Somatic Mutations and Clonal Hematopoiesis

Unexpected Potential New Drivers of Age-Related Cardiovascular Disease

José J. Fuster, Kenneth Walsh

Abstract: **Increasing evidence shows that conventional cardiovascular risk factors are incompletely predictive of cardiovascular disease, particularly in elderly individuals, suggesting that there may still be unidentified causal risk factors. Although the accumulation of somatic DNA mutations is a hallmark of aging, its relevance in cardiovascular disease or other age-related conditions has been, with the exception of cancer, largely unexplored. Here, we review recent clinical and preclinical studies that have identified acquired mutations in hematopoietic stem cells and subsequent clonal hematopoiesis as a new cardiovascular risk factor and a potential major driver of atherosclerosis. Understanding the mechanisms underlying the connection between somatic mutation-driven clonal hematopoiesis and cardiovascular disease will be highly relevant in the context of personalized medicine, as it may provide key information for the design of diagnostic, preventive, or therapeutic strategies tailored to the effects of specific somatic mutations. (*Circ Res*. 2018;122:523-532. DOI: 10.1161/CIRCRESAHA.117.312115.)**

Key Words: aging ■ atherosclerosis ■ cardiovascular disease ■ CHIP ■ inflammation ■ risk factors

Aging and Cardiovascular Disease, an Incompletely Understood Connection

Cardiovascular/cerebrovascular disease (CVD) is the leading cause of morbidity and mortality in both industrialized and low- to middle-income countries, particularly in the elderly population. Furthermore, this situation is expected to worsen as the world population is aging and CVD incidence steeply increases with age, accounting for >40% of total deaths and >85% of chronic disease deaths in those older than 70 years.[1] Although epidemiological studies clearly show that advanced age is the most important risk factor for CVD, we have an incomplete understanding of how it promotes disease progression, in particular in the context of atherosclerosis. Although the effect of aging on atherosclerotic CVD reflects to a great extent the cumulative exposure to many traditional risk factors (eg, hypercholesterolemia, diabetes mellitus, hypertension, smoking),[2] clinical evidence suggests that additional, still unidentified age-related factors may contribute to atherosclerosis development. Indeed, age remains an independent risk factor for CVD even after statistical adjustment for traditional risk factors,[3,4] and most CVD events occur in elderly individuals who are at low or intermediate risk based on traditional risk factors. For example, an international study that included >120 000 atherosclerotic CVD patients revealed that ≈65% of patients older than 65 years old had either none or just one conventional cardiovascular risk factor.[5] Consistent with this observation, imaging studies show that most individuals at low cardiovascular risk based on conventional risk algorithms exhibit substantial subclinical atherosclerosis.[6–9] In addition, emerging evidence suggests that the effects of some conventional risk factors on cardiovascular risk differ between young and elderly individuals. For example, the impact of hypercholesterolemia on cardiovascular risk has been shown to gradually decrease with aging in several studies,[10–12] suggesting the existence of alternative nonconventional risk factors that contribute to the pathogenesis of atherosclerotic CVD in the elderly population. Consistent with these results, recent clinical trials of treatments with high-intensity statin regimens or new cholesterol-lowering drugs (eg, PCSK9 [proprotein convertase subtilisin/kexin type 9] inhibitors) show that, despite massive reductions in blood cholesterol levels, atherosclerotic plaques progress in a significant proportion of individuals[13,14] and a substantial level of residual cardiovascular risk remains.[15,16] Therefore, although the major contribution to atherosclerosis of the cumulative exposure to conventional risk factors—particularly hypercholesterolemia—is undeniable,[17] these aforementioned studies lead inevitably to the question: what else is driving atherosclerotic CVD in the elderly? Emerging evidence from human and mouse studies suggests that the acquisition of somatic mutations in hematopoietic cells may be part of the answer to this question.

Somatic Mutations in the Hematopoietic System and Atherosclerotic CVD: Evidence From Human Studies

Over the past 2 decades, research on the impact of human genetics on CVD has focused mostly on genetic variation transmitted through the germ line.[18,19] Although these studies have unveiled a contribution of many inherited variants to CVD, recent evidence suggests that noninherited, acquired mutations that affect somatic cells may also be important players in CVD and

From the Molecular Cardiology Unit, Whitaker Cardiovascular Institute, Boston University School of Medicine, MA.
Correspondence to José J. Fuster, PhD, Boston University School of Medicine, 700 Albany St, W-611, Boston, MA 02118, E-mail jjfuster@bu.edu; or Kenneth Walsh, PhD, Boston University School of Medicine, 700 Albany St, W-611, Boston, MA 02118, E-mail kxwalsh@bu.edu

Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/CIRCRESAHA.117.312115**

Nonstandard Abbreviations and Acronyms

ASXL1	additional sex combs like 1
BM	bone marrow
CHD	coronary heart disease
CVD	cardiovascular disease
DNMT3A	DNA methyltransferase 3 α
HSPC	hematopoietic stem/progenitor cell
IFN-γ	interferon-γ
IL-1β	interleukin-1β
JAK2	Janus kinase 2
TET2	tet methylcytosine dioxygenase 2
TP53	tumor protein p53

potentially other age-related disorders. These somatic mutations occur randomly, beginning in prenatal development.[20–22] In most cases, they have no or little phenotypic consequences, as most mutations have no effect on cellular function or in some cases are toxic or even lethal to the mutant cell, leading to its quick disappearance. However, in a few instances, a somatic mutation confers a competitive advantage to the cell, which leads to the progressive expansion of the mutant clone. Consequently, individuals become a mosaic of cells with different genotypes over time.[21,23,24] Accumulating evidence reveals that this somatic genome mosaicism is indeed an inevitable consequence of the normal aging process in many tissues.[25,26] This is particularly true in highly proliferative tissues, such as the hematopoietic system, which generates >100 billion cells daily. Recent evidence shows that individual hematopoietic stem/progenitor cells (HSPCs) accumulate somatic mutations as a function of age, even in healthy individuals. Human HSPCs have been calculated to develop 0.13±0.02 exonic mutations per year of life, and thus, it can be estimated that by age 50, an individual would accumulate an average of 5 coding gene mutations within each HSPC.[24] This sets the stage for a robust Darwinian selection of mutations that provide a competitive advantage to the mutant HSPC by promoting its self-renewal, proliferation, or survival. Such a competitive advantage eventually leads to the clonal expansion of the mutant HSPC in the hematopoietic system and, through its cellular progeny, in the blood and other tissues infiltrated by blood cells. Early studies documented the occurrence of age-related clonal hematopoiesis based on the results of X chromosome inactivation studies,[27–30] and a substantial body of evidence has now demonstrated that this process of somatic mutation-driven clonal hematopoiesis (Figure [A]) is common in healthy elderly individuals.[31–38] In the case of extreme age, there is sometimes a marked expansion of a few clones and a collapse of HSPC diversity caused by the acquisition of somatic mutations in key hematopoiesis regulators or other mechanisms of clonal expansion.[39] For example, deep whole-genome sequencing of the peripheral blood cells from a 115-year-old woman revealed that the majority of her white blood cells were derived from just 2 related HSPC clones.[40]

Clonal hematopoiesis is often driven by a single mutation in a cancer-related gene, and, accordingly, it is associated with increased risk of hematologic malignancies.[35,36] Despite this, epidemiological studies show that most individuals carrying cancer-related somatic mutations that drive clonal expansion of the mutant cell will never develop blood cancer because this is a rare condition requiring the accumulation of multiple additional oncogenic gene mutations (Figure [B]).[35] On the basis of these observations, this phenomenon is frequently referred by hematologists as clonal hematopoiesis of indeterminate potential (CHIP).[41,42] Although large chromosomal rearrangements[43–46] and point mutations in >100 cancer-related genes[31–38,47] have been identified as potential drivers of clonal hematopoiesis, most somatic mutations linked to this phenomenon reported to date affect a small subset of genes known to play important roles in hematopoiesis and hematologic cancers (Table). Notably, most somatic mutations associated with clonal hematopoiesis occur in 3 genes (DNA methyltransferase 3α [*DNMT3A*], tet methylcytosine dioxygenase 2 [*TET2*], and additional sex combs like 1 [*ASXL1*]) that encode for epigenetic regulators that have been implicated in the control of hematopoiesis.[48–55] It can be estimated that at least 10% to 20% of individuals older than 65 years old exhibit a significant fraction of white blood cells (eg, >4%) that harbor somatic mutations in these or other known driver genes.[34–36] Furthermore, highly sensitive sequencing techniques that allow the detection of mutations in a smaller percentage of blood cells suggest that low levels of clonal hematopoiesis may be even more prevalent, trending toward inevitability in the very elderly.[31,33,37] Despite these observations, the relevance of clonal hematopoiesis in the context of age-associated disorders other than cancer has

Table. Fifteen Most Frequently Mutated Genes Identified as Candidate Drivers of Clonal Hematopoiesis in Cancer-Free Individuals in Several Human Studies

Gene	References
DNMT3A	31–37,47
TET2	31,32,34–38,47
ASXL1	31,32,34–37,47
JAK2	31–37,47
TP53	31,32,34–37,47
PPM1D	31,34,36,47
IDH2	31–37,47
CBL	31,32,34–37,47
SF3B1	31,33–37,47
SRSF2	31,33,35–37,47
GNAS	31,34,35,37,47
KRAS	32,33,35,37,47
GNB1	31,35,37,47
NRAS	31–33,35,37
MYD88	31,35–37

ASXL1 indicates additional sex combs like 1; CBL, Casitas B-lineage lymphoma; DNMT3A, DNA methyltransferase 3α; GNAS, guanine nucleotide-binding protein, alpha stimulating; GNB1, guanine nucleotide-binding protein (G protein), beta 1; IDH2, isocitrate dehydrogenase 2 ($NADP^+$); JAK2, Janus kinase 2; KRAS, Kirsten rat sarcoma viral oncogene homolog; MYD88, myeloid differentiation primary response gene 88; NRAS, neuroblastoma ras oncogene; PPM1D, protein phosphatase 1D magnesium-dependent, delta isoform; SF3B1, splicing factor 3b subunit 1; SRSF2, serine- and arginine-rich splicing factor 2; TET2, tet methylcytosine dioxygenase 2; and TP53, tumor protein p53.

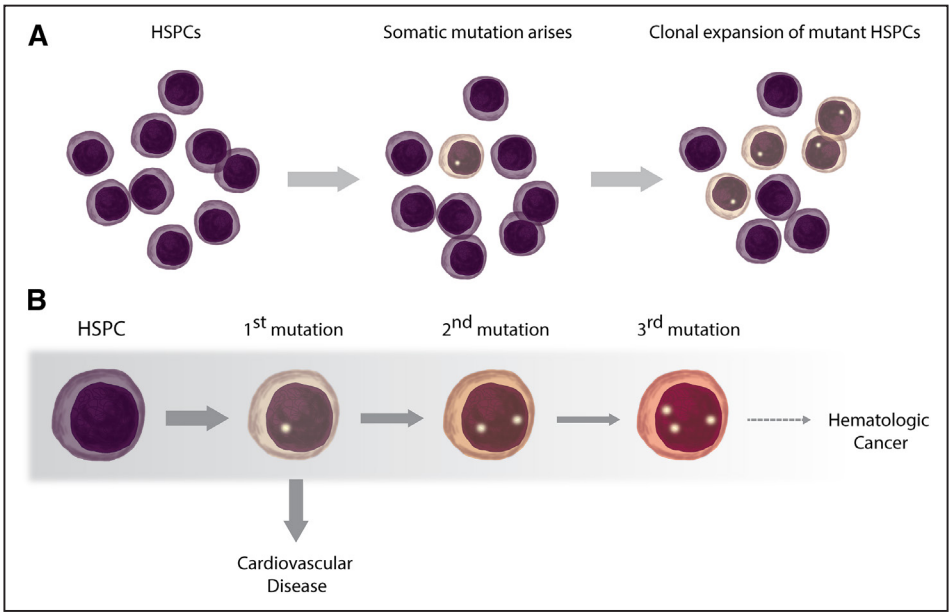

Figure. Somatic mutations in blood cells as a shared mechanism of hematologic cancer and cardiovascular disease. A, The accumulation of somatic mutations in hematopoietic progenitor and stem cells is an inevitable consequence of the process of aging. Some of these random mutations confer a competitive advantage to the mutant cells, leading to its clonal expansion. This phenomenon can be defined as somatic mutation-driven clonal hematopoiesis. **B**, Most individuals exhibiting somatic mutation-driven clonal hematopoiesis only carry 1 driver mutation (eg, in DNMT3A, TET2, ASXL1, or JAK2). Although this situation greatly increases the risk of acquiring additional driver mutations and eventually developing a hematologic cancer, this condition is infrequent, even in individuals with clonal hematopoiesis. The main cause of death in individuals exhibiting somatic mutation-driven clonal hematopoiesis is atherosclerotic cardiovascular disease. Grey shade in the figure indicates decreasing frequency. HSPC indicates hematopoietic stem/progenitor cell.

been largely neglected. The scarce investigation of the clinical consequences of this phenomenon is somewhat surprising considering that mutations in HSPCs are likely to affect the function of their progeny—immune cells, red blood cells, and platelets—whose dysfunction is at the center of most age-related chronic disorders. This situation is bound to change as evidence has emerged supporting the concept that age-associated somatic mosaicism in the hematopoietic system and clonal hematopoiesis may have major pathophysiological implications in the setting of CVD and other chronic diseases.

Early evidence of a connection between a specific somatic mutation in the hematopoietic system and the development of CVD in the absence of cancer can be found in some small studies that reported the presence of the Janus kinase 2 (JAK2) V617F mutation in blood cells of a higher than expected proportion of cancer-free patients with venous thrombosis,[56,57] stable coronary heart disease (CHD),[58] or peripheral artery disease.[59] Although limited by their small sample size and retrospective nature, these studies, among others, opened the door to the possibility of a connection between somatic mutations in blood cells and CVD. This possibility was reinforced when whole-exome sequencing of blood cells of >5000 cancer-free individuals in 7 independent cohorts revealed that the presence of single-nucleotide variants and small insertions or deletions in any of 160 analyzed hematopoiesis-related genes is associated with a ≈40% increase in all-cause mortality.[35] Although the presence of 1 somatic mutation in any of these genes was associated with an ≈11-fold increase in the future risk of hematologic cancer, this only affected 0.5% to 1% of mutation carriers each year and did not explain the marked increase in all-cause mortality. Unexpectedly, the increased mortality was associated with a substantial increase in deaths caused by myocardial infarction or stroke among individuals exhibiting a relative frequency of the mutant allele (variant allele fraction) >10%, typically indicative of the presence of >20% blood cells carrying the mutation in heterozygosis. Consistent with this finding, a significant association was found between the detection of somatic mutations in blood cells and the future incidence of atherosclerotic conditions (CHD and stroke): ≈45% of mutation carriers developed atherosclerotic CVD during the study, compared with ≈20% of noncarriers. Thus, carrying somatic mutations in hematopoiesis-related genes increased >2-fold the risk of developing atherosclerotic CVD, an effect that was comparable or even greater than that of various conventional risk factors.[35] Although this study reported an association between CVD and a somatic mutation in any of 160 genes, mutations in just 4 genes, namely *DNMT3A*, *TET2*, *ASXL1*, and *JAK2*, accounted for >70% of the detected mutations. A more recent study has confirmed the existence of independent associations between CHD and mutations in each of these 4 genes with median variant allele fraction ranging from 10% to 20%.[47] However, it is likely that larger studies with longer follow-up periods will identify additional mutated genes linked to CHD and that significant associations will be observed even with lower variant allele fraction values. Indeed, this latter study also reported an association of CHD with any of 56 different somatic mutations in less frequently mutated genes,[47] supporting the possibility that other mutated genes may be connected to age-related CVD. Somatic mutations may also be linked to the premature development of CVD in young and middle-aged individuals, as a retrospective case–control analysis of individuals <50 years old revealed a

remarkable 4-fold higher risk of early-onset myocardial infarction in individuals exhibiting somatic mutation-driven clonal hematopoiesis.[47] Furthermore, the presence of somatic mutations in blood cells was also found to be associated with a 3-fold higher risk of exhibiting a high degree of coronary artery calcification, a marker of subclinical atherosclerosis burden. Remarkably, the increased risk of coronary artery calcification was 12-fold in individuals exhibiting a variant allele fraction >10%.[47]

Overall, these human data suggest a strong and unexpected connection between age-related somatic mutations in hematopoietic cells, clonal hematopoiesis, and atherosclerosis. However, it must be noted that the descriptive nature of these types of epidemiological studies does not allow cause–effect relationships to be established. The associations between somatic mutation-driven clonal hematopoiesis and atherosclerotic CVD could simply reflect shared consequences of the aging process or be secondary to confounding factors. Furthermore, these studies do not fully address the issue of directionality, that is, is clonal hematopoiesis a driver of CVD or are low-grade chronic inflammation and metabolic dysfunction, frequent in subjects at high cardiovascular risk, driving somatic mutagenesis or clonal hematopoiesis as suggested by some researchers?[43,60] Answering these questions requires the combination of additional human data and carefully designed experimental studies in animal models.

Somatic Mutations in the Hematopoietic System and Atherosclerotic CVD: Evidence From Mouse Studies, the Case of TET2

As described above, most reported somatic mutations associated with clonal hematopoiesis and CVD occur in a small number of genes, particularly in 4 genes (*DNMT3A*, *TET2*, *ASXL1*, and *JAK2*), which are known to modulate hematopoiesis,[48–55,61–63] but whose role in CVD remained mostly unexplored until recently. The first of these genes reported to exhibit somatic mutations in blood cells in individuals with clonal hematopoiesis without blood cancer was *TET2*.[38] More than 130 different mutations have been reported in this gene in blood cells of cancer-free individuals,[31,32,34–38,47] which are considered to result in loss of gene function given that most are small insertions/deletions, nonsense mutations, or missense mutations leading to the substitution of key amino acids in the catalytic domain. TET2 is a multifaceted transcriptional regulator that can facilitate both transcriptional activation and repression depending on the molecular/cellular context. It is able to catalyze the conversion of 5-methylcytosine into 5-hydroxymethylcytosine, a process that facilitates DNA demethylation and transcriptional activation.[64–66] Conversely, TET2 can also mediate transcriptional repression by recruiting histone deacetylases to gene promoters.[67] Mouse studies have shown that TET2 deletion or haploinsufficiency results in increased HSPC self-renewal and a bias toward differentiation into the myeloid lineage,[53–55,68] consistent with human data linking TET2 mutations with clonal hematopoiesis. However, despite the broad expression pattern of TET2,[69] the relevance of this protein in pathophysiological settings other than stem cell biology or cancer has just recently begun to be explored. In this regard, a recent study from our group demonstrated that TET2 loss-of-function–driven clonal hematopoiesis accelerates atherosclerosis in hyperlipidemic mice, providing experimental evidence in support of the causal contribution of somatic mutation-driven clonal hematopoiesis to atherosclerotic CVD.[70] In this study, experimental conditions in mice recapitulated many features of clonal hematopoiesis linked to somatic TET2 mutations in humans. Competitive bone marrow (BM) transplantation experiments were used to introduce a small number of TET2-deficient cells in atherosclerosis-prone *Ldlr*$^{-/-}$ mice and mimic the scenario of clonal hematopoiesis, where the mutation is initially present in a small clone that expands over time. In agreement with previous studies,[53–55] TET2-deficient cells expanded progressively in BM, spleen, and blood in this experimental setting, and they showed a mild myeloid bias, with a preferential expansion into the Ly-6C-high classical monocyte population.[70] Importantly, this did not affect total numbers of white blood cells or their distribution in different subsets, consistent with human studies on cancer-free individuals carrying TET2 mutations.[35,38] The expansion of *Tet2*$^{-/-}$ cells in these conditions accelerated atherosclerosis substantially, leading to the formation of ≈60% larger plaques. A similar, albeit milder, effect was observed in equivalent transplantation experiments with *Tet2*$^{+/-}$ cells, which may mimic better the human scenario given that somatic TET2 mutations are likely to be in heterozygosis in most individuals. In addition, atherosclerotic plaque size was also increased when TET2 ablation was restricted to myeloid cells.[70] These findings were recently validated in independent studies in mice exhibiting full hematopoietic ablation of TET2.[47] Collectively, these experimental studies provide strong support to the existence of a causal connection between somatic mutations in this gene and the development of atherosclerotic CVD.

From a mechanistic point of view, in vitro and in vivo studies suggest that accelerated atherosclerosis in conditions of TET2 loss-of-function–driven clonal hematopoiesis is mainly because of the exacerbated expression of proinflammatory cytokines and chemokines in TET2-deficient macrophages,[47,70,71] particularly because of the overproduction of the proinflammatory cytokine IL-1β (interleukin-1β).[70] Interestingly, this mechanism is consistent with the possibility that a small number of TET2-mutant cells in the plaque may be sufficient to drive exacerbated vascular inflammation and atherosclerosis by promoting proinflammatory changes in both mutant and wild-type cells. In this regard, it was found that exacerbated IL-1β production by TET2-deficient plaque cells in mice promotes P-selectin expression and endothelial cell activation in the plaque, leading to increased monocyte recruitment regardless of the *Tet2* genotype of recruited monocytes.[70] Furthermore, IL-1β has been reported to stimulate its own expression by an autoregulatory feedback loop,[72–76] and therefore, it is likely that overproduction of IL-1β by TET2-deficient plaque cells promotes further expression of IL-1β in both TET2-deficient and wild-type cells. Hence, it can be speculated that a few TET2-mutant cells in the plaque act in a catalytic manner, amplifying and perpetuating vascular inflammation and atherosclerosis. Consistent with this few bad apples spoil the barrel scenario,

the progressive expansion of *Tet2*$^{-/-}$ cells in a competitive BM transplantation setting seems to have a comparable effect on atherosclerosis than that of the full ablation of TET2 in all BM cells.[47,70] This relatively surprising finding likely reflects the complexity of the actions of TET2 in the hematopoietic system, and it suggests that the effects of TET2 deficiency on atherosclerosis may be dependent on cell type and stage of plaque development. It also provides valuable information on the variety of research strategies available to model clonal hematopoiesis in mice. Although conventional full BM transplantation strategies are the most straightforward strategy to study the effects on atherosclerosis of clonal hematopoiesis-related mutations, the use of cell-type–specific approaches or competitive BM transplantation strategies with low percentages of mutant cells may be advantageous in some instances. This may be the case when the mutant HSPCs exhibit a clear bias to differentiate into specific lineages or when the ubiquitous expression of the mutated gene in hematopoietic progenitors leads to hematologic abnormalities or malignancies not present in most cancer-free individuals exhibiting clonal hematopoiesis (eg, *JAK2* gain-of-function mutations[61–63]).

The use of mouse studies tailored to the human scenario of clonal hematopoiesis may provide clinically relevant information. For instance, the findings in our mouse model of TET2 mutation-mediated clonal hematopoiesis suggest that IL-1β blockade may be particularly effective for the prevention/treatment of CVD in individuals carrying somatic mutations in TET2. Supporting this possibility, pharmacological blockade of NLRP3 (NLR family pyrin domain-containing 3) inflammasome-mediated IL-1β secretion had greater atheroprotective effects in conditions of TET2 loss of function than in wild-type controls and completely suppressed differences in atherosclerosis development between genotypes.[70] These findings are especially relevant in the context of the results of the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study), which demonstrated the efficacy of a neutralizing antibody against IL-1β (Canakinumab) in the secondary prevention of atherosclerotic CVD in high-risk patients exhibiting evidence of systemic inflammation.[77] Even though Canakinumab has shown efficacy against atherosclerotic CVD, its clinical use poses substantive challenges in the setting of chronic disorders, as it also increases the risk of infections.[77] Therefore, this drug, and probably any anti-inflammatory drug, should probably be administered exclusively to those patients who are predicted to show the greatest response to this therapy and to obtain the most atheroprotection from it. Currently, the analysis of hsCRP (high-sensitivity C-reactive protein), a marker of systemic inflammation, is the best available tool to identify these patients and distinguish between responders and nonresponders to anti-inflammatory therapies.[78] Although no conclusive data have yet been provided, it can be speculated that clonal hematopoiesis driven by TET2 loss of function or other preleukemic mutations may be associated with increased systemic inflammation in some individuals and that the genetic analysis of blood cells may be used in the future to identify responders to anti-inflammatory therapies in the context of precision medicine strategies.[79] In this regard, our preclinical data suggest that individuals with TET2 mutations may respond more favorably than the general population to IL-1β/NLRP3-targeted therapies, thus creating the basis for a personalized therapy for the prevention of accelerated atherosclerotic CVD in individuals carrying somatic mutations in this gene. This notion could be clinically validated through a genetic screening for somatic TET2 mutations in the CANTOS cohort. Furthermore, given that IL-1β plays a pivotal role in vascular inflammation[80] and TET2 also affects the expression of other immunomodulatory molecules,[47,70,71] it may be of interest to apply similar strategies in ongoing clinical trials with other broad-spectrum anti-inflammatory compounds such as methotrexate[81] or colchicine.[82]

Somatic Mutation-Driven Clonal Hematopoiesis and Atherosclerotic CVD Beyond TET2: Other Genes and Mechanisms?

TET2 was the first gene reported to carry somatic mutations in cancer-free individuals exhibiting clonal hematopoiesis,[38] and mutations in this gene have been the first to be causally linked to an age-associated disorder other than cancer, atherosclerotic CVD.[35,47,70] However, human evidence suggests that many other mutated genes may contribute to this condition.[35,47] As discussed above, independent associations with atherosclerotic CVD have been reported for 3 additional genes, *DNMT3A*, *ASXL1*, and *JAK2*, and mutations in other less frequently mutated genes also seem to be associated with increased risk of atherosclerotic CVD.[35,47] It is reasonable to speculate that these additional mutated genes will differentially affect CVD because of their unique functions and mechanisms of action. Some mutations may induce qualitative changes in key immune cells, whereas others may affect the differentiation of HSPCs to specific lineages, leading to quantitative changes in specific immune cell subsets, particularly in individuals who exhibit large mutant clones and a reduced HSPC repertoire. Whether somatic mutations in clonal hematopoiesis-related genes other than *TET2* contribute to atherosclerosis remains unexplored, and additional experimental studies are warranted. However, previous research allows for some speculation on the different mechanisms by which some of these mutated genes may promote CVD.

Accumulating experimental evidence suggests that *DNMT3A*, the most frequently mutated gene in cancer-free individuals who exhibit clonal hematopoiesis, is an important modulator of both hematopoiesis and inflammatory responses. This gene encodes for a methyl transferase enzyme that catalyzes DNA methylation and modulates gene transcription in multiple scenarios. Supporting a major role of DNMT3A mutations in clonal hematopoiesis in humans, mouse HSPCs exhibiting heterozygotic DNMT3A loss of function develop a competitive advantage and myeloid skewing over time.[83] Importantly, DNMT3A deficiency has also been reported to lead to several potentially proatherogenic phenotypes in immune cells, including exacerbated proinflammatory activation of mast cells,[84] increased IFN (interferon)-γ production by T cells,[85–87] and restrained immunosuppressive function in myeloid-derived suppressor cells.[88] In contrast, DNMT3A inhibition has also been shown to increase the expression of IL-13 in T cells[87] and to limit the production of type I interferons in macrophages,[89] which could potentially protect against

atherosclerosis development.[90,91] Therefore, given the complexity of the immunomodulatory functions of DNMT3A, carefully designed experimental studies will be required to determine the potential contribution of somatic mutations in this gene to CVD.

Another frequently mutated gene for which previous data support a role in CVD is *JAK2*, which encodes for an important signaling kinase. Indeed, early evidence linking somatic mutations to CVD was provided by the observation that essential thrombocytopenia and polycythemia vera patients carrying the JAK2 V617F gain-of-function mutation exhibit a higher risk of thrombosis.[92–100] Increased blood counts of leukocytes, red blood cells, and platelets are potential contributors to the increased cardiovascular risk in these patients. However, additional qualitative changes in blood cells are also likely to play a role, as increased cardiovascular risk has been observed in individuals carrying this mutation and exhibiting clonal hematopoiesis with little or no changes in blood cell counts.[35,101] Some mouse studies suggest that JAK2 V617F-driven clonal hematopoiesis exhibits a clear myeloid, particularly granulocytic, bias,[102] and thus, it is possible that the effects of this somatic mutation on the cardiovascular system are mediated by phenotypic changes in myeloid cells. Given that findings in various experimental models support a role for JAK2 in the modulation of the proinflammatory activities of macrophages and neutrophils,[103–105] it will be of interest to evaluate the impact of the JAK2 V617F mutation on CVD in the absence of hematologic malignancies.

Beyond the above-mentioned genes, it must be noted that most other mutated genes associated with clonal hematopoiesis have never been evaluated for their role in CVD in any context. Furthermore, controversy exists on whether some of the observed mutations lead to loss or gain of gene function, as is the case of ASXL1 truncation mutations.[51,52,106–108] On the other hand, it is tempting to speculate that some of the somatic mutations that lead to clonal hematopoiesis may be benign or exhibit protective rather than pathogenic effects on the cardiovascular system. In other words, some types of clonal hematopoiesis may be uncoupled from CVD or other age-associated chronic diseases, and these forms of clonal hematopoiesis might be overrepresented in individuals who exhibit exceptional longevity.

Somatic Mutation-Driven Clonal Hematopoiesis: Many Open Questions

Beyond Atherosclerosis, a Role in Other Age-Related Conditions?

The link between acquired somatic mutation-driven clonal hematopoiesis and atherosclerosis development is now supported by both clinical and preclinical data. However, the possibility that mutations driving clonal hematopoiesis also modulate other forms of CVD or other age-related chronic conditions remains largely unexplored and deserves attention. Indeed, recent preclinical studies in mouse models suggest that TET2 loss-of-function–driven clonal hematopoiesis may accelerate heart failure, a condition that is common in the elderly.[109] Furthermore, human studies have suggested a potential connection between various forms of somatic mutations in blood cells and type 2 diabetes mellitus, chronic pulmonary disease, or psychiatric conditions.[31,32,35,43] Future experimental studies are warranted to examine the nature of these connections, especially given that many of the known mutations that drive clonal hematopoiesis affect immune cells and pathways that are at the center of many age-related chronic disorders.

Beyond Mutations, What Else Modulates Clonal Hematopoiesis?

As indicated above, prior exome sequencing studies have suggested that 10% to 20% of individuals older than 65 years old exhibit somatic mutation-driven clonal hematopoiesis leading to a substantial percentage (eg, >4%) of mutant cells in the blood.[34–36] However, more recent studies using highly sensitive sequencing techniques that allow the detection of mutations in a smaller fraction of blood cells found that the prevalence of clonal hematopoiesis is probably at least double than that initially reported.[31,33,37] Furthermore, the seeds for somatic mutation-driven clonal hematopoiesis may be present even in younger individuals, as one recent study using targeted error-corrected sequencing found that somatic mutations in driver genes (most frequently *DNMT3A* and *TET2*) are present in a very small fraction of blood cells (eg, 1/3000) in 95% of individuals between 50 and 60 years of age.[110] Similarly, it has been estimated that almost half of healthy individuals at 50 years of age carry at least 1 HSPC with a randomly generated mutation in tumor protein p53 (*TP53*), another potential driver of clonal hematopoiesis.[111] Thus, the presence of low levels of hematopoietic cells with somatic mutations may be ubiquitous by middle age, but only a fraction of individuals may advance to a condition where the mutant clone undergoes marked expansion. Overall, these observations suggest the existence of additional factors, perhaps genetic or lifestyle factors, that regulate the clonal expansion of mutant HSPCs. For example, some germ line variants, most notably in the *JAK2* and *TERT* genes, have been associated with increased prevalence of clonal hematopoiesis associated with the JAK2 V617F mutation.[112] Furthermore, smoking has also been found to be associated with clonal hematopoiesis.[31,36,113] Additional investigations of the effect of these and other factors on somatic mutation-driven clonal hematopoiesis may lead to a better understanding of the complex molecular mechanisms that regulate the dynamics of this process, which could contribute to the development of therapies to prevent it.

Somatic Mutations in BM Cells: A New Factor to Consider in the Application of Cell Therapies?

Numerous adult stem cell therapies are being tested in clinical trials or are offered to the public by commercial entities.[114–116] These therapies often involve the delivery of autologous or allogeneic BM cells or cells from tissues that contain a substantial fraction of BM-derived cells. In addition, some of these procedures use cells that are selected based on the expression of cell surface markers, such as CD34, that lead to the enrichment in HSPCs. Given the realization that somatic mutations in HSPCs can promote their clonal expansion and alter the function of their cellular progeny, questions can be raised about the long-term safety of some of these adult stem cell procedures. It is theoretically possible that the delivery

of cell fractions from elderly individuals, who may exhibit clonal hematopoiesis, will nullify the therapeutic effect of the transplanted cells or even contribute to adverse outcomes because of the transplantation of hematopoietic cells that harbor somatic mutations. Furthermore, one cannot rule out the possibility that the adoptive transfer of mutant HSPCs could further accelerate their clonal expansion and contribute to disease processes as the patient ages. Future studies are therefore warranted to evaluate whether it might be appropriate to examine transplanted cell populations for their clonal hematopoiesis status before use.

Clonal Hematopoiesis Without Known Driver Mutations: What Are We Missing?

Most research efforts to date in the context of clonal hematopoiesis have been focused on the identification of mutations in blood cancer-related genes previously known to modulate hematopoiesis. However, it is noteworthy that ≈50%, or greater, of cases of clonal hematopoiesis occur without any known driver gene mutation.[31,36] Although this may be related to technical issues in some cases (eg, sequencing depth or specific blood cell populations being sequenced), these results strongly suggest the existence of additional, still unidentified somatic mutations that can drive clonal hematopoiesis and perhaps contribute to age-related disorders. The analysis of chromosomal rearrangements or mutations in nonprotein coding genes may deserve special attention in this context. Indeed, a significant association between the presence of clonally expanded blood cells carrying acquired large chromosomal rearrangements and the occurrence of vascular complications of diabetes mellitus has been reported.[43] Alternatively, nonmutational mechanisms may also contribute to clonal hematopoiesis. Computer simulations suggest that, at least in some cases, clonal hematopoiesis can arise in the absence of a driver mutation simply because of stochastic neutral drift in the HSPC population.[31] Among other nonmutational mechanisms,[117] it is possible, for instance, that epigenetic alterations and the associated cell-to-cell variability in gene expression lead to the dominance of specific HSPC populations. Similarly, the heterogeneous distribution of extracellular signals in the BM niche could lead to the preferential expansion of some HSPCs because of their local environment. Interestingly, clonal hematopoiesis without candidate driver gene mutations has also been associated with an increase in all-cause mortality,[31] although its potential association with CVD remains unexplored. Be it somatic mutations or nonmutational mechanisms, extensive investigation will be required to identify new drivers of clonal hematopoiesis and understand their clinical relevance.

Conclusions

The accumulation of random mutations in the hematopoietic system is an unavoidable consequence of the aging process. Although the pathophysiologic relevance of these acquired somatic mutations has been traditionally restricted to the cancer field, emerging evidence suggests that these mutations are important drivers of CVD and potentially many other age-related disorders. A combination of clinical and preclinical studies will be required to understand the pathogenic effects (or lack of) of the various mutated genes, as well as the molecular mechanisms that govern the expansion of mutant hematopoietic stem cells. This information could eventually lead to the design of personalized therapies or preventive strategies for individuals carrying specific somatic mutations in blood cells.

Acknowledgments

The illustration was provided by Shraddha Nayak.

Sources of Funding

This work was funded by National Institutes of Health grants HL126141, HL131006, HL132564, and HL138014 to K. Walsh and by American Heart Association Scientist Development Grant 17SDG33400213 to J.J. Fuster.

Disclosures

None.

References

1. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1459–1544. doi: 10.1016/S0140-6736(16)31012-1.
2. Sniderman AD, Furberg CD. Age as a modifiable risk factor for cardiovascular disease. *Lancet*. 2008;371:1547–1549. doi: 10.1016/S0140-6736(08)60313-X.
3. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117:743–753. doi: 10.1161/CIRCULATIONAHA.107.699579.
4. Kannel WB, Vasan RS. Is age really a non-modifiable cardiovascular risk factor? *Am J Cardiol*. 2009;104:1307–1310. doi: 10.1016/j.amjcard.2009.06.051.
5. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA*. 2003;290:898–904. doi: 10.1001/jama.290.7.898.
6. Baber U, Mehran R, Sartori S, Schoos MM, Sillesen H, Muntendam P, Garcia MJ, Gregson J, Pocock S, Falk E, Fuster V. 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.
7. 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. doi: 10.1161/CIRCULATIONAHA.114.014310.
8. Laclaustra M, Casasnovas JA, Fernández-Ortiz A, Fuster V, León-Latre M, Jiménez-Borreguero LJ, Pocovi M, Hurtado-Roca Y, Ordovas JM, Jarauta E, Guallar E, Ibañez B, Civeira F. Femoral and carotid subclinical atherosclerosis association with risk factors and coronary calcium: the AWHS Study. *J Am Coll Cardiol*. 2016;67:1263–1274. doi: 10.1016/j.jacc.2015.12.056.
9. Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, García-Ruiz JM, Mendiguren J, Bueno H, Pocock S, Ibáñez B, Fernández-Ortiz A, Sanz J. Normal LDL-cholesterol levels are associated with subclinical atherosclerosis in the absence of risk factors. *J Am Coll Cardiol*. 2017;70:2979–2991. doi: 10.1016/j.jacc.2017.10.024.
10. Gränsbo K, Almgren P, Nilsson PM, Hedblad B, Engström G, Melander O. Risk factor exposure in individuals free from cardiovascular disease differs according to age at first myocardial infarction. *Eur Heart J*. 2016;37:1977–1981. doi: 10.1093/eurheartj/ehw026.
11. Sniderman AD, Islam S, McQueen M, Pencina M, Furberg CD, Thanassoulis G, Yusuf S. Age and cardiovascular risk attributable to apolipoprotein B, low-density lipoprotein cholesterol or non-high-density lipoprotein cholesterol. *J Am Heart Assoc*. 2016;5:e003665.
12. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R; Prospective Studies Collaboration. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with

55,000 vascular deaths. *Lancet*. 2007;370:1829–1839. doi: 10.1016/S0140-6736(07)61778-4.
13. Nicholls SJ, Ballantyne CM, Barter PJ, Chapman MJ, Erbel RM, Libby P, Raichlen JS, Uno K, Borgman M, Wolski K, Nissen SE. Effect of two intensive statin regimens on progression of coronary disease. *N Engl J Med*. 2011;365:2078–2087. doi: 10.1056/NEJMoa1110874.
14. Nicholls SJ, Puri R, Anderson T, et al. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV Randomized Clinical Trial. *JAMA*. 2016;316:2373–2384. doi: 10.1001/jama.2016.16951.
15. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet*. 2010;376:1670–1681. doi: 10.1016/S0140-6736(10)61350-5.
16. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR; FOURIER Steering Committee and Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713–1722. doi: 10.1056/NEJMoa1615664.
17. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364:937–952. doi: 10.1016/S0140-6736(04)17018-9.
18. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat Rev Genet*. 2017;18:331–344. doi: 10.1038/nrg.2016.160.
19. O'Donnell CJ, Nabel EG. Genomics of cardiovascular disease. *N Engl J Med*. 2011;365:2098–2109. doi: 10.1056/NEJMra1105239.
20. Poduri A, Evrony GD, Cai X, Walsh CA. Somatic mutation, genomic variation, and neurological disease. *Science*. 2013;341:1237758. doi: 10.1126/science.1237758.
21. Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, Lee S, Chittenden TW, D'Gama AM, Cai X, Luquette LJ, Lee E, Park PJ, Walsh CA. Somatic mutation in single human neurons tracks developmental and transcriptional history. *Science*. 2015;350:94–98. doi: 10.1126/science.aab1785.
22. Ju YS, Martincorena I, Gerstung M, et al. Somatic mutations reveal asymmetric cellular dynamics in the early human embryo. *Nature*. 2017;543:714–718. doi: 10.1038/nature21703.
23. Martincorena I, Roshan A, Gerstung M, et al. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science*. 2015;348:880–886.
24. Welch JS, Ley TJ, Link DC, 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.
25. Forsberg LA, Gisselsson D, Dumanski JP. Mosaicism in health and disease - clones picking up speed. *Nat Rev Genet*. 2017;18:128–142. doi: 10.1038/nrg.2016.145.
26. Vijg J. Somatic mutations, genome mosaicism, cancer and aging. *Curr Opin Genet Dev*. 2014;26:141–149. doi: 10.1016/j.gde.2014.04.002.
27. Fey MF, Liechti-Gallati S, von Rohr A, Borisch B, Theilkäs L, Schneider V, Oestreicher M, Nagel S, Ziemiecki A, Tobler A. Clonality and X-inactivation patterns in hematopoietic cell populations detected by the highly informative M27 beta DNA probe. *Blood*. 1994;83:931–938.
28. Gale RE, Fielding AK, Harrison CN, Linch DC. 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.
29. Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, Maragh M, Gilliland DG. Nonrandom X-inactivation patterns in normal females: lyonization ratios vary with age. *Blood*. 1996;88:59–65.
30. Shlush LI. Age related clonal hematopoiesis (ARCH) [published online ahead of print November 15, 2017]. *Blood*. doi: 10.1182/blood-2017-07-746453. http://www.bloodjournal.org/content/early/2017/11/15/blood-2017-07-746453.long.
31. 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. doi: 10.1182/blood-2017-02-769869.
32. Buscarlet M, Provost S, Zada YF, Barhdadi A, Bourgoin V, Lépine G, Mollica L, Szuber N, Dubé MP, Busque L. 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.
33. McKerrell T, Park N, Moreno T, et al; Understanding Society Scientific Group. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep*. 2015;10:1239–1245. doi: 10.1016/j.celrep.2015.02.005.
34. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014;20:1472–1478. doi: 10.1038/nm.3733.
35. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371:2488–2498. doi: 10.1056/NEJMoa1408617.
36. Genovese G, Kähler AK, Handsaker RE, 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.
37. Acuna-Hidalgo R, Sengul H, Steehouwer M, van de Vorst M, Vermeulen SH, Kiemeney LALM, Veltman JA, Gilissen C, Hoischen A. 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.
38. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012;44:1179–1181. doi: 10.1038/ng.2413.
39. Goodell MA, Rando TA. Stem cells and healthy aging. *Science*. 2015;350:1199–1204. doi: 10.1126/science.aab3388.
40. Holstege H, Pfeiffer W, Sie D, et al. Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. *Genome Res*. 2014;24:733–742. doi: 10.1101/gr.162131.113.
41. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, Ebert BL. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126:9–16. doi: 10.1182/blood-2015-03-631747.
42. Bejar R. CHIP, ICUS, CCUS and other four-letter words. *Leukemia*. 2017;31:1869–1871. doi: 10.1038/leu.2017.181.
43. Bonnefond A, Skrobek B, Lobbens S, Eury E, Thuillier D, Cauchi S, Lantieri O, Balkau B, Riboli E, Marre M, Charpentier G, Yengo L, Froguel P. Association between large detectable clonal mosaicism and type 2 diabetes with vascular complications. *Nat Genet*. 2013;45:1040–1043. doi: 10.1038/ng.2700.
44. Forsberg LA, Rasi C, Razzaghian HR, et al. Age-related somatic structural changes in the nuclear genome of human blood cells. *Am J Hum Genet*. 2012;90:217–228. doi: 10.1016/j.ajhg.2011.12.009.
45. Jacobs KB, Yeager M, Zhou W, et al. Detectable clonal mosaicism and its relationship to aging and cancer. *Nat Genet*. 2012;44:651–658. doi: 10.1038/ng.2270.
46. Laurie CC, Laurie CA, Rice K, 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.
47. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–121. doi: 10.1056/NEJMoa1701719.
48. Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet*. 2011;44:23–31. doi: 10.1038/ng.1009.
49. Guryanova OA, Lieu YK, Garrett-Bakelman FE, et al. Dnmt3a regulates myeloproliferation and liver-specific expansion of hematopoietic stem and progenitor cells. *Leukemia*. 2016;30:1133–1142. doi: 10.1038/leu.2015.358.
50. Fisher CL, Pineault N, Brookes C, Helgason CD, Ohta H, Bodner C, Hess JL, Humphries RK, Brock HW. Loss-of-function additional sex combs like 1 mutations disrupt hematopoiesis but do not cause severe myelodysplasia or leukemia. *Blood*. 2010;115:38–46. doi: 10.1182/blood-2009-07-230698.
51. Abdel-Wahab O, Gao J, Adli M, et al. Deletion of Asxl1 results in myelodysplasia and severe developmental defects in vivo. *J Exp Med*. 2013;210:2641–2659. doi: 10.1084/jem.20131141.
52. Wang J, Li Z, He Y, et al. Loss of Asxl1 leads to myelodysplastic syndrome-like disease in mice. *Blood*. 2014;123:541–553. doi: 10.1182/blood-2013-05-500272.
53. Ko M, Bandukwala HS, An J, Lamperti ED, Thompson EC, Hastie R, Tsangaratou A, Rajewsky K, Koralov SB, Rao A. Ten-eleven-translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc Natl Acad Sci USA*. 2011;108:14566–14571.
54. Moran-Crusio K, Reavie L, Shih A, 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.
55. Quivoron C, Couronné L, Della Valle V, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent

event during human lymphomagenesis. *Cancer Cell*. 2011;20:25–38. doi: 10.1016/j.ccr.2011.06.003.
56. De T, Prabhakar P, Nagaraja D, Christopher R. Janus kinase (JAK) 2 V617F mutation in Asian Indians with cerebral venous thrombosis and without overt myeloproliferative disorders. *J Neurol Sci*. 2012;323:178–182. doi: 10.1016/j.jns.2012.09.012.
57. De Stefano V, Fiorini A, Rossi E, Za T, Farina G, Chiusolo P, Sica S, Leone G. Incidence of the JAK2 V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. *J Thromb Haemost*. 2007;5:708–714. doi: 10.1111/j.1538-7836.2007.02424.x.
58. Muendlein A, Gasser K, Kinz E, Stark N, Leiherer A, Rein P, Saely CH, Grallert H, Peters A, Drexel H, Lang AH. Evaluation of the prevalence and prospective clinical impact of the JAK2 V617F mutation in coronary patients. *Am J Hematol*. 2014;89:295–301. doi: 10.1002/ajh.23632.
59. Muendlein A, Kinz E, Gasser K, Leiherer A, Rein P, Saely CH, Grallert H, Peters A, Fraunberger P, Drexel H, Lang AH. Occurrence of the JAK2 V617F mutation in patients with peripheral arterial disease. *Am J Hematol*. 2015;90:E17–E21. doi: 10.1002/ajh.23874.
60. Hasselbalch HC. Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer? *Blood*. 2012;119:3219–3225. doi: 10.1182/blood-2011-11-394775.
61. Li J, Spensberger D, Ahn JS, et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood*. 2010;116:1528–1538. doi: 10.1182/blood-2009-12-259747.
62. Mullally A, Lane SW, Ball B, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell*. 2010;17:584–596. doi: 10.1016/j.ccr.2010.05.015.
63. Akada H, Yan D, Zou H, Fiering S, Hutchison RE, Mohi MG. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood*. 2010;115:3589–3597. doi: 10.1182/blood-2009-04-215848.
64. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*. 2011;333:1300–1303. doi: 10.1126/science.1210597.
65. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, Rao A. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010;468:839–843. doi: 10.1038/nature09586.
66. He YF, Li BZ, Li Z, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science*. 2011;333:1303–1307. doi: 10.1126/science.1210944.
67. 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. doi: 10.1038/nature15252.
68. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, Yang FC, Xu M. Deletion of TET2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood*. 2011;118:4509–4518. doi: 10.1182/blood-2010-12-325241.
69. Nagase T, Kikuno R, Nakayama M, Hirosawa M, Ohara O. Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. *DNA Res*. 2000;7:273–281.
70. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355:842–847. doi: 10.1126/science.aag1381.
71. Cull AH, Snetsinger B, Buckstein R, Wells RA, Rauh MJ. TET2 restrains inflammatory gene expression in macrophages. *Exp Hematol*. 2017;55:56.e13–70.e13. doi: 10.1016/j.exphem.2017.08.001.
72. Manson JC, Symons JA, Di Giovine FS, Poole S, Duff GW. Autoregulation of interleukin 1 production. *Eur J Immunol*. 1989;19:261–265. doi: 10.1002/eji.1830190207.
73. Hiscott J, Marois J, Garoufalis J, D'Addario M, Roulston A, Kwan I, Pepin N, Lacoste J, Nguyen H, Bensi G. Characterization of a functional NF-kappa B site in the human interleukin 1 beta promoter: evidence for a positive autoregulatory loop. *Mol Cell Biol*. 1993;13:6231–6240.
74. Warner SJ, Auger KR, Libby P. Interleukin 1 induces interleukin 1. II. Recombinant human interleukin 1 induces interleukin 1 production by adult human vascular endothelial cells. *J Immunol*. 1987;139:1911–1917.
75. Dinarello CA, Ikejima T, Warner SJ, Orencole SF, Lonnemann G, Cannon JG, Libby P. Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. *J Immunol*. 1987;139:1902–1910.
76. Warner SJ, Auger KR, Libby P. Human interleukin 1 induces interleukin 1 gene expression in human vascular smooth muscle cells. *J Exp Med*. 1987;165:1316–1331.
77. Ridker PM, Everett BM, Thuren T, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914.
78. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ; 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 [published online ahead of print November 13, 2017]. *Lancet*. doi: http://dx.doi.org/10.1016/S0140-6736(17)32814-3. http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)32814-3/fulltext.
79. Baylis RA, Gomez D, Mallat Z, Pasterkamp G, Owens GK. The CANTOS Trial: one important step for clinical cardiology but a giant leap for vascular biology. *Arterioscler Thromb Vasc Biol*. 2017;37:e174–e177. doi: 10.1161/ATVBAHA.117.310097.
80. 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.
81. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, Gupta M, Clearfield M, Libby P, Hasan AA, Glynn RJ, Ridker PM. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. *Am Heart J*. 2013;166:199.e15–207.e15. doi: 10.1016/j.ahj.2013.03.018.
82. Tardif JC, L'Allier PL. Colchicine Cardiovascular Outcomes Trial (COLCOT). https://www.clinicaltrials.gov/ct2/show/NCT02551094?term=colcot&rank=1. Accessed January 2, 2018.
83. 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. doi: 10.1172/JCI93041.
84. Leoni C, Montagner S, Rinaldi A, Bertoni F, Polletti S, Balestrieri C, Monticelli S. Dnmt3a restrains mast cell inflammatory responses. *Proc Natl Acad Sci USA*. 2017;114:E1490–E1499. doi: 10.1073/pnas.1616420114.
85. 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. doi: 10.4049/jimmunol.0802960.
86. Pham D, Yu Q, Walline CC, Muthukrishnan R, Blum JS, Kaplan MH. Opposing roles of STAT4 and Dnmt3a in Th1 gene regulation. *J Immunol*. 2013;191:902–911. doi: 10.4049/jimmunol.1203229.
87. Yu Q, Zhou B, Zhang Y, Nguyen ET, Du J, Glosson NL, Kaplan MH. DNA methyltransferase 3a limits the expression of interleukin-13 in T helper 2 cells and allergic airway inflammation. *Proc Natl Acad Sci USA*. 2012;109:541–546. doi: 10.1073/pnas.1103803109.
88. Rodríguez-Ubreva J, Català-Moll F, Obermajer N, Álvarez-Errico D, Ramirez RN, Company C, Vento-Tormo R, Moreno-Bueno G, Edwards RP, Mortazavi A, Kalinski P, Ballestar E. Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human myeloid-derived suppressor cells. *Cell Rep*. 2017;21:154–167. doi: 10.1016/j.celrep.2017.09.018.
89. Li X, Zhang Q, Ding Y, Liu Y, Zhao D, Zhao K, Shen Q, Liu X, Zhu X, Li N, Cheng Z, Fan G, Wang Q, Cao X. Methyltransferase Dnmt3a upregulates HDAC9 to deacetylate the kinase TBK1 for activation of antiviral innate immunity. *Nat Immunol*. 2016;17:806–815. doi: 10.1038/ni.3464.
90. Cardilo-Reis L, Gruber S, Schreier SM, Drechsler M, Papac-Milicevic N, Weber C, Wagner O, Stangl H, Soehnlein O, Binder CJ. Interleukin-13 protects from atherosclerosis and modulates plaque composition by skewing the macrophage phenotype. *EMBO Mol Med*. 2012;4:1072–1086. doi: 10.1002/emmm.201201374.
91. Goossens P, Gijbels MJ, Zernecke A, Eijgelaar W, Vergouwe MN, van der Made I, Vanderlocht J, Beckers L, Buurman WA, Daemen MJ, Kalinke U, Weber C, Lutgens E, de Winther MP. Myeloid type I interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. *Cell Metab*. 2010;12:142–153. doi: 10.1016/j.cmet.2010.06.008.
92. Campbell PJ, Scott LM, Buck G, et al; United Kingdom Myeloproliferative Disorders Study Group; Medical Research Council Adult Leukaemia Working Party; Australasian Leukaemia and Lymphoma Group. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*. 2005;366:1945–1953. doi: 10.1016/S0140-6736(05)67785-9.

93. Cheung B, Radia D, Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2 V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. *Br J Haematol*. 2006;132:244–245. doi: 10.1111/j.1365-2141.2005.05858.x.
94. Finazzi G, Rambaldi A, Guerini V, Carobbo A, Barbui T. Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2 V617F mutation status. *Haematologica*. 2007;92:135–136.
95. Hsiao HH, Yang MY, Liu YC, Lee CP, Yang WC, Liu TC, Chang CS, Lin SF. The association of JAK2V617F mutation and leukocytosis with thrombotic events in essential thrombocythemia. *Exp Hematol*. 2007;35:1704–1707. doi: 10.1016/j.exphem.2007.08.011.
96. Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, Li CY, Wu W, Ketterling RP, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. *Cancer*. 2007;109:2279–2284. doi: 10.1002/cncr.22663.
97. Vannucchi AM, Antonioli E, Guglielmelli P, Longo G, Pancrazzi A, Ponziani V, Bogani C, Ferrini PR, Rambaldi A, Guerini V, Bosi A, Barbui T; MPD Research Consortium. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. *Leukemia*. 2007;21:1952–1959. doi: 10.1038/sj.leu.2404854.
98. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. *Blood*. 2007;110:840–846. doi: 10.1182/blood-2006-12-064287.
99. Dahabreh IJ, Zoi K, Giannouli S, Zoi C, Loukopoulos D, Voulgarelis M. Is JAK2 V617F mutation more than a diagnostic index? A meta-analysis of clinical outcomes in essential thrombocythemia. *Leuk Res*. 2009;33:67–73. doi: 10.1016/j.leukres.2008.06.006.
100. Xavier SG, Gadelha T, Rezende SM, Zalcberg IR, Spector N. JAK2V617F mutation in patients with thrombosis: to screen or not to screen? *Int J Lab Hematol*. 2011;33:117–124. doi: 10.1111/j.1751-553X.2010.01275.x.
101. Jaiswal S, Natarajan P, Ebert BL. Clonal hematopoiesis and atherosclerosis. *N Engl J Med*. 2017;377:1401–1402. doi: 10.1056/NEJMc1710381.
102. Lundberg P, Takizawa H, Kubovcakova L, Guo G, Hao-Shen H, Dirnhofer S, Orkin SH, Manz MG, Skoda RC. Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing JAK2-V617F. *J Exp Med*. 2014;211:2213–2230. doi: 10.1084/jem.20131371.
103. Okugawa S, Ota Y, Kitazawa T, Nakayama K, Yanagimoto S, Tsukada K, Kawada M, Kimura S. Janus kinase 2 is involved in lipopolysaccharide-induced activation of macrophages. *Am J Physiol Cell Physiol*. 2003;285:C399–C408. doi: 10.1152/ajpcell.00026.2003.
104. Oku S, Takenaka K, Kuriyama T, Shide K, Kumano T, Kikushige Y, Urata S, Yamauchi T, Iwamoto C, Shimoda HK, Miyamoto T, Nagafuji K, Kishimoto J, Shimoda K, Akashi K. JAK2 V617F uses distinct signalling pathways to induce cell proliferation and neutrophil activation. *Br J Haematol*. 2010;150:334–344. doi: 10.1111/j.1365-2141.2010.08249.x.
105. Desai HR, Sivasubramaniyam T, Revelo XS, et al. Macrophage JAK2 deficiency protects against high-fat diet-induced inflammation. *Sci Rep*. 2017;7:7653. doi: 10.1038/s41598-017-07923-0.
106. Abdel-Wahab O, Adli M, LaFave LM, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell*. 2012;22:180–193. doi: 10.1016/j.ccr.2012.06.032.
107. Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, Lähdesmäki H, Rao A. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. *Nat Commun*. 2015;6:7307. doi: 10.1038/ncomms8307.
108. Yang H, Kurtenbach S, Guo Y, et al. Gain-of-function of ASXL1 truncating protein in the pathogenesis of myeloid malignancies [published online ahead of print November 7, 2017]. *Blood*. doi: 10.1182/blood-2017-06-789669. http://www.bloodjournal.org/content/early/2017/11/07/blood-2017-06-789669.long.
109. Sano S, Oshima K, Wang Y, MacLauchlan S, Katanasaka Y, Sano M, Zuriaga MA, Yoshiyama M, Goukassian D, Cooper MA, Fuster JJ, Walsh K. Tet2-mediated clonal hematopoiesis accelerates experimental heart failure through an IL-1β/NLRP3 inflammasome mechanism. *J Am Coll Cardiol*. 2018. In press.
110. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016;7:12484. doi: 10.1038/ncomms12484.
111. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*. 2015;518:552–555. doi: 10.1038/nature13968.
112. Hinds DA, Barnholt KE, Mesa RA, Kiefer AK, Do CB, Eriksson N, Mountain JL, Francke U, Tung JY, Nguyen HM, Zhang H, Gojenola L, Zehnder JL, Gotlib J. 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.
113. Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, Hyman DM, Solit DB, Robson ME, Baselga J, Arcila ME, Ladanyi M, Tallman MS, Levine RL, Berger MF. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell*. 2017;21:374.e4–382.e4. doi: 10.1016/j.stem.2017.07.010.
114. Behfar A, Crespo-Diaz R, Terzic A, Gersh BJ. Cell therapy for cardiac repair–lessons from clinical trials. *Nat Rev Cardiol*. 2014;11:232–246. doi: 10.1038/nrcardio.2014.9.
115. Turner L, Knoepfler P. Selling stem cells in the USA: assessing the direct-to-consumer industry. *Cell Stem Cell*. 2016;19:154–157. doi: 10.1016/j.stem.2016.06.007.
116. Afzal MR, Samanta A, Shah ZI, Jeevanantham V, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy for ischemic heart disease: evidence and insights from randomized controlled trials. *Circ Res*. 2015;117:558–575. doi: 10.1161/CIRCRESAHA.114.304792.
117. Klein AM, Simons BD. Universal patterns of stem cell fate in cycling adult tissues. *Development*. 2011;138:3103–3111. doi: 10.1242/dev.060103.

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Somatic Mutations and Clonal Hematopoiesis: Unexpected Potential New Drivers of Age-Related Cardiovascular Disease

José J. Fuster and Kenneth Walsh

Circ Res. 2018;122:523-532
doi: 10.1161/CIRCRESAHA.117.312115
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571