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Associations between Blood Phenotypes, Somatic Mutations in Blood Cells and Atherosclerotic Coronary Artery Disease

Airong Li * and Zhikun Guo#

Henan Key Laboratory of Tissue Regeneration, Xinxiang Medical University, Xinxiang, Henan 453003, P.R. China

***Corresponding Author:** Airong Li, Henan Key Laboratory of Tissue Regeneration, Xinxiang Medical University, Xinxiang, Henan 453003, P.R. China.

#Corresponding Author: Zhikun Guo, Henan Key Laboratory of Tissue Regeneration, Xinxiang Medical University, Xinxiang, Henan 453003, P.R. China.

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Abstract

Coronary artery disease (CAD) is the most common type of heart disease and the leading cause of death. CAD results from atherosclerosis damage in coronary arteries.

Genetic studies have identified causal genes and mutations that underlie atherosclerotic CAD. Germline mutations have been identified and characterized for rare monogenic familial CAD and lipoprotein disorders. However, the potential causal role of somatic mutations in the development of atherosclerosis CAD remains unclear. This review summarizes the recent findings in associations between blood phenotypes and incident CAD and exploring the role of *TET2* somatic mutations in blood cells in atherosclerosis CAD. By whole-exome sequencing of DNA from the peripheral-blood cells of Caucasians in population-based cohorts somatic mutations leading to clonal outgrowth of hematopoietic cells were frequently found. The most commonly mutated gene was *DNMT3A*, *TET2* and *ASXL1*. Individuals carrying a somatic mutation as compared with those without a mutation had a 2 fold increase for incident CAD by multivariable analyses. *TET2* encodes an epigenetic regulatory enzyme that catalyzes the oxidation of 5-methylcytosine (5 mC) in DNA to 5-hydroxymethylcytosine (5 hmC). *TET2* mutations have been associated with increased CAD incidence and all-cause mortality. Clonal hematopoiesis induced by Tet2 loss-of-function plays an important role in atherosclerosis. These findings may provide new clues for the underlying mechanism, risk prediction and prevention of CAD.

Keywords: *Coronary artery disease; Atherosclerosis; Mutation; Hematopoiesis; Gene*

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Introduction

Coronary artery disease (CAD), also known as ischemic heart disease (IHD), is the most common type of heart disease and the leading cause of death [1]. CAD results from atherosclerosis damage or diseased coronary arteries that supply blood to the heart. Depending on partially or totally block of the blood flow of coronary arteries, patients with CAD exhibit a range of clinical presentations including asymptomatic subclinical atherosclerosis, angina pectoris, myocardial infarction (MI) or sudden cardiac death. Known key risk factors for CAD include high blood pressure, smoking, elevated total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein cholesterol (HDL-C) and triglyceride [2].

Genetics plays an important role in the etiology of CAD and MI [3]. Genetically, CAD is heterogeneous. Genetic studies using various approaches, including candidate gene, family-based linkage analysis, genome-wide association studies (GWAS) and high throughput sequencing have identified causal genes and mutations that underlie atherosclerotic CAD [2]. Germline mutations are inherited from parents and detected by sequencing analysis of genomic DNA that are typically 50% of allele reads; whereas somatic mutations occur in somatic tissues that arise spontaneously during the process of aging and are detected by analysis of tissue DNA with less than 50% allele reads. Germline mutations have been identified and well characterized in *ST6GALNAC5, CYP27A1, MEF2A, LRP6* as causal for rare monogenic familial CAD; in *LDL receptor, PCSK9, ApoB-100, LDLRAP1* and *ARH* for high LDL; and in monogenic lipid disorders: in *ABCA1* and *LCAT* for low *HDL*; and in *Apo C-II* for high triglyceride [3-4]. It is known that accumulation of somatic DNA mutations has been associated with a range of human diseases including future development of cancer [5]. However, the potential causal role of somatic mutations in the development of atherosclerosis CAD remains unclear. This review summarizes the recent findings in associations between blood phenotypes and incident CAD and exploring the role of *TET2* somatic mutations in blood cells in atherosclerosis CAD.

Associations between blood phenotypes and atherosclerotic CAD

A meta-analysis of prospective cohort study of 1,764 CAD cases from 30,374 individuals, reported associations between total white blood cell, granulocyte, and neutrophil counts and incident CAD [6]. People with higher baseline total leukocyte counts have about a 40% increased risk of CAD than these with lower total leukocyte count [7]. In an effort to identify causal genes and pathways in hematological traits, the International Human Epigenome Consortium (IHEC) analyzed a total of 173,480 European ancestry individuals from three large-scale UK studies which reveals associations between blood cell indices and various common diseases, including CAD. GWAS interrogates 36 traits across the hematopoietic system and identified a total of 2,706 associated variants [8]. A multivariable Mendelian randomization analysis identified weak but significant positive association between hemolysis and CHD risk. Lymphocyte count had positive associations with 10% increase in incidence of CAD and 17% increase in schizophrenia, 28% increase in multiple sclerosis, as well as 20% lower incidence of asthma and 25% lower incidence of celiac disease. Removal of the major histocompatibility complex (MHC) region made the association with CAD as well as schizophrenia and asthma disappear and only the associations with multiple sclerosis and celiac disease were robust. These data strongly suggest that genes within MHC predominantly are the major driver of the association of CAD and hemolysis. Moreover, there was an association of 1.12 fold increased CHD risk with reticulocyte indices and an association of 8% reduced CHD risk with mean platelet volume (MPV) [8]. Reticulocyte count and percentage from erythrocyte turnover are increased with hemolysis to release more circulating free hemoglobin. Cell-free hemoglobin-based blood substitutes were associated with a 30% increase in mortality and a nearly threefold rise in the rate of MI in a meta-analysis [9]. Free hemoglobin reduces nitrous oxide and increases vasoconstriction, which may contribute to the increased risk of MI.

Somatic mutations in the blood cells of atherosclerosis CAD

Accumulation of somatic DNA mutations in hematopoietic progenitor and stem cells throughout life is a feature of aging, most of which are nonpathogenic but some somatic mutations may cause clonal hematopoiesis. Single-nucleotide variants (SNVs) and small indels in the blood of the elderly who are not known to have any hematologic abnormalities were examined by whole-exome sequencing

of DNA in the peripheralblood cells of 17,182 Caucasians in 22 population-based cohorts in three consortia. Somatic mutations leading to clonal outgrowth of hematopoietic cells were frequently found in 10% of persons older than 70 years of age carried these lesions, a median of 18% of peripheral-blood leukocytes were part of the abnormal clone. Longitudinal analysis showed that clonal hematopoiesis with somatic mutations persisted over a time period of 4 to 8 years [5].

The most commonly mutated gene was *DNMT3A* (403 variants), *TET2* (72 variants) and *ASXL1* (62 variants) [5]. Notably, clonal expansions are detected in these three genes that are most frequently involved somatic mutations and previously been implicated in hematologic cancers. Clonal hematopoiesis was a strong risk factor for subsequent hematologic cancer (hazard ratio, 12.9; 95% confidence interval, 5.8 to 28.7) and present more than 6 months before a first diagnosis of cancer in approximately 42% of hematologic cancers in a large cohort of 12,380 subjects [10]. In addition, metaanalysis of the two cohorts adjusted for age, sex, and status with respect to type 2 diabetes, clonal hematopoiesis led to an 11 fold increase of the incidence of hematologic cancers. In particular, among persons with a variant allele fraction of 0.10 or greater (indicating a higher proportion of cells in the blood carrying the mutation), the risk of a hematologic cancer was increased nearly 50 fold [5]. However, the majority of persons with clonal mutations in peripheral blood did not develop the myelodysplastic syndrome or some other hematologic cancers. Surprisingly, individuals who had clonal hematopoiesis with somatic mutations as compared with those without exhibited a significant 1.4 fold increase in all-cause mortality, which could not be accounted for alone by the increase in death from a hematologic cancer. Overall, a hematologic cancer developed during the study period only in approximately 4% of persons with a mutation. This indicates the absolute risk to develop a hematologic cancer for these with clonal hematopoiesis with somatic mutations is small.

Somatic mutations in genes known to cause hematologic cancers were also significantly associated with a 30% increase in risk of type 2 diabetes, even after adjustment for potential confounding variables. These who had clonal hematopoiesis with somatic mutations demonstrated an increased cumulative incidence of both CAD and ischemic stroke, even in the presence of traditional risk factors of smoking, total cholesterol level, and HLD cholesterol level [5]. In multivariable analyses including age, sex, status with respect to type 2 diabetes, systolic blood pressure, and body-mass index as covariates, individuals carrying a somatic mutation as compared with those without a mutation had a 2 fold increase for incident CAD and 2.6 fold for ischemic stroke, respectively. A cause-specific mortality analysis revealed that individuals with mutations had a higher risk of death from cardiovascular causes but not from cancer.

Red blood cell distribution width (RDW) is a measure of red blood cell volume variations. The presence of a somatic mutation was significantly associated with a higher RDW. An elevated RDW has been associated with 3.7 fold increased risk of death as compared with those who had a normal RDW and did not have mutations [5,11]. Clonal expansion in individuals with somatic mutations may lead to perturbation of hematopoiesis and high RDW; patients with higher RDW values had decreased red blood cell deformability and impaired blood flow through the microcirculation contributing to the pathophysiology of CAD and MI. Studies have shown that RDW is a predictor of poor clinical outcomes in patients with CAD [12]. Furthermore, red blood cells count (RBC) and HDL-C level were evaluated in 3,534 CAD patients who were performed coronary angiography. Lower HDL-C increased CAD incidence 55%; higher RBC levels can reduce the risk of CAD in patients with lower HDL-C levels, which suggest RBC may play an atheroprotective role in patients with coronary atherosclerosis [13].

TET2 mutations and atherosclerosis CAD

TET2 encodes an epigenetic regulatory enzyme that catalyzes the oxidation of 5 methylcytosine (5 mC) in DNA to 5-hydroxymethylcytosine (5 hmC) [14-15]. *TET2* mutations have been associated with increased CAD incidence and all-cause mortality [5]. Over 70 mutations in *TET2* have been identified, which were specific to individuals with clonal hematopoiesis without hematological malignancies [16]. However, the molecular mechanism of a *TET2* mutation leading to CAD pathogenesis remains unclear. For the purpose of investigating how clonal expansion of Tet2-deficient hematopoietic cells contributes to atherosclerosis, atherosclerosis-prone Ldlr−/− chimeric

mice with a small proportion of Tet2-deficient HSPCs were generated with a competitive bone marrow transplantation (BMT) strategy. This strategy mimics those human cells carrying somatic *TET2* mutations are transplanted into immune-deficient mice [17].

Mice with clonal expansion of Tet2-deficient BM cells on high fat/high cholesterol (HFHC) diet exhibited a significantly increased atherosclerosis with 60% larger plaques in the aortic root than controls, which was independent of alterations in systemic metabolism, changes in blood cell counts or macrophage proliferation or apoptosis in the plaque. Tet2-heterozygous cell expansion was slower yet sufficient to accelerate atherosclerosis. In atherosclerosis-prone mice exhibiting Tet2 deficiency restricted to myeloid cells, partial inactivation of Tet2 in BM-derived macrophages led to an increase in plaque size in the aortic root of HFHC-fed mice [17]. These findings provide strong evidence that Tet2-deficiency in myeloid cells promotes atherogenesis.

Tet2-deficient hematopoietic stem and progenitor cell (HSPC) expanded preferentially into the macrophage population in the atherosclerotic vascular wall.

However, *Tet2* deficiency did not affect macrophage proliferation, apoptosis, oxidized LDL (oxLDL) uptake or the expression of cholesterol trafficking regulators. It was reported previously that *Tet2* selectively mediates active repression of interleukin-6 (IL-6) transcription during inflammation resolution in innate myeloid cells [18]. Affymetrix microarray and quantitative RT-PCR analyses revealed that genes in these classes with known pro-inflammatory actions were mostly up-regulated in *Tet2*-deficient macrophages. Loss of function of *Tet2* affects NLRP3-mediated IL-1β secretion [17], which is essential for the atherogenic consequences of clonal expansion of *Tet2* deficient cells. P-selectin expression was significantly correlated with IL-1β in the aorta and significantly increased in Tet2-deficient BM cells. These support that IL-1β contributes to the aortic expression of P-selectin which recruits monocytes to the atherosclerotic plaque [17]. *Tet2* deficiency led to increased IL-6 levels in macrophage culture supernatants [17], which further supports that *Tet2* represses pro-inflammatory responses. The repression of *Tet2* on IL-6 and pro-inflammatory responses and resolving inflammation may result from the gene-specific transcription repression activity of Tet2 via histone deacetylation [17-18]. In addition, *TET2* is necessary master epigenetic regulator of smooth muscle cell differentiation [19]. Moreover, *TET2* plays a central role in mast cell differentiation, cytokine production, and proliferation, and loss of *TET2* leads to extensive changes in transcriptome and 5 hmC landscape [20]. *TET2* somatic mutations may be associated to the etiology of CAD by impairing epigenetic regulation and/or inflammation responses.

Collectively, these findings strengthen support that blood cell properties are important contributors for CAD and that somatic mutations in blood cells have been linked to CAD. Clonal hematopoiesis induced by *Tet2* loss-of-function plays an important role in atherosclerosis. Studies on evaluating the impact of somatic mutations in *TET2* and other genes to the overall incidence of CAD are warranted. The findings may provide new clues for the underlying mechanism, risk prediction and prevention of CAD, which will facilitate the development of effective novel therapies or preventive care strategies for atherosclerosis CAD.

References

1. Mozaffarian D., *et al*. "Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association". *Circulation* 133.4 (2015): e38-360.
2. Fryar CD., *et al*. "Prevalence of uncontrolled risk factors for cardiovascular disease: United States, 1999-2010". *NCHS Data Brief* 103 (2012): 1-8.
3. Dai X., *et al*. "Genetics of coronary artery disease and myocardial infarction". *World Journal of Cardiology* 8.1 (2016): 1-23.
4. Assimes TL and Roberts R. "Genetics: Implications for Prevention and Management of Coronary Artery Disease". *Journal of the American College of Cardiology* 68.25 (2016): 2797-2818.
5. Jaiswal S., *et al.* "Age-related clonal hematopoiesis associated with adverse outcomes". *The New England Journal of Medicine* 371.26 (2014): 2488-2498.

6. Wheeler JG., *et al*. "Associations between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30,374 individuals". *European Heart Journal* 25.15 (2004): 1287-1292.
7. Danesh J., *et al.* "Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies". *JAMA* 279.18 (1998): 1477-1482.
8. Astle WJ., *et al.* "The Allelic Landscape of Human Blood Cell Trait Variation and Links to Common Complex Disease". *Cell* 167.5 (2016): 1415-1429.
9. Natanson C., *et al*. "Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis". *JAMA* 299.19 (2008): 2304-2312.
10. Genovese G., *et al.* "Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence". *The New England Journal of Medicine* 371.26 (2014): 2477-2487.
11. Akin F., *et al*. "Relation between red cell distribution width and severity of coronary artery disease in patients with acute myocardial infarction". *Angiology* 64.8 (2013): 592-596.
12. Bujak K., *et al*. "The Prognostic Role of Red Blood Cell Distribution Width in Coronary Artery Disease: A Review of the Pathophysiology". *Disease Markers* 2015 (2015): 824624.
13. Schaffer A., *et al*. "Impact of red blood cells count on the relationship between high density lipoproteins and the prevalence and extent of coronary artery disease: a single centre study [corrected]". *Journal of Thrombosis and Thrombolysis* 40.1 (2015): 61-68.
14. Tahiliani M., *et al*. "Conversion of 5-methylcytosine to 5hydroxymethylcytosine in mammalian DNA by MLL partner TET1". *Science* 324.5929 (2009): 930-935.
15. Ito S., *et al.* "Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification". *Nature* 466.7310 (2010): 1129-1133.
16. Busque L., *et al*. "Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis". *Nature Genetics* 44.11 (2012): 1179-1181.
17. Fuster JJ., *et al*. "Clonal hematopoiesis associated with Tet2 deficiency accelerates atherosclerosis development in mice". *Science* 355.6327 (2017): 842-847.
18. Zhang Q., et al. "*Tet2* is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6". *Nature* 525.7569 (2015): 389-393.
19. Liu R., *et al*. "Teneleven translocation-2 (*TET2*) is a master regulator of smooth muscle cell plasticity". *Circulation* 128.18 (2013): 2047-2057.
20. Montagner S., *et al*. "*TET2* Regulates Mast Cell Differentiation and Proliferation through Catalytic and Non-catalytic Activities". *Cell Reports* 15.7 (2016): 1566-1579.