REVIEW

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Clonal hematopoiesis driven by DNMT3A and TET2 mutations: role in monocyte and macrophage biology and atherosclerotic cardiovascular disease

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Purpose of review
Clonal hematopoiesis of indeterminate potential (CHIP), defined by the presence of somatic mutations in hematopoietic cells, is associated with advanced age and increased mortality due to cardiovascular disease. Gene mutations in *DNMT3A* and TET2 are the most frequently identified variants among patients with CHIP and provide selective advantage that spurs clonal expansion and myeloid skewing. Although *DNMT3A* and TET2 appear to have opposing enzymatic influence on DNA methylation, mounting data has characterized convergent inflammatory pathways, providing insights to how CHIP may mediate atherosclerotic cardiovascular disease (ASCVD).

Recent findings
We review a multitude of studies that characterize aberrant inflammatory signaling as result of *DNMT3A* and TET2 deficiency in monocytes and macrophages, immune cells with prominent roles in atherosclerosis. Although specific DNA methylation signatures associated with these known epigenetic regulators have been identified, many studies have also characterized diverse modulatory functions of DNTM3A and TET2 that urge cell and context-specific experimental studies to further define how *DNMT3A* and TET2 may nonenzymatically activate inflammatory pathways with clinically meaningful consequences.

Summary
CHIP, common in elderly individuals, provides an opportunity understand and potentially modify age-related chronic inflammatory ASCVD risk.

Keywords
atherosclerosis, cardiovascular disease, clonal hematopoiesis

INTRODUCTION

Age-related clonal hematopoiesis is a recently identified risk factor for atherosclerotic cardiovascular disease

Despite significant advances in contemporary therapies targeted at traditional risk factors for atherosclerotic cardiovascular disease (ASCVD), it remains the main cause of morbidity and mortality in western societies [1,2]. With an expanding elderly population worldwide, age, a potent and independent risk factor for ASCVD [3–5], accounts partly for the global burden of disease. In addition, ASCVD risk modification is limited by an incomplete understanding of how to treat nontraditional risk factors such as chronic inflammation safely and effectively [6]. Through an increasing body of research over the past decade, somatic mutations found in hematopoietic cells, termed clonal hematopoiesis of indeterminate potential (CHIP), has emerged as an age-related ASCVD risk factor that also offers important insights toward clinically relevant pathways mediating chronic inflammation.

Somatic mutations are an inevitable consequence of ageing and contribute to cellular heterogeneity in

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KEY POINTS

- Clonal hematopoiesis, driven by somatic mutations and increasingly prevalent with age, is an independent risk factor for ASCVD.
- The majority of mutations associated with clonal hematopoiesis are found in *DNMT3A* and TET2, despite the antagonistic biochemical functions of their encoded proteins.
- *DNMT3A* and TET2 converge in regulation of pro-inflammatory pathways in monocytes and macrophages, cell types highly relevant to the development and progression of atherosclerosis.

tissues [7–11]. These mutations are particularly apparent in highly proliferative tissues and tissues exposed to environmental or chemical factors such as the hematopoietic system, skin and esophagus [12–15] but is also observed in tissues with low-proliferative rate like the pancreas [16–18]. Somatic mutations conveying a selective advantage and subsequent clonal expansion of hematopoietic cells in apparently healthy individuals is known as CHIP. The prevalence of CHIP increases with age with more than 15–20% of septuagenarians affected. Although mutations in more than 100 genes are found in CHIP, several of the most commonly mutated genes associated with ASCVD play roles as epigenetic or transcriptional regulators involved in diverse aspects of cellular development and function, including *DNMT3A*, *TET2*, *ASXL1* and *JAK2* [19–26]. Although the overlap between mutations associated with hematologic malignancies and CHIP had been long appreciated, the indeterminant potential of CHIP reflected the low (0.5–1%/year) risk for developing a hematologic malignancy particularly for those with *DNMT3A*, *TET2* and *ASXL1*. Long-term follow-up of patients with CHIP revealed an increased risk in mortality that was largely due to CVD [26].

Both systemic and local inflammation have important roles in the development and progression of atherosclerosis, resulting in risk for myocardial infarction (MI), ischemic heart failure, stroke, and peripheral arterial disease [6,27]. Monocytes and macrophages are key mediators of these disease processes. Activation of monocytes in the setting of ASCVD risk factors lead to their extravasation into the arterial intima, where these cells interact with modified and retained apolipoprotein B (apoB) containing lipoproteins and differentiate into macrophage foam cells. Foam cells secrete various cytokines and chemokines that perpetuates arterial inflammation and disease progression, but also destabilize the plaque and promote thrombotic events associated with adverse clinical outcomes [28,29].

A major focus of inquiry is to understand how CHIP, resulting from somatic mutations in more than 100 functionally diverse genes and affecting only a fraction of hematopoietic cells may contribute to inflammation and atherosclerosis. Here, we consider this question with respect to *DNMT3A* and *TET2*, which together account for approximately 50% of the mutations associated with CHIP in ASCVD patients. *DNMT3A* and *TET2* are of particular interest because they possess antagonistic enzymatic activities. *DNMT3A* is an enzyme that catalyzes de novo DNA methylation of cytosine, a modification often associated with gene silencing. Conversely, *TET2* encodes a methyl cytosine dioxygenase that initiates a sequence of reactions leading to cytosine demethylation. If loss of the enzymatic activities of *DNMT3A* or *TET2* were the critical determinant of increased risk of ASCVD, a common phenotype would be expected to be due to effects on different genes and potentially different cell types, which nevertheless have convergent effects on ASCVD pathogenesis. Alternatively, the apparent paradoxical contribution of *DNTM3A* and *TET2* mutations in CHIP raises the possibility of an alternative mechanism that is independent in changes in DNA methylation. Moreover, despite displaying antagonistic enzymatic activities, loss-of-function of *DNMT3A* or *TET2* in murine models show overlapping phenotypes in terms of increased Hematopoietic stem cell (HSC) fitness [30] suggesting a common program regulated by *DNMT3A* and *TET2*.

Role of *DNMT3A* and *TET2* in the development of clonal hematopoiesis of indeterminate potential and atherosclerosis

DNMT3A and *TET2* are the most commonly identified mutated genes associated with CHIP [20,22–24]. Both genes are widely expressed in hematopoietic stem and progenitor cells (HSPC) and have been implicated in their expansion and differentiation. Mutations in these genes can bestow a selective advantage to affected HSPCs resulting in clonal expansion. *DNMT3A* was shown to repress the stem cell program of HSC and activate their transcriptional differentiation program [31,32]. In addition, *DNMT3A* has high expression in macrophages [33]. *TET2* expression is higher during bone marrow-derived macrophages (BMDM) differentiation [34] and TET2 regulates osteoclasts differentiation by interacting with RUNX1 [35] indicating a role during differentiation of myeloid cells.

Consistent with a causal role of *DNMT3A* mutations in CHIP and ASCVD, murine HSPCs exhibiting heterozygotic *DNMT3A* loss of function develop a competitive advantage and myeloid skewing over time [36]. In patients with CHIP solely driven by *DMNT3A* mutations, genotyping of fluorescent-activated sorted blood cells revealed presence of the driver mutation in lymphocytes as well as in myeloid cells, suggesting multipotent lineage involvement [37]. Importantly, *DNMT3A* deficiency has to lead to several potentially pro-atherogenic phenotypes in a variety of immune cell populations, including pro-inflammatory activation of mast cells [38] increased IFNγ production by T cells and restrained immunosuppressive function in myeloid-derived suppressor cells [39]. On the contrary, *DNMT3A* inhibition has been shown to increase the expression of IL-13 in T cells [40] and to limit the production of type I interferons in macrophages [33], which could potentially protect against atherosclerosis development [40,41]. These findings are consistent with diverse modulatory functions of DNTM3A and call for cell/context-specific experimental studies to determine the contribution of somatic mutations in *DNMT3A* to the development of ASCVD.

In contrast to *DNMT3A*, *TET2* mutations were predominantly restricted to myeloid lineages in blood from individuals with CHIP and single *TET2* mutations [37]. Murine studies have shown that *TET2* deletion or haploinsufficiency result in increased HSPC self-renewal and a bias toward differentiation into the myeloid lineage [42–44]. However, despite the broad expression pattern of *TET2* [45], the relevance of this protein in pathophysiological settings other than stem cell biology or cancer has just recently begun to be explored. The expansion of Tet2 deficient cells, and to a lesser extent for Tet2 heterozygous cells, in these conditions accelerated atherosclerosis in murine models substantially, leading to the formation of ∼60% larger plaques. In addition, atherosclerotic plaque size was also increased when Tet2 ablation was restricted to myeloid cells [46]. These findings were recently validated in independent studies in mice exhibiting full hematopoietic ablation of Tet2 [26]. Collectively, these experimental studies provide strong support to the existence of causal connection between somatic mutations in this gene and the development of atherosclerotic ASCVD. In addition, Tet2's role specifically in myeloid cells suggests mechanisms beyond expansion of progenitor cells.

DNMT3A and TET2 in monocyte biology

DNMT3A and TET2 have important roles in the biology of monocytes. Using single cell RNA-Seq analysis, monocytes from patients with a recent MI and heart failure with or without detectable *DNMT3A* mutations were recently compared. Monocytes isolated from patients carrying *DNMT3A* mutations had an increased expression of pro-inflammatory genes compared with monocytes from patients without *DNMT3A* mutations. These genes include inflammatory cytokines *IL1B*, *IL6* and *IL8*, *CCL3* and *CCL4*, inflammasome components (*NLRP3*) and resistin (*RETN*), which promotes monocyte adhesion to endothelial cells [47▪,48]. These findings remain to be confirmed in a larger and more diverse patient population with high-throughput assessment (e.g., flow cytometry or cytometry by time of flight, CyTOF of inflammatory cells subtypes), but are consistent with a pro-atherogenic role for CHIP with *DNMT3A* mutations. Moreover, *DNMT3A* mutations have been associated with increased risk for hospitalization or death in patients with heart failure secondary to ASCVD, but also heart failure due to nonischemic etiologies [49–51]. Further studies are required to evaluate the combinatorial impact of mutation frequency and mutation site location on transcriptional profiles, as different mutations within the same gene may have divergent effects on the coding molecule's functional outcome and subsequent physiological outcomes.

The methylcytosine dioxygenase TET2 also plays an important role in monocyte biology. Small interference RNA (siRNA)-mediated *TET2* knockdown in primary monocytes were shown to prevent active DNA demethylation, providing evidence that TET2-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine initiates targeted, active DNA demethylation in a mature postmitotic myeloid cell type [52]. Similarly, familial germline *TET2* loss in seven individuals, three of whom had a diagnosis of nodular lymphocyte-predominant lymphoma, and additionally a de novo *TET2* mutation in an unrelated individual were all associated with hematopoietic cell hypermethylation that was especially prominent at active enhancer regions [53]. Significantly, the regions displayed reduced methylation relative to all open chromatin regions in four *DNMT3A* germline mutation carriers, potentially due to TET2-mediated oxidation. These results indicate that the perturbation in hematopoiesis caused by reduced TET2 function appears to relate to aberrations in DNA methylation that require synergistic actions of TET2 and master transcription factors involved in hematopoiesis and enhancer activation. Significantly, contrary to the effects of *DNMT3A* in monocytes, familial germline *TET2*-mutant monocytes did not display neither unusual predisposition to atherosclerosis nor abnormal pro-inflammatory cytokine or chemokine expression [53]. More work is required to understand the potential differential effects of germline vs. somatic as well as unique *TET2* mutations and their relationship to atherosclerosis.

DNMT3A and TET2 in macrophage biology

DNMT3A regulates inflammatory pathways in macrophages in a context-specific manner. In a genome-wide association study, the single nucleotide polymorphism (SNP) g.25498283A>C in the intronic region of *DNMT3A* gene was associated with protection against recurrent methicillin-resistant *Staphylococcus aureus* bacteremia and reduced levels of the anti-inflammatory cytokine IL-10 [54]. Moreover, *DNMT3A* expression knockdown using siRNA in human macrophages increased IL-10 production in response to S. aureus stimulation. Supporting the importance of *DNMT3A* methyltransferase activity, macrophages treated with the methylation inhibitor 5-aza-2′-deoxycytidine produced higher levels of IL-10. The g.25498283A>C SNP does not appear to have impact on expression of *DNMT3A* mRNA but is associated with higher levels of methylation in gene-regulatory CpG.

In contrast to *DNMT3A*'s role in restraining an anti-inflammatory response to bacterial infections, *DNMT3A* activates the antiviral immune response of macrophages through upregulation of Histone deacetylase 9 (HDAC9) to deacetylase Tank binding kinase 1 (TBK1) [33]. In this regard, DNTM3A inhibition leads to lower production of type I IFNs in mouse peritoneal macrophages (PM) triggered by pattern-recognition receptors but does not reduce the expression of other inflammatory genes such as *Tnf* or *Il6* [33]. These apparent paradoxical effects exemplify the diverse modulatory functions of DNTM3A, and further urge cell/context-specific experimental studies to determine the role of *DNMT3A* in specific cell populations. In addition, transcriptional regulation of *DNMT3A* and *TET2* needs to be further characterized. Recent work by Li *et al.* [55] provided insight to the transcriptional regulation of *DNTM3A* by long noncoding RNAs (lncRNAs) *Dnmt3aos* (*DNMT3A*, opposite strand), located on the antisense strand of *Dnmt3a*. Cellular assays and functional experiments confirmed that *Dnmt3aos* regulates *DNMT3A* mRNA and protein expression and that reduced *DNMT3A* expression by lower *Dnmt3aos* leads to an exacerbated response to lipopolysaccharide (LPS) and IFNy and an aberrant response to IL4 through alterations in DNA methylation [55]. These examples of transcriptional regulation highlight the importance of *DNMT3A* in regulating macrophage biology including regulation of inflammatory programs and response to stimuli.

Several studies have also supported the role of TET2 loss of function in promoting macrophage inflammation relevant to atherosclerosis. Tet2 deficiency in murine macrophages results in inflammasome activation and an enhanced secretion of IL-1B and IL-6 [26,46]. Moreover, activation of type I interferons by Interferon Response Factor 3 (IRF3), known to promote an adverse response to MI [56], is regulated by NLRP3, TET2 and nuclear factor erythroid 2-related factor 2 (NFE2L2 or NRF2) (Calcagno *et al.*, 2020, BioRxive) [57]. The distinctions between Tet2 deficient BMDM and PM support context-specific roles of TET2 in macrophages, similar to observations with *DNMT3A*. In addition to the activation of inflammatory pathways during formation of atherosclerosis, macrophages that scavenge excessive lipid content becomes foam cells that lead to plaque formation [58–60] and further, impaired phagocytic capacity of these macrophages [61]. In addition, up-regulation of Tet2 by CEBPA during transdifferentiation of pre-B cells to macrophages is required for upregulation of macrophage markers as well as phagocytic capacity, indicating a role for TET2 regulating phagocytosis of macrophages [62].

CONCLUSION

Summary and unanswered questions

Somatic mutations in *DNMT3A* and *TET2*, the most commonly affected genes in individuals with CHIP, are associated with increased ASCVD risk. Accumulating evidence support the role of *DNMT3A* and *TET2* in promoting inflammation in monocytes and macrophages and have provided a potential global mechanistic basis for how CHIP may promote development and progression of atherosclerosis. However, there remain significant gaps in understanding of the potentially multiple pathways involved in ASCVD risk associated with *DNMT3A* and *TET2* driven CHIP.

The apparent opposing enzymatic activities of *DNMT3A* and *TET2* on DNA methylation and the role of their nonenzymatic activities in promoting atherosclerosis remain incompletely understood. One possibility is that enzymatic activities of *DNMT3A* and *TET2* both require maintaining a homeostatic DNA methylation status and that loss of either promotes a DNA methylation pattern that promotes disease. In support of this theory, dynamic DNA methylation of *DNMT3A*-maintained enhancers in B-cells is determined by the coincident activity of *DNMT3A* and TET enzymes [63▪,64]. On the other hand, and not mutually exclusive with a role in DNA methylation, increasing evidence implicates *DNMT3A* and TET2 in activation of inflammatory programs independent of their catalytic activities. The lack of correlation between methylation and differential gene expression in murine bone marrow is consistent with other studies including *Dnmt3a*-null HSC [32] and human samples of acute myeloid leukemia [31]. TET2's role beyond

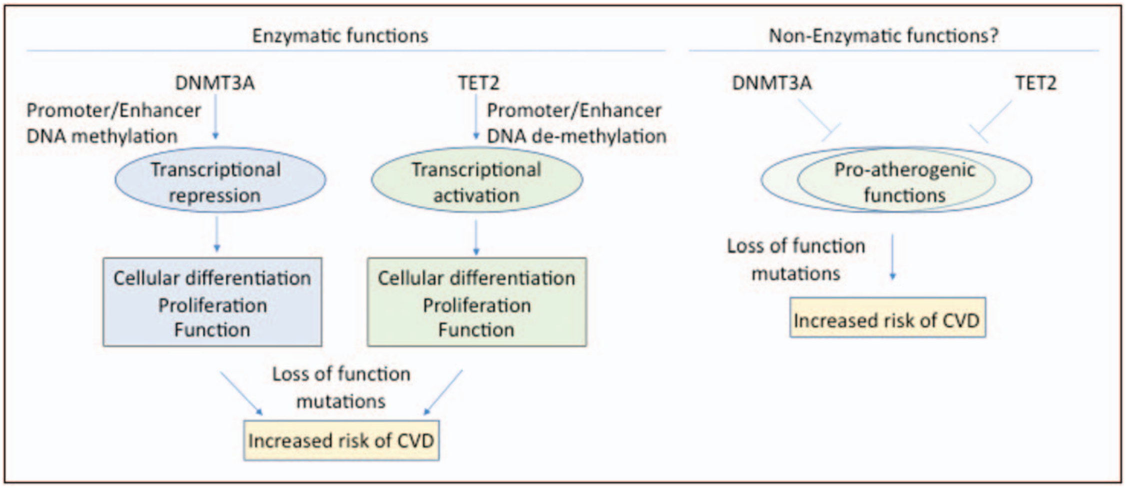

FIGURE 1. Schematic of the potential functional consequences of mutations in DNMT3A and TET2 in macrophages during atherosclerotic cardiovascular disease.

DNA methylation is consistent with previous work showing noncatalytic mechanisms mediated by TET2 are crucial for HSPC homeostasis [32]. This opens the possibility of additional mechanisms such as complexing with other transcription factors, co-activator and co-regulators of gene expression or by regulating the three dimensional structure of the genome (Fig. 1).

Another incompletely understood concept is how CHIP, resulting in clonal expansion affecting only a minority of circulating immune cells, may contribute robustly to ASCVD risk. CHIP is characterized by its driver mutation(s) and the percentage of alleles sequenced containing the mutation, or variant allele frequency (VAF). The number of nucleated blood cells carrying the mutation is approximately equal to double the VAF (i.e., a VAF of 10% affects 20% of cells in whole blood sequenced). CHIP with a VAF of as little as 10% was sufficiently associated with ASCVD risk [65]. More remarkably, CHIP due to mutations in *DNMT3A* or TET2 with a VAF 2% or less was associated with incident heart failure and also worse prognosis with heart failure in a dose dependent manner [49,50,66]. A potential explanation for how a minor population of immune cells carrying a *DNMT3A* or *TET2* mutation may influence the local environment (e.g., atherosclerotic plaque) through expansion and/or remodeling noncell autonomously through secretion of inflammatory mediators. As a hypothetical example, *DNMT3A* or *TET2* mutant macrophages would active neighboring immune, endothelial, or smooth muscle cells thereby accelerating and perpetuating tissue inflammation driving the progression of atherosclerosis.

DNMT3A and *TET2* may also mediate ASCVD risk through regulating lipid and glucose metabolism. CHIP is independently associated with an increased risk for type 2 diabetes [23,67]. *DNMT3A*, for instance, is significantly increased in adipose tissue-derived macrophages but not PM from mice fed with high-fat diet and that is sufficient to mediate insulin resistance in cultured mouse and human adipocytes [68]. Similarly, a recent report indicates that clonal expansion in Tet2 deficient cells exacerbates insulin resistance, obesity and ageing in mice [69▪]. The role of CHIP in other processes highly relevant to ASCVD such as cholesterol regulation, early plaque formation and phagocytosis of dying and dead cells at the plaque has not been well investigated.

A highly clinically important question to be answered is whether CHIP represents an opportunity to modify age-related ASCVD risk in those affected. A preliminary secondary analysis of the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) demonstrated a more potent CVD risk reduction with IL-1B inhibition in those with CHIP compared with the overall trial population. Although promising, CVD outcomes studies randomizing larger and more elderly populations with CHIP to anti-inflammatory therapies with favorable safety profiles will be needed to confirm and validate the finding from CANTOS (AHA Journal, Abstract 15111).

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Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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