clonal hematopoiesis carry single ‘driver’ mutations in preleukemic genes including *DNA methyltransferase 3a*, *ten-eleven translocation 2*, *additional sex combs like 1*, and *Janus kinase 2*. Experimental studies show that these mutations in some of these genes can alter the inflammatory properties of the leukocyte and contribute to the pathogenesis of ASCVD.

Summary—We review recent epidemiological and experimental findings on the association between age-related clonal hematopoiesis and ASCVD by focusing on prevalent driver gene mutations.

Keywords

additional sex combs like 1; atherosclerosis; clonal hematopoiesis; *DNA methyltransferase 3a*; driver genes; *Janus kinase 2*; *ten-eleven translocation 2*

Correspondence to Kenneth Walsh, PhD, Hematovascular Biology Center, Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine, 415 Lane Rd, Suite 1010, PO Box 801394, Charlottesville, VA 22908, USA. Tel: +1 434 243 8303; kw9ar@virginia.edu.

Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Despite large declines in mortality associated with atherosclerotic cardiovascular disease (ASCVD) [1], it is still the leading cause of disease burden and deaths globally [1-4]. ASCVD is particularly prevalent in the elderly population, and the social and financial burden attributable to ASCVD will continue to rise due to the projected population growth of the elderly over the next several decades [5]. Atherosclerosis is a multifactorial disease, which involves the complex interplay of genetic and environmental factors over the course of one's life. The impacts of traditional risk factors such as age, sex, type 2 diabetes mellitus, elevated blood pressure, smoking, high levels of LDL cholesterol, and low levels of HDL cholesterol on ASCVD risk have been well documented [6]. However, increasing evidence shows that conventional cardiovascular risk factors are incompletely predictive of ASCVD, particularly in elderly individuals [7,8]. For instance, almost 60% of elderly patients with ASCVD have either no conventional risk factors (e.g., hypertension, hypercholesterolemia, etc.) or just one risk factor [8]. In light of these considerations, it is widely recognized that aging *per se* is a powerful risk factor for ASCVD [9,10]. Despite this strong association between aging and the risk of ASCVD, the biological mechanisms by which aging promotes ASCVD remain incompletely understood.

A major hallmark of aging is the accumulation of somatic DNA mutations in multiple tissues throughout the body [11-14]. In most cases, these sporadic mutations have no effect on cellular function or they confer a selective disadvantage to the mutant cell leading to its quick demise [15]. However, occasionally one of these mutations may confer a competitive advantage to the cell, such as by promoting its proliferation, self-renewal, survival, or some combination of these, and this may result in the expansion of the mutant cell at a disproportionate rate compared with other cells (i.e., clonal expansion) [16].

Recent epidemiological studies have revealed that normal human aging is associated with clonal expansion of hematopoietic cells harboring an initiating preleukemic driver mutation [17-20]. This condition has been referred to as clonal hematopoiesis and more recently as clonal hematopoiesis of indeterminate potential or age-related clonal hematopoiesis to distinguish it from the therapy-related clonal hematopoiesis in which the preexisting small mutant clones expand selectively after chemo or radiation therapy [17,21]. The initial studies in this field used whole-exome DNA sequencing of peripheral blood to estimate the prevalence of clonal hematopoiesis in the general population [17,18]. It was found that mutations rarely accumulated in individuals less than 40 years old but became more frequent with each successive decade of life thereafter. Using this approach, it has been estimated that the prevalence of clonal hematopoiesis is between 5 and 10% by 70 years of age. However, more recent studies using more sensitive detection methods estimate a much higher prevalence of clonal hematopoiesis in this population [17-20].

Although age-related clonal hematopoiesis is associated with increase in the risk of future hematologic malignancies [17,18], the absolute risk of these conditions is still relatively low (approximately 0.5–1.0% per year) [17]. Unexpectedly, this phenomenon has been strongly linked with an increase in both all-cause mortality and incidence and death due to ASCVD including myocardial infarction (MI) and ischemic stroke [17,22]. While many different

candidate driver genes have been associated with clonal hematopoiesis in the elderly, most of the known mutations occur in just four genes, specifically *DNA methyltransferase 3a* (*DNMT3A*), *ten eleven translocation 2* (*TET2*), *additional sex combs like 1* (*ASXL1*), and *Janus kinase 2* (*JAK2*) [17-19,22]. Studies indicate that most individuals with clonal hematopoiesis only harbor a mutation in a single gene, rather than multiple genes, which would otherwise substantially increase the likelihood of the individual developing a hematological malignancy. It should also be noted that clonal hematopoiesis can occur without the presence of a known driver mutation, perhaps by genetic drift in the hematopoietic stem/progenitor cell (HSPC) pool during normal aging, by a mutation in an unidentified driver gene, or by a heritable epigenetic trait [20,23,24]. In fact, studies estimate that mutations in unknown driver genes account for a substantial proportion of clonal events within the aged hematopoietic system [18,20]; however, these types of mutations are outside the scope of the current review. In this review, we summarize the current evidence on the association between age-related clonal hematopoiesis and ASCVD by focusing on four of the most commonly known driver gene mutations.

GENES COMMONLY MUTATED IN AGE-RELATED CLONAL HEMATOPOIESIS AND THEIR RELEVANCE WITH ATHEROSCLEROSIS

Ten-eleven translocation 2

TET2 is the second most commonly mutated known driver gene observed in elderly individuals without hematological malignancies [17,19,22] and was the first genetic mutation studied to assess the causality between age-related clonal hematopoiesis and cardiovascular disease [25]. *TET2* encodes an epigenetic regulatory enzyme that converts 5-methylcytosine to 5-hydroxymethylcytosine, generally resulting in a reduction in DNA methylation and transcriptional activation [26,27]. On the other hand, it has also been shown that *TET2* can promote the transcriptional repression of genes by recruiting histone deacetylases (HDAC) to gene promoter regions [25,28]. Thus, *TET2* is a multifaceted epigenetic regulator that can facilitate either transcriptional activation and repression of many genes and this appears to largely depend on the cellular and molecular environment [26-28].

Based on the epidemiological association between clonal hematopoiesis and increased incidence and mortality due to ASCVD [17,22], our group recently explored the potential cardiovascular consequences of *TET2*-mediated clonal hematopoiesis in an experimental system. To study clonal hematopoiesis in ASCVD, Fuster *et al.* [25] used a competitive bone marrow transplant approach in which a small number of *Tet2*-deficient cells were introduced to atherosclerosis prone *Ldlr*^{-/-} mice. This experimental approach models the human setting of *TET2*-clonal hematopoiesis, as these mutations are initially carried by a small number of HSPCs, which will gradually expand over time [25]. It was found that partial bone marrow reconstitution with *Tet2*-deficient cells led to clonal expansion of the mutant cells over a 12-week study period and this expansion was associated with a marked increase in atherosclerotic plaque size [25]. Crucially, the accelerated effect of *Tet2*-deficient cells on atherosclerosis was independent of alterations in systemic metabolism such as body weight and insulin sensitivity as well as changes in blood cell counts. This is analogous to what is

observed in cancer-free individuals who exhibit clonal hematopoiesis associated with a *TET2* mutation. During clonal expansion of the mutant cells, a slight myeloid bias was observed and, these cells appeared to expand preferentially into macrophages in the atherosclerotic vascular wall. To determine if *Tet2*-deficient myeloid cells were playing a role in driving the progression of atherosclerosis, Fuster *et al.* [25] also generated a mouse model in which *Tet2* was specifically ablated in myeloid cells using a LysM (lysozyme M)-Cre (cre recombinase)/LoxP (locus of X-over P1) strategy. These mice displayed a similar profile of accelerated atherosclerosis to the animals that underwent a competitive bone marrow transplant, indicating that cells of myeloid origin and likely macrophages were the key drivers of the augmented lesion size in this experimental setting [25]. These findings were later validated by an independent study in which hematopoietic cell ablation and myeloid-specific ablation of *Tet2* led to accelerated atherosclerosis in mice [22]. Collectively, these experimental studies provided the first causal link between somatic mutations in the hematopoietic system and ASCVD.

From a mechanistic point of view, in vitro and in vivo studies suggested that the accelerated atherosclerosis in conditions of hematopoietic *Tet2* loss-of-function were mainly driven by elevated expression of proinflammatory cytokines in *Tet2*-deficient macrophages [25,29]. While genome wide expression profiling studies found that the expression of nearly 500 genes was altered by *Tet2*-deficiency in macrophages, it was noted that these cells exhibited increased production of the proinflammatory cytokine IL-1 β , a key driver of local and systemic immune responses in ASCVD [30]. In the aortas of mice transplanted with *Tet2* deficient cells it was noted that the elevated expression of IL-1 β was accompanied by the upregulation of NLRP3 (nucleotide binding domain leucine-rich repeat-containing receptor (NLR) pyrin domain-containing 3) expression and caspase-1 activity [25], both of which are components of the inflammasome that converts IL-1 β to its active form [31]. In-vitro experiments extended these findings and noted that there was greater histone H3 acetylation at the *IL-1 β* promoter under the conditions of *Tet2* deficiency, which led to the upregulation of NLRP3 expression and caspase-1 activity [25]. This upregulation of inflammasome components could thus at least in part account for the observed increase in IL-1 β production [25]. Consistent with these findings, treatment with a HDAC inhibitor increased IL-1 β expression and abolished expression differences between *Tet2*-deficient and wild-type (WT) macrophages. Importantly, treatment with the selective NLRP3 inhibitor, MCC950 abrogated IL-1 β production and eliminated differences in atherosclerotic lesion between the mice that received WT bone marrow or *Tet2*-deficient bone marrow [25], confirming a role for IL-1 β and the NLRP3 inflammasome in the augmented lesion size.

The above-mentioned findings on *TET2*-mediated clonal hematopoiesis may have implications for understanding the outcomes of the recent CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcome Study) clinical trial. CANTOS was a large, placebo controlled clinical trial that examined the effects of escalating doses of a neutralizing IL-1 β antibody, in patients who had a previous MI and sustained levels of inflammation as measured by high-sensitivity C-reactive protein (hsCRP). It was found that targeting IL-1 β at the middle dose (150mg every 3 months) led to a modest but significant reduction in recurrent cardiovascular events compared with the placebo group. Despite the evidence of a preventive effect of canakinumab on recurrent ASCVD in individuals with a high degree of

systemic inflammation, its widespread use could be challenging because of potential adverse events such as increased risk of infection. Thus, a secondary analysis was performed to determine whether there was a clinical subgroup that were more responsive to the therapy [32]. It was found that individuals with hsCRP level that dropped below 2 mg/l had a reduction of 25, 31, and 31% in major adverse cardiovascular events, cardiovascular mortality and all-cause mortality events, respectively. Individuals which did not experience a reduction in hsCRP below 2mg/l displayed no reduction in these end points [32]. Given the preclinical causal link between *TET2*-mediated clonal hematopoiesis and accelerated atherosclerosis through the IL-1 β /NLRP3 pathway [25], it can be speculated that individuals with *TET2* mutations may respond more favorably than the general study population to IL-1 β -targeted therapy. Recently, an exploratory analysis of CANTOS supported this speculation, in which treatment with canakinumab decreased the relative risk of major cardiovascular events by 64% in carriers of *TET2* mutations (detected by targeted deep sequencing in blood genomic DNA) compared with more modest reductions in the overall cohort [33]. Although this result should be further validated in prospective studies, these data suggest the possible clinical utility of testing clonal hematopoiesis for a personalized anti-inflammatory therapy for the prevention of recurrent ASCVD.

Finally, the results of the ASCVD model were recently corroborated and extended by a second study that examined the role of *Tet2* in models of heart failure. Sano *et al.* [34] demonstrated that hematopoietic or myeloid *Tet2* deficiency worsened cardiac remodeling and function in response to pressure overload as well as chronic ischemia. These greater cardiac dysfunctions also resulted from the excessive production of IL-1 β by *Tet2*-deficient myeloid cell progeny. Overall, these findings further indicate that clonal hematopoiesis associated with *Tet2* mutations is a novel important player which can lead to accelerated cardiovascular disease.

Janus kinase 2

JAK2 is another commonly mutated gene that has been associated with age-related clonal hematopoiesis, although the frequency of this mutation is less than that of mutations in *DNMT3A*, *TET2*, and *ASXL1* [17-20,22]. *JAK2* is a nonreceptor tyrosine kinase that transmits intracellular signals downstream of various cytokine receptors [35]. The most common type of *JAK2* mutation associated with clonal hematopoiesis is the V617F mutation, which results in a change in the amino acid, valine to phenylalanine at the 617 position, thus this mutation is commonly denoted as *JAK2*^{V617F} [36]. The mutation is believed to cause changes to the autoinhibitory site of *JAK2* kinase, resulting in a constitutively active protein; thus, this type of mutation is considered a gain-of-function mutation [37]. The *JAK2*^{V617F} mutation is also the most frequently acquired mutation in patients with myeloproliferative neoplasms (MPNs), including polycythemia vera, essential thrombocytosis, and myelofibrosis, that are generally associated with the aberrant production of red blood cells, platelets and leukocytes, respectively [38-40]. Major causes of morbidity and mortality in MPNs are represented by arterial and venous thrombotic complications in addition to their progression to myelofibrosis and acute leukemia [38-40]. Abnormalities of blood cells arising from the clonal proliferation of *JAK2* mutated-hematopoietic stem cells are known to involve not only quantitative changes but also

qualitative modifications that characterize the switch of these cells from a resting to a procoagulant phenotype [40]. For example, enhanced *JAK2^{V617F}*-driven neutrophil extracellular trap formation has been suggested to be responsible for the increased incidence of thrombosis in patients with MPNs [41].

Recently, the role of the hematopoietic *JAK2^{V617F}* mutation in the development of atherosclerosis was examined [42]. In this study, lethally irradiated *Ldlr^{-/-}* mice were transplanted with either *JAK2^{V617F}* or WT bone marrow cells. Mice transplanted with *JAK2^{V617F}* bone marrow cells showed accelerated atherosclerotic lesions despite lower levels of plasma cholesterol [42]. It was suggested that elevated erythrophagocytosis (phagocytosis of red blood cells by macrophages) and defective efferocytosis (the process by which dying/dead cells are removed by phagocytic cells) were the underlying mechanisms by which *JAK2^{V617F}* accelerates atherosclerosis in this experimental setting [42]. However, in this study, mice transplanted *JAK2^{V617F}* mutant cells displayed marked erythrocytosis, thrombocytosis and neutrophilia [42], and thus the enhanced atherogenesis could be due to the confounding effects of the MPN phenotype, which is not representative of individuals with age-related clonal hematopoiesis.

Despite their role in MPNs, *JAK2^{V617F}* mutations frequently occur in individuals with clonal hematopoiesis who exhibit no abnormalities in blood cell counts [17-20,22,43-45]. In light of this consideration, it is warranted to adopt appropriate experimental strategies which can delineate the role of *JAK2^{V617F}* in cardiovascular disease under the condition of normal blood cell counts, thereby excluding the *JAK2^{V617F}*-associated MPN phenotypes (i.e., high blood cell counts and the prothrombotic complications). Our group recently developed a system to restrict *JAK2^{V617F}* expression only to differentiated blood myeloid cells [46[■]]. This was accomplished by transplantation of lineage-negative *JAK2^{V617F}* bone marrow cells that were generated by a lentiviral vector and the *JAK2* mutation was expressed under the control of the myeloid specific SP147-gp91 promoter-enhancer. This system was developed to avoid the expression of *JAK2^{V617F}* in the erythroid and megakaryocyte populations that would lead to changes in blood cell numbers [46[■]]. Notably, these mice exhibited normal levels of hemoglobin and platelet counts after transplantation and did not show any MPN-like phenotypes. These mice exhibited greater myocardial inflammation and accelerated heart failure when subjected to experimental cardiac injury. Although these mice were studied in view of pathological remodeling in murine heart failure, it is possible that these results might be relevant with the pathogenesis of atherosclerosis considering its mechanism of action through the enhanced inflammation by *JAK2^{V617F}* in myeloid cells [46[■]]. Regardless, further research is required to elucidate the roles of *JAK2^{V617F}* in the context of clonal hematopoiesis and atherosclerosis.

Other genes (*DNA methyltransferase 3a* and *additional sex combs like 1*)

DNMT3A is the most frequently mutated known driver gene in age-related clonal hematopoiesis [17-20,22]. *DNMT3A* acts as a de novo DNA methyltransferase and its mutation is frequently detected in myeloid malignancies [47-49]. Mutations of *DNMT3A* are generally believed to result in loss of its enzymatic activity [48], which leads to enhanced self-renewal of HSPCs and thus facilitates their clonal expansion [49]. The role of

hematopoietic mutations in *DNMT3A* in the pathogenesis of cardiovascular disease has been less extensively studied compared with the aforementioned genes, *TET2* and *JAK2*. Our group has demonstrated that the hematopoietic *Dnmt3a* loss-of-function leads to greater cardiac dysfunction following experimental pressure overload [50[■]]. Notably, this preclinical role for *DNMT3A* mutations in experimental heart failure was followed up by the independent analysis in a human cohort comprised 200 patients with chronic ischemic heart failure [51]. These data suggested that the expansion of *DNMT3A* somatic mutations (along with *TET2* mutations) in hematopoietic cells was associated with the progression of chronic heart failure [51]. Although these studies on *DNMT3A* have been performed in the context of heart failure [50[■],51], *DNMT3A* mutations may also contribute to atherosclerosis considering its multiple roles in the regulation of inflammation. For instance, *DNMT3A* deficiency has been shown to result in increased IFN- γ production by T cells [52,53], higher mast cell activity [54], and higher activity of IL-6 in synovial fibroblasts [55]. In line with these previous results, our group has also shown that *Dnmt3a* deficiency promotes inflammation via the upregulation of specific cytokines (i.e., IL-6) and chemokines including Cxcl1, Cxcl2 and Ccl5 in macrophages [50[■]]. Finally, a preliminary report by Rauch *et al.* [56] indicates that hematopoietic loss-of-function mutations in *Dnmt3a* can promote atherosclerosis in *Ldlr*^{-/-} mice. In toto, accumulating preclinical evidence suggests that hematopoietic *DNMT3A* mutation may promote ASCVD.

ASXL1 also encodes for an epigenetic regulator [57], and it is frequently mutated in individuals with clonal hematopoiesis [17-20,22]. As a chromatin regulator, *ASXL1* plays an important role in regulating gene expression involved in either differentiation or proliferation by methylation and acetylation of histone that results in activating or repressing the transcription of genes [57]. *ASXL1* is mutated at a high frequency in multiple forms of myeloid malignancies and this is associated with poor prognosis [57,58]. Most *ASXL1* mutants that have been identified in myeloid malignancies harbor nonsense or frameshift mutations localized near the 5' end of the last exon [59]. Presumably, these mutations should escape nonsense-mediated decay of mRNA and produce a gain-of-function protein truncated at the C terminus [58]. To date, however, mechanistic evidence about the contribution of *ASXL1* mutations to cardiovascular disease including atherosclerosis is lacking and further studies are warranted.

FUTURE PERSPECTIVES

Although, to date, studies of clonal hematopoiesis have provided novel insights into mechanisms by which aging may promote cardiovascular disease, this research represents the 'tip-of-the-iceberg' with regard to defining the role that hematopoietic mutations have on the cardiovascular system. First, dozens of candidate driver genes have been associated with clonal hematopoiesis, yet only three (*TET2*, *DNMT3A*, and *JAK2*^{V617F}) have been investigated in experimental models of cardiovascular diseases [22, 25,42,46[■],50[■],56]. Second, considering the numerous mechanistic differences between the proteins encoded by these driver genes, it is highly possible that mutations in these genes will confer divergent functions on hematopoietic cells. Therefore, some mutant driver genes will be highly pathological, whereas others could be relatively benign or perhaps even protective with respect to cardiovascular disease. Third, a nonbiased whole genome analysis has shown that

known driver genes contribute to a relatively small fraction (~20%) of all clinically observed clonal hematopoiesis [20]. Notably, mutations in unknown driver genes have also been associated with an increase in all-cause mortality and conceivably some of these deaths could be due to cardiovascular conditions. Therefore, identifying these unknown drivers may will be an important next step. Fourth it is possible that the same mutation may affect components of the cardiovascular system differently, and, thus, future experimental studies should examine the effects of clonal hematopoiesis on cardiovascular diseases beyond heart failure and atherosclerosis. Therefore, many research opportunities exist to better understand how clonal hematopoiesis contributes to and shapes ASCVD and potentially other age-associated diseases.

CONCLUSION

While aging is the major risk factor for overall ASCVD, we have a poor understanding of how aging promotes disease progression. Several epidemiological studies have led to the recognition that somatic mutations in blood cells, that is, clonal hematopoiesis, is a previously unrecognized risk factor of cardiovascular disease [17,22,29,51]. However, the descriptive nature of these human studies does not reveal a cause–effect relationship, as these associations could be secondary to confounding factors or simply reflect a shared consequence of the process of aging. Although several experimental studies examining the casual role of candidate driver gene mutations including *TET2*, *JAK2*^{V617F} and *DNMT3A* on the pathogenesis of cardiovascular disease have been conducted (Fig. 1), there is a clear need for additional experimental studies, particularly those examining other driver mutations and disease models beyond heart failure and atherosclerosis. These studies could conceivably lead to the identification of new pathogenic mechanisms that underlie ASCVD and contribute to the design of personalized therapies for individuals with specific somatic mutations in their white blood cells.

Acknowledgments

Financial support and sponsorship

The current work was supported by NIH grants HL138014, HL142650, HL141256 and HL139819 (K.W.); American Heart Association grant 20POST35210098 (M.A.E.); and the Basic Science Research Program, National Research Foundation of Korea (NRF), Ministry of Education grant 2015R1D1A1A01057720 (C.J.).

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest

1. Roth GA, Johnson CO, Abate KH, et al. The burden of cardiovascular diseases among us states, 1990–2016. *JAMA Cardiol* 2018; 3:375–389. [PubMed: 29641820]
2. Vos T, Abajobir AA, Abbafati C, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic

analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; 390:1211–1259. [PubMed: 28919117]

3. Naghavi M, Abajobir AA, Abbafati C, et al. 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. [PubMed: 28919116]
4. Wang H, Abajobir AA, Abate KH, et al. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017; 390:1084–1150. [PubMed: 28919115]
5. Yazdanyar A, Newman AB. The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. *Clin Geriatr Med* 2009; 25:563–577. [PubMed: 19944261]
6. Fruchart JC, Nierman MC, Stroes ESG, et al. New risk factors for atherosclerosis and patient risk assessment. *Circulation* 2004; 109:III15–III19. [PubMed: 15198961]
7. Khot UN, Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease. *J Am Med Assoc* 2003; 290:898–904.
8. Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort the PESA (Progression of Early Subclinical Atherosclerosis) study. *Circulation* 2015; 131:2104–2113. [PubMed: 25882487]
9. D'Agostino RB, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham heart study. *Circulation* 2008; 117:743–753. [PubMed: 18212285]
10. Kannel WB, Vasan RS. Is age really a non-modifiable cardiovascular risk factor? *Am J Cardiol* 2009; 104:1307–1310. [PubMed: 19840582]
11. Forsberg LA, Gisselsson D, Dumanski JP. Mosaicism in health and disease-clones picking up speed. *Nat Rev Genet* 2017; 18:128–142. [PubMed: 27941868]
12. Zhang L, Dong X, Lee M, et al. Single-cell whole-genome sequencing reveals the functional landscape of somatic mutations in B lymphocytes across the human lifespan. *Proc Natl Acad Sci USA* 2019; 116:9014–9019. [PubMed: 30992375]
13. Milholland B, Auton A, Suh Y, Vijg J. Age-related somatic mutations in the cancer genome. *Oncotarget* 2015; 22:24627–24635.
14. Yizhak K, Aguet F, Kim J, et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science* 2019; 364:970.
15. Fuster JJ, Walsh K. Somatic mutations and clonal hematopoiesis: unexpected potential new drivers of age-related cardiovascular disease. *Circ Res* 2018; 122:523–532. [PubMed: 29420212]
16. Wagner W, Bork S, Horn P, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. *PLoS One* 2009; 4:e5846. [PubMed: 19513108]
17. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; 371:2488–2498. [PubMed: 25426837]
18. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014; 371:2477–2487. [PubMed: 25426838]
19. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; 20:1472–1478. [PubMed: 25326804]
20. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* 2017; 130:742–752. [PubMed: 28483762]
21. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with nonhematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell* 2017; 21:374–382. [PubMed: 28803919]
22. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med* 2017; 377:111–121. [PubMed: 28636844]
23. Shlush LI. Age-related clonal hematopoiesis. *Blood* 2018; 131:496–504. [PubMed: 29141946]
24. Loh PR, Genovese G, Handsaker RE, et al. Insights into clonal haematopoiesis from 8,342 mosaic chromosomal alterations. *Nature* 2018; 559:350–355. [PubMed: 29995854]

25. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 2017; 355:842–847. [PubMed: 28104796]
26. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009; 324:930–935. [PubMed: 19372391]
27. Iyer LM, Tahiliani M, Rao A, Aravind L. Prediction of novel families of enzymes involved in oxidative and other complex modifications of bases in nucleic acids. *Cell Cycle* 2009; 8:1698–1710. [PubMed: 19411852]
28. Zhang Q, Zhao K, Shen Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature* 2015; 525:389–393. [PubMed: 26287468]
29. Bick AG, Pirruccello JP, Griffin GK, et al. Genetic interleukin 6 signaling deficiency attenuates cardiovascular risk in clonal hematopoiesis. *Circulation* 2020; 141:124–131. [PubMed: 31707836]
30. Libby P Interleukin-1 beta as a target for atherosclerosis therapy: biological basis of CANTOS and beyond. *J Am Coll Cardiol* 2017; 70:2278–2289. [PubMed: 29073957]
31. Grebe A, Hoss F, Latz E. NLRP3 inflammasome and the IL-1 pathway in atherosclerosis. *Circ Res* 2018; 122:1722–1740. [PubMed: 29880500]
32. Ridker PM, MacFadyen JG, Everett BM, et al. 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. [PubMed: 29146124]
33. Svensson E, Madar A, Campbell C, et al. TET2-driven clonal hematopoiesis predicts enhanced response to canakinumab in the CANTOS trial: an exploratory analysis. *Circulation* 2018; 138:A15111.
34. Sano S, Oshima K, Wang Y, et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1beta/NLRP3 inflammasome. *J Am Coll Cardiol* 2018; 71:875–886. [PubMed: 29471939]
35. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev* 2009; 228:273–287. [PubMed: 19290934]
36. Shammo JM, Stein BL. Mutations in MPNs: prognostic implications, window to biology, and impact on treatment decisions. *Sci Transl Med* 2018; 10:eaan8292. [PubMed: 29643232]
37. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005; 7:387–397. [PubMed: 15837627]
38. Spivak JL. Myeloproliferative neoplasms. *N Engl J Med* 2017; 376: 2168–2181. [PubMed: 28564565]
39. Tefferi A, Pardanani A. Myeloproliferative neoplasms: a contemporary review. *JAMA Oncol* 2015; 1:97–105. [PubMed: 26182311]
40. Barbui T, Finazzi G, Falanga A. Myeloproliferative neoplasms and thrombosis. *Blood* 2013; 122:2176–2184. [PubMed: 23823316]
41. Wolach O, Sellar RS, Martinod K, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med* 2018; 10:eaan8292. [PubMed: 29643232]
42. Wang W, Liu W, Fidler T, et al. Macrophage inflammation, erythrophagocytosis, and accelerated atherosclerosis in JAK2V617F mice. *Circ Res* 2018; 123:e35–e47. [PubMed: 30571460]
43. Nielsen C, Birgens HS, Nordestgaard BG, Bojesen SE. Diagnostic value of JAK2 V617F somatic mutation for myeloproliferative cancer in 49 488 individuals from the general population. *Br J Haematol* 2013; 160:70–79. [PubMed: 23116358]
44. Nielsen C, Birgens HS, Nordestgaard BøG, et al. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. *Haematologica* 2011; 96:450–453. [PubMed: 21160067]
45. Sidon P, El Housni H, Dessars B, Heimann P. The JAK2V617F mutation is detectable at very low level in peripheral blood of healthy donors. *Leukemia* 2006; 20:1622. [PubMed: 16775613]
46. ■ Sano S, Wang Y, Yura Y, et al. JAK2V617F-mediated clonal hematopoiesis accelerates pathological remodeling in murine heart failure. *JACC Basic Transl Sci* 2019; 4:684–697 [PubMed: 31709318] The first mechanistic study to demonstrate that *JAK2*^{V617F} clonal

hematopoiesis can lead to cardiovascular disease without *JAK2*^{V617F}-associated myeloproliferative neoplasms phenotypes.

47. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 2012; 44:23–31.
48. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; 99:247–257. [PubMed: 10555141]
49. Cole CB, Russler-Germain DA, Ketkar S, et al. Haploinsufficiency for DNA methyltransferase 3A predisposes hematopoietic cells to myeloid malignancies. *J Clin Invest* 2017; 127:3657–3674. [PubMed: 28872462]
50. ■ Sano S, Oshima K, Wang Y, et al. CRISPR-mediated gene editing to assess the roles of Tet2 and Dnmt3a in clonal hematopoiesis and cardiovascular disease. *Circ Res* 2018; 123:335–341 [PubMed: 29728415] The first study to demonstrate that inactivating mutations in *ten-eleven translocation 2* and *DNA methyltransferase 3a* differ in their ability to promote hematopoietic cell expansion *in vivo* and in inflammatory gene patterns, suggesting gene-specific actions.
51. Dorsheimer L, Assmus B, Rasper T, et al. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. *JAMA Cardiol* 2019; 4:25–33. [PubMed: 30566180]
52. Gamper CJ, Agoston AT, Nelson WG, Powell JD. Identification of DNA Methyltransferase 3a as a T cell receptor-induced regulator of Th1 and Th2 differentiation. *J Immunol* 2009; 183:2267–2276. [PubMed: 19625655]
53. Yu Q, Zhou B, Zhang Y, et al. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc Natl Acad Sci U S A* 2012; 109:541–546. [PubMed: 22190484]
54. Leoni C, Montagner S, Rinaldi A, et al. Dnmt3a restrains mast cell inflammatory responses. *Proc Natl Acad Sci U S A* 2017; 114:E1490–E1499. [PubMed: 28167789]
55. Yang F, Zhou S, Wang C, et al. Epigenetic modifications of interleukin-6 in synovial fibroblasts from osteoarthritis patients. *Sci Rep* 2017; 7:43592. [PubMed: 28262826]
56. Rauch PJ, Silver AJ, Gopakumar J, et al. Loss-of-function mutations in Dnmt3a and Tet2 lead to accelerated atherosclerosis and convergent macrophage phenotypes in mice. *Blood* 2018; 132:745.
57. Gelsi-Boyer V, Brecqueville M, Devillier R, et al. Mutations in ASXL1 are associated with poor prognosis across the spectrum of malignant myeloid diseases. *J Hematol Oncol* 2012; 5:12. [PubMed: 22436456]
58. Yang H, Kurtenbach S, Guo Y, et al. Gain of function of ASXL1 truncating protein in the pathogenesis of myeloid malignancies. *Blood* 2018; 131:328–341. [PubMed: 29113963]
59. Kitamura T ASXL1 mutations gain a function. *Blood* 2018; 131:274–275. [PubMed: 29348310]

KEY POINTS

- Traditional cardiovascular risk factors are incompletely predictive of ASCVD, particularly in elderly individuals.
- Recent epidemiological studies have shown that human aging is associated with an increased frequency of somatic mutations in the hematopoietic system, some of which provide a competitive advantage to a mutant cell, thus allowing for its clonal expansion, a phenomenon known as clonal hematopoiesis.
- This phenomenon has been strongly linked with increased all-cause mortality and ASCVD including myocardial infarction and ischemic stroke.
- Notably, many individuals exhibiting clonal hematopoiesis carry single ‘driver’ mutations in preleukemic genes including *DNA methyltransferase 3a*, *ten-eleven translocation 2*, *additional sex combs like 1*, and *Janus kinase 2*.
- Here, we review recent epidemiological and experimental findings on the association between age-related clonal hematopoiesis and ASCVD by focusing on these prevalent driver gene mutations.

FIGURE 1.

Age-related clonal hematopoiesis contributes to accelerated atherosclerosis through driver gene-specific mechanisms.