Journal of Taibah University Medical Sciences (2017) 12(3), 199–204

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Review Article

The 9p21.3 risk locus for coronary artery disease: A 10-year search for its mechanism

Naif A.M. Almontashiri, PhD

Department of Pathology, Massachusetts General Hospital, Harvard University, Boston, USA

Received 1 January 2017; revised 3 March 2017; accepted 5 March 2017; Available online 25 April 2017

الملخص

يعتبرالموضع الخطر للقطعة رقم پ21.3 على كروموسوم رقم ٩ أول موضع يوصف لارتباطه بزيادة خطر الإصابة بالحوادث ذات الصلة بأمراض الشرايين التاجية، وعدد من الظواهر الأخرى. تحتوي هذه القطعة على ٥٩ من النوكليوتيدات المنفردة المتعددة الأشكال في منطقة، مع العديد من المحفزات بعيدة المدى، ومناطق طويلة من الحمض النووي الريبي بلا رموز، التي تؤثر على التعبير عن الجينات القريبة، والسيكلين المعتمد على الكيناز 2أ و2ب والمطلوبة للتحكم في تنظيم الانقسام المتسارع، وشيخوخة الخلايا العضلية الملساء الموجودة في الأوعية الدموية. أجريت عدة دراسات لتحديد الآلية الصحيحة بدقة لكيفية ممارسة هذا الموضع تأثيره المرضي لزيادة خطر الإصابة بالحوادث ذات الصلة بأمراض الشرايين التاجية. في هذه المراجعة، سوف نقوم بتسليط الضوء على أبرز ما تم إنجازه، ومعرفته فيما يتعلق بارتباط النمط الجيني-المظهري على الصعيدين الميكانيكي والمظهري. نظرا إلى الخطر العالي على السكان الذي يعزى للموضع الخطر للقطعة رقم پ21.3 على كروموسوم رقم ٩، وآلية المعرفة التي حصلنا عليها حتى الآن، بالإضافة إلى الجهود المستمرة، قد تساعد في تصميم جزيئات علاجية جديدة لتقليل خطر أمراض الشرايين التاجية والحوادث ذات الصلة.

الكلمات المفتاحية: القطعة رقم پ21.3 على كروموسوم رقم 9؛ مرض الشرايين التاجية؛ CDKN2A و CDKN2B؛ الخلايا العضلية الملساء؛ موضع خطر

Abstract

The 9p21.3 risk locus is the first locus to be associated with an increased risk of coronary artery disease (CAD)-related events and many other phenotypes. This locus contains 59 single nucleotide polymorphisms (SNPs) in a region with multiple long range enhancers and long non-coding RNAs (lncRNAs) that affect the expression of neighbouring genes, cyclin-dependent kinase 2A and 2B (CDKN2A and CDKN2B), which are required for controlling vascular smooth muscle cell proliferation and ageing. Several studies have attempted to identify the precise mechanism by which this locus exerts its pathogenic effect to increase the risk of CAD-related events. In this review, we will highlight the major advances in our understanding of the genotype–phenotype correlation at the mechanistic and phenotypic levels. The high population attributable risk of the 9p21.3 risk locus, mechanistic knowledge acquired thus far, and ongoing research efforts could facilitate the design of novel therapeutic molecules to reduce the risk of CAD and its related events.

Keywords: 9p21.3; CDKN2A and CDKN2B; Coronary artery disease; Risk locus; Smooth muscle cell

Introduction

The major cause of CAD is atherosclerosis, which results from the plaque build-up and narrowing of the inner walls of the coronary arteries that supply heart muscle with blood, thereby leading to limited blood flow and eventual ischaemia. Plaque primarily comprises fat (cholesterol and fatty acids)-laden macrophages, vascular smooth muscle cells (VSMCs), cellular debris, and minerals, such as calcium. The white blood cell (WBC) component of plaque produces inflammatory cytokines that recruit inflammatory cells to the site of

Corresponding address: 65 Landsdowne Street, Suite 350, Cambridge, MA 02139, USA.
E-mail: nalmontashiri@partners.org

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jtumed.2017.03.001

the plaque. Although this process is meant to be protective, it further contributes to the plaque size and inflammation.[1] CAD is a common and complex chronic disease with traditional and genetic risk factors. The traditional risk factors of CAD include age, gender, obesity, dyslipidaemia, diabetes, hypertension and smoking. These factors act independently or in concert with each other to increase the risk of CAD. Controlling for the known risk factors of CAD, such as smoking, randomized clinical trials have shown that hypercholesterolaemia[2] is associated with an approximately 30–40% reduction in clinical events, such as myocardial infarction and subsequent death.[3] The other 40–60% of the risk of CAD is heritable according to epidemiological, twin and family studies. The heritability of CAD is the component of the CAD risk explained by genetic factors.[4] In a landmark case-control study, CAD was shown to have strong heritability, ranging from 56% (when patients with monogenic heart disease are excluded) to 63% (including patients with monogenic heart diseases).[5] Moreover, first-degree relatives showed a higher risk index for ischemic heart disease and stroke (3 and 1, respectively) than second-degree relatives (1 and 0.5, respectively). The heritability in this study was calculated using Falconer's method to determine the heritability of certain traits or phenotypes based on the difference between twins. Among first-degree relatives with CAD, heritability was estimated at approximately 100% in patients under age 46; whereas, heritability ranged from 15% to 30% in late onset cases of CAD.[6] The younger the CAD patient at the diagnosis of the first event of MI, the more common was CAD in his relatives of parents and siblings.

The pathophysiology of atherosclerosis

Atherosclerosis is an asymptomatic chronic late onset disease. Atherosclerotic plaques are divided into stable or unstable plaques. Stable plaques are often asymptomatic and contain collagen-rich extracellular matrix that is primarily produced primarily smooth muscle cells. The collagen-rich extracellular matrix (ECM) generates a stabilizing fibrous cap that separates the plaque from the lumen of the vessel. By contrast, unstable plaques are rich in foam cells and collagen-poor ECM, making the unstable and vulnerable to rupture. The rupture of the plaque and release of its thrombogenic components trigger thrombosis and formation of blood clots that occlude arteries and induce MI.[7]

Cell proliferation in atherosclerosis

The process of cell proliferation is regulated by a group of proteins called cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs). Activation of cell cycle phase-specific CDKs drives progression through this phase. For example, the CKIs p15 and p16 interact with and inactivate CDK4 and CDK6, preventing them from binding and activating cyclin D. Inactive cyclin D cannot phosphorylate the cytoplasmic retinoblastoma protein (RB) and release the sequestered E2 promoter binding factor 1 (E2F1) to enter the nucleus, thereby activating transcription of genes involved in the progression from G1 to S phase.[8,9]

Epigenetic modifications, such as hypermethylation, were enriched in the aorta and PBMCs, playing causative roles in the process of atherosclerosis development in a mouse model of CAD.[10] Hypermethylation is significantly associated with the risk of CAD.[11,12] Risk factors, such as dyslipidaemia and hyperhomocysteinaemia, are key mediators of the hypermethylation observed in patients with CAD.[10,12] Hypermethylation of the upstream regions of the TGFBR3 genes was significantly enriched in those patients.[11] Perturbed expression of *CDKN2A* and *CDKN2B* has been associated with several tumours.[13–15] Methylation of *CDKN2A* and *CDKN2B* is associated with CAD in humans.[16] Excessive cell proliferation within the walls of the arteries contributes to the enlargement of the plaque and restenosis after angioplasty.[17] VSMCs and macrophages are the main proliferating cell types in human atherosclerotic plaques.[18]

TGFβ and VSMC proliferation in atherosclerosis

Proliferation of VSMCs is critical for repair and healing after vascular injury or insult. However, if the insult persists, as in atherosclerosis, then the mitogenic stimulus continues and the proliferation of VSMCs becomes atherogenic.[19] VSMCs produce ECM, which stabilizes the plaque, and the migration and proliferation of arterial smooth-muscle cells enlarge the atherosclerotic lesion.[17] TGFβ inhibits VSMC migration and proliferation and induces collagen-rich ECM production.[20,21] In the walls of normal vessels, VSMCs primarily express type II TGFβ receptor (*TGFBR*), as opposed to type I TGFβ receptor, which is produced in VSMCs from atherosclerotic vessels.[20] In response to TGFβ, normal VSMCs expressing type II TGFβ receptor greatly induce the expression of contractile proteins and minimal production of the ECM. By contrast, diseased VSMCs expressing type I TGFβ receptor increase the expression of collagen rich-ECM but fail to induce the expression of contractile proteins in response to TGFβ. If fresh VSMCs are maintained in TGFβ-containing media, then these cells maintain type II TGFβ receptor.[22] Low concentrations of TGFβ increase the proliferation of VSMCs, consistent with the fact that low levels of plasma TGFβ are associated with a poor outcome in CAD.[23,24] Conversely, at higher concentrations, TGFβ inhibits VSMC proliferation[23] and reduces atherosclerosis.[25] The levels of plasma TGFβ are reduced at sites of lesion development in the intima of the coronary arteries and human aorta.[26,27] In sum, these studies demonstrate that TGFβ is critical for maintaining the contractility of VSMCs and inhibiting their proliferation and migration to the intima of arteries. Therefore, impaired or a lack of TGFβ signalling is atherogenic. Mutations in the *TGFBR1* and *TGFBR2* genes that cause congenital heart disease and arterial aneurysms are associated with increased VSMC proliferation, increased collagen expression and reduced contractile protein (SMC α-actin, β-myosin, and calponin) expression.[28,29]

TGFβ induces the expression of p16 and p15 through Smad proteins, such as Smad2 and Smad3, to control the cell cycle and induce cellular senescence.[8,30,31] Smad3 interacts with the TEA-domain (TEAD) family of transcription factors, particularly TEAD3 and TEAD4.[32] The TEAD family has a

common N-terminal domain that enables these proteins to bind a specific DNA element called M-CAT (5′-CATTCC-3′) and a transactivation domain to interact with co-activators, such as Smad proteins. TEAD transcription factors play key roles in the expression of cardiac, smooth and skeletal muscle-specific genes (such as SMC α-actin and β-myosin).[33] These factors play a major role in tumour suppression and cell cycle control.[34] The major role of TEAD factors in VSMCs and cardiac development explains their involvement in congenital and developmental heart diseases.[35]

9p21.3 CAD risk locus as the first hit of a GWAS

Using microarrays of SNPs to genotype large numbers of cases and controls, the first common genetic variants at chromosome 9p21.3 conferring a risk for CAD were identified.[36] This risk locus has also been associated with other diseases, such as type 2 diabetes,[37] CAD-associated MI, abdominal aortic and intracranial aneurysm.[38] Several other large GWASs have confirmed this association with CAD.[39,40] The minor allele frequency (MAF) in different populations is as follows: European (50%), Sub-Saharan African (50%), African American (24%), Asian (47%), and Han Chinese (37%). The mechanism whereby these genetic variants contribute to the risk of CAD has remained elusive.[41] The 9p21 risk alleles predict the severity of CAD according to the burden of arterial atherosclerosis: the 9p21 risk allele was more frequently observed in patients with a narrowing of 3 coronary arteries than in those with a single affected artery.[42] No significant association with the frequency of the 9p21.3 risk locus was observed between CAD patients with or without MI,[42] suggesting that the 9p21.3 risk locus functions through plaque development and not rupture. The 9p21.3 locus contains 59 linked SNPs located 100,000 base pairs upstream of the cell cycle suppressor genes *CDKN2A* (codes for p16 and p14) and *CDKN2B* (codes for p15). This locus overlaps with the 3′ region of *ANRIL* (antisense noncoding RNA at the ink4 locus non-coding gene). The 9p21.3 risk variants overlapping the 3′ region of *ANRIL* are associated with the induced expression of different splicing isoforms of ANRIL and reduced expression of *CDKN2A* and *CDKN2B*.[43] Polycomb repressor complex 1 and 2 (PRC1 and PRC2) and polycomb complex protein EZH2 are recruited to *ANRIL*, which in turn leads to the recruitment of DNA methyltransferase (*DNMT1*), further increasing DNA methylation and inactivation of the *CDKN2A* locus.[44] However, the increased expression of the *ANRIL* transcript and methylation of the *CDKN2A* and *CDKN2B* loci were significantly associated with CAD in angiographic-defined patients, but not those with the 9p21.3 risk locus.[16]

Several studies have demonstrated reduced expression of p16 and p15 in the presence of the 9p21.3 CAD risk locus in aortic smooth muscle cells (AoSMCs).[43,45,46] The reduced expression of p16 and p15 at the risk locus is associated with increased HAoSMC proliferation and a failure to enter senescence. Indeed, p16 and p15 are well-known tumour suppressors and cellular senescence markers that function through the retinoblastoma pathway. Thus, the 9p21 risk allele may promote the deposition of atherosclerotic plaques in the coronary arteries, likely through accumulation of fat-laden foam cells and proliferation of VSMCs in the intima, rather than the weakening of the extracellular matrix to cause plaque rupture and myocardial infarction.

The regulation of gene expression at the 9p21.3 CAD risk locus

The 9p21.3 risk locus is as complex as the associated phenotypes. The 9p21.3 risk locus contains several enhancers with defined risk haplotypes linked to distinct phenotypes, suggesting that the 9p21.3 risk locus exerts tissue- and disease-specific effects.[47,48] For example, the CAD risk haplotype tagged by rs1333049 is associated with CAD and atherosclerosis burden but not with MI in patients with CAD compared to patients without CAD, suggesting a phenotype-specific enhancer effect.[49]

Gene transcript profiling showed no association of the CAD risk variants with genes in the vicinity of 9p21.3 in donor macrophages.[50,51] Primary HAoSMCs from atherosclerotic plaques showed reduced expression of p16 and p15 proteins and increased proliferation when homozygous for the risk allele (65). Knockout of the 9p21.3 orthologous sequences in mice resulted in a significant reduction of CDKN2A and CDKN2B expression and increased aortic smooth muscle cell proliferation as well as failure to enter senescence, with the strongest effect observed in aortic tissues.[46] Consistent with these findings, we showed that the 9p21.3 CAD risk locus is associated with reduced expression of p15 and p16, increased proliferation of HAoSMCs and failure to enter senescence.[52] Given these data, the increased proliferation and reduced expression p15 and p16 are the established biological phenotypes linked to the 9p21.3 CAD risk locus (Figure 1). Overall, these data suggest phenotype- and tissue-specific effects of the variants at the 9p21.3 locus.

The VSMC proliferation observed with the 9p21.3 risk locus is involved in the pathogenesis of atherosclerosis and plaque growth. However, to intervene with the pathogenesis of this locus, we have to determine the mechanisms by which the p15 and p16 levels are reduced to affect VSMC proliferation. Several attempts have been made to identify this mechanism. Knockout of the mouse orthologue has provided a model to study the disease in an animal context.[46] This model supported previous findings of reduced p15 and p16 expression in patients with CAD.[43] Notably, 45% of the knockout mice that developed tumours and neoplasms were not reported to associate with the 9p21.3 CAD risk locus according to GWASs. In an attempt to determine the mechanism, another study claimed that interferon-γ long-range induction of p15 and p16 as well as other genes is disrupted by the risk variant that disrupts STAT1 binding at the locus.[48] However, our work did not support this hypothesis and showed that p15 and p16 were induced by interferon-γ, independent of the 9p21.3 risk locus.[53] We used a larger sample size and many different types of cells. Consistent with our findings, *Erridge* et al. (2013) showed that the interferon-γ signalling to the interferon family of genes is not disrupted by the presence of the 9p21.3 risk locus.[50]

Pilbrow et al. (2013) showed that expression of genes involved in the TGFβ pathway was affected by the 9p21.3

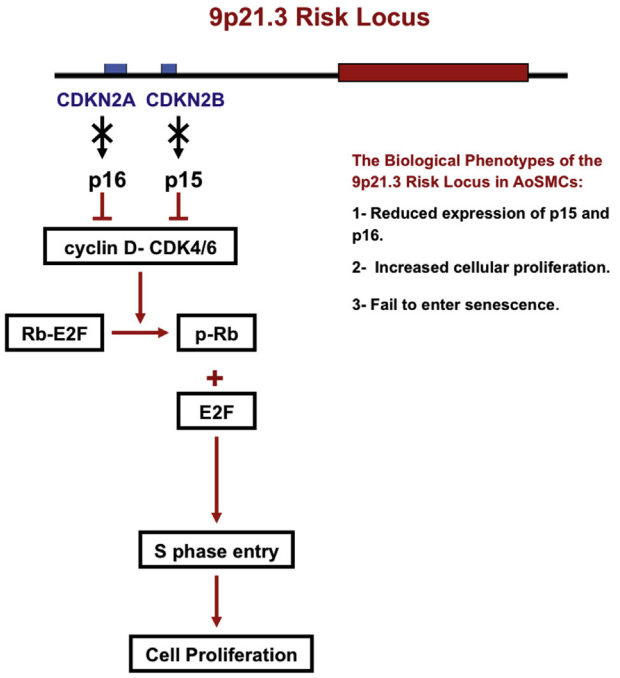

Figure 1: Schematic diagram of the effect of the 9p21.3 risk locus on cell cycle suppressor genes and cell proliferation.

risk locus in multiple human tissues.[54] Using the knockout mouse model of the 9p21.3 risk locus, *Loinard* et al. (2014) showed reduced TGFβ-dependent Smad2 signalling associated with reduced p15 expression as well as increased proliferation in AoSMCs in the knockout mice. Moreover, these authors also showed that these mice were susceptible to aneurysm and plaque rupture; these effects were preventable using CDK inhibitors. Our unbiased scanning approach for the disruption of transcription factor binding at the 9p21.3 locus identified TEAD3 as a mediator of TGFβ induction of p16 (p15 was not affected), which is disrupted by the presence of the risk locus.[52] Our findings are consistent with those of *Pilbrow* et al. (2013) with regard to the involvement of the TGFβ pathway in the risk mechanism. However, our work did not support the findings of *Loinard* et al. (2014), as we showed contrasting findings. This contradiction could reflect species differences between mice and humans and also models differences. These previous studies used a knockout mouse model, whereas we used primary HAoSMCs that carry CAD-linked SNPs, which is more biologically and clinically relevant to the 9p21.3 risk locus. The reduction in the level of p15 expression may reflect the post-transcriptional effect of the *ANRIL* anti-sense transcript, which is up-regulated with the risk locus and has been shown to repress expression of the *CDKN2B* locus and its transcript.[16,43,44] Our recent work provided a novel mechanism by which TEAD3 and TEAD4 induce p16 expression and mediate TGFβ induction of p16 at the 9p21.3 locus.

Conclusion

A peptide that activates TEADs factors leading to control of the cell cycle and suppression of gastric cancer growth in vivo has been developed.[55] Future studies can employ similar strategies to prevent or mitigate atherosclerosis in CAD patients who are not homozygotes for the 9p21.3 risk allele. Notably, the mechanism by which the 9p21.3 risk locus affects the expression of CDKN2A and smooth muscle proliferation does not explain or account for the ability of 9p21.3 to confer a risk of coronary artery[56] and aortic calcification,[57] and this mechanism awaits future studies.

Author's contribution

NA wrote initial and final draft of the review. NA has approved the final draft and is responsible for the content of the manuscript.

Conflict of interest

The author has no conflict of interest to declare.

References

1. Libby P. Inflammation in atherosclerosis. **Nature 2002**; 420: 868–874.
2. Almontashiri NA. Usefulness of genome-wide association studies to identify novel genetic variants underlying the plasma lipoprotein metabolism as risk factors for CAD. **J Taibah Univ Med Sci 2015**; 10: 266–270.
3. Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. **BMJ 2003**; 326: 1419.
4. Lusis AJ. Atherosclerosis. **Nature 2000**; 407: 233–241.
5. Nora JJ, Lortscher RH, Spangler RD, Nora AH, Kimberling WJ. Genetic–epidemiologic study of early-onset ischemic heart disease. **Circulation 1980**; 61: 503–508.
6. Rissanen AM. Familial occurrence of coronary heart disease: effect of age at diagnosis. **Am J Cardiol 1979**; 44: 60–66.
7. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. **Arterioscler Thromb Vasc Biol 2010**; 30: 1282–1292.
8. Hannon GJ, Beach D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. **Nature 1994**; 371: 257–261.
9. Hara E, Smith R, Parry D, Tahara H, Stone S, Peters G. Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. **Mol Cell Biol 1996**; 16: 859–867.
10. Lund G, Andersson L, Lauria M, et al. DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. **J Biol Chem 2004**; 279: 29147–29154.
11. Sharma P, Garg G, Kumar A, et al. Genome wide DNA methylation profiling for epigenetic alteration in coronary artery disease patients. **Gene 2014**; 541: 31–40.
12. Sharma P, Kumar J, Garg G, et al. Detection of altered global DNA methylation in coronary artery disease patients. **DNA Cell Biol 2008**; 27: 357–365.
13. Krishnamurthy J, Torrice C, Ramsey MR, et al. Ink4a/Arf expression is a biomarker of aging. **J Clin Invest 2004**; 114: 1299–1307.
14. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. **Genes Dev 2010**; 24: 2463–2479.
15. Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. **Int J Cancer 2012**; 130: 1715–1725.
16. Zhuang J, Peng W, Li H, et al. Methylation of p15INK4b and expression of ANRIL on chromosome 9p21 are associated with coronary artery disease. **PLoS One 2012**; 7: e47193.

17. Ross R. Atherosclerosis—an inflammatory disease. **N Engl J Med 1999**; 340: 115—126.
18. Rekhter MD, Gordon D. Active proliferation of different cell types, including lymphocytes, in human atherosclerotic plaques. **Am J Pathol 1995**; 147: 668—677.
19. Kragel AH, Reddy SG, Wittes JT, Roberts WC. Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death. **Circulation 1989**; 80: 1747—1756.
20. Grainger DJ. Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. **Arterioscler Thromb Vasc Biol 2004**; 24: 399—404.
21. Kojima S, Harpel PC, Rifkin DB. Lipoprotein (a) inhibits the generation of transforming growth factor beta: an endogenous inhibitor of smooth muscle cell migration. **J Cell Biol 1991**; 113: 1439—1445.
22. Grainger DJ, Metcalfe JC, Grace AA, Mosedale DE. Transforming growth factor-beta dynamically regulates vascular smooth muscle differentiation in vivo. **J Cell Sci 1998**; 111(Pt 19): 2977—2988.
23. Berk BC. Vascular smooth muscle growth: autocrine growth mechanisms. **Physiol Rev 2001**; 81: 999—1030.
24. Tashiro H, Shimokawa H, Sadamatu K, Yamamoto K. Prognostic significance of plasma concentrations of transforming growth factor-beta in patients with coronary artery disease. **Coron Artery Dis 2002**; 13: 139—143.
25. Reifenberg K, Cheng F, Orning C, et al. Overexpression of TGF-ss1 in macrophages reduces and stabilizes atherosclerotic plaques in ApoE-deficient mice. **PLoS One 2012**; 7: e40990.
26. Borkowski P, Robinson MJ, Kusiak JW, Borkowski A, Brathwaite C, Mergner WJ. Studies on TGF-beta 1 gene expression in the intima of the human aorta in regions with high and low probability of developing atherosclerotic lesions. **Mod Pathol 1995**; 8: 478—482.
27. Jiang X, Zeng HS, Guo Y, Zhou ZB, Tang BS, Li FK. The expression of matrix metalloproteinases-9, transforming growth factor-beta1 and transforming growth factor-beta receptor I in human atherosclerotic plaque and their relationship with plaque stability. **Chin Med J (Engl) 2004**; 117: 1825—1829.
28. Inamoto S, Kwartler CS, Lafont AL, et al. TGFBR2 mutations alter smooth muscle cell phenotype and predispose to thoracic aortic aneurysms and dissections. **Cardiovasc Res 2010**; 88: 520—529.
29. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. **Nat Genet 2005**; 37: 275—281.
30. Reynisdottir I, Polyak K, Iavarone A, Massague J. Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. **Genes Dev 1995**; 9: 1831—1845.
31. Vijayachandra K, Higgins W, Lee J, Glick A. Induction of p16ink4a and p19ARF by TGFbeta1 contributes to growth arrest and senescence response in mouse keratinocytes. **Mol Carcinog 2009**; 48: 181—186.
32. Fujii M, Toyoda T, Nakanishi H, et al. TGF-beta synergizes with defects in the Hippo pathway to stimulate human malignant mesothelioma growth. **J Exp Med 2012**; 209: 479—494.
33. Stewart AF, Suzow J, Kubota T, Ueyama T, Chen HH. Transcription factor RTEF-1 mediates alpha1-adrenergic reactivation of the fetal gene program in cardiac myocytes. **Circ Res 1998**; 83: 43—49.
34. Pobbati AV, Hong W. Emerging roles of TEAD transcription factors and its coactivators in cancers. **Cancer Biol Ther 2013**; 14: 390—398.
35. Yoshida T. MCAT elements and the TEF-1 family of transcription factors in muscle development and disease. **Arterioscler Thromb Vasc Biol 2008**; 28: 8—17.
36. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. **Science 2007**; 316: 1488—1491.
37. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. **Nature 2007**; 447: 661—678.
38. Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. **Nat Genet 2008**; 40: 217—224.
39. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. **Science 2007**; 316: 1491—1493.
40. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. **N Engl J Med 2007**; 357: 443—453.
41. Musunuru K. Enduring mystery of the chromosome 9p21.3 locus. **Circ Cardiovasc Genet 2013**; 6: 224—225.
42. Dandona S, Stewart AF, Chen L, et al. Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. **J Am Coll Cardiol 2010**; 56: 479—486.
43. Jarinova O, Stewart AF, Roberts R, et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. **Arterioscler Thromb Vasc Biol 2009**; 29: 1671—1677.
44. Yap KL, Li S, Munoz-Cabello AM, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. **Mol Cell 2010**; 38: 662—674.
45. Motterle A, Pu X, Wood H, et al. Functional analyses of coronary artery disease associated variation on chromosome 9p21 in vascular smooth muscle cells. **Hum Mol Genet 2012**; 21: 4021—4029.
46. Visel A, Zhu Y, May D, et al. Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. **Nature 2010**; 464: 409—412.
47. Chen HH, Almontashiri NA, Antoine D, Stewart AF. Functional genomics of the 9p21.3 locus for atherosclerosis: clarity or confusion? **Curr Cardiol Rep 2014**; 16: 502.
48. Harismendy O, Notani D, Song X, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response. **Nature 2011**; 470: 264—268.
49. Fan M, Dandona S, McPherson R, et al. Two chromosome 9p21 haplotype blocks distinguish between coronary artery disease and myocardial infarction risk. **Circ Cardiovasc Genet 2013**; 6: 372—380.
50. Erridge C, Gracey J, Braund PS, Samani NJ. The 9p21 locus does not affect risk of coronary artery disease through induction of type 1 interferons. **J Am Coll Cardiol 2013**; 62: 1376—1381.
51. Zollbrecht C, Grassl M, Fenk S, et al. Expression pattern in human macrophages dependent on 9p21.3 coronary artery disease risk locus. **Atherosclerosis 2013**; 227: 244—249.
52. Almontashiri NA, Antoine D, Zhou X, et al. 9p21.3 coronary artery disease risk variants disrupt TEAD transcription factor-dependent transforming growth factor beta regulation of p16 expression in human aortic smooth muscle cells. **Circulation 2015**; 132: 1969—1978.
53. Almontashiri NA, Fan M, Cheng BL, Chen HH, Roberts R, Stewart AF. Interferon-gamma activates expression of p15 and p16 regardless of 9p21.3 coronary artery disease risk genotype. **J Am Coll Cardiol 2013**; 61: 143—147.
54. Pilbrow AP, Folkersen L, Pearson JF, et al. The chromosome 9p21.3 coronary heart disease risk allele is associated with altered gene expression in normal heart and vascular tissues. **PLoS One 2012**; 7: e39574.

55. Jiao S, Wang H, Shi Z, et al. A peptide mimicking VGLL4 function acts as a YAP antagonist therapy against gastric cancer. **Cancer Cell 2014**; 25: 166–180.
56. O'Donnell CJ, Kavousi M, Smith AV, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. **Circulation 2011**; 124: 2855–2864.
57. van Setten J, Isgum I, Smolonska J, et al. Genome-wide association study of coronary and aortic calcification implicates risk loci for coronary artery disease and myocardial infarction. **Atherosclerosis 2013**; 228: 400–405.

How to cite this article: Almontashiri NAM. The 9p21.3 risk locus for coronary artery disease: A 10-year search for its mechanism. J Taibah Univ Med Sc 2017;12(3): 199–204.