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Review

Molecular genetics of coronary artery disease and ischemic stroke

Yoshiji Yamada [a, b, *]

[a] Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Japan
[b] CREST, Japan Science and Technology Agency, Tokyo, Japan

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ABSTRACT

Coronary artery disease (CAD) and ischemic stroke are important clinical problems because they are associated with high mortality rates. The main causal and treatable risk factors of CAD and ischemic stroke include hypertension, dyslipidemia, diabetes mellitus, chronic kidney disease, and smoking. In addition, recent studies have highlighted the importance of genetic factors and their interactions with environmental factors in the development of CAD and ischemic stroke. Disease prevention is an important strategy for reducing the overall burden of CAD and ischemic stroke, and identification of markers of disease risk can help in risk prediction and the use of accurate interventions can help decrease the risk of these events. Although genetic linkage analyses and candidate gene association studies have implicated several loci and candidate genes in predisposition to CAD or ischemic stroke, these loci and genes have not been identified definitively. Recent genome-wide association studies (GWASs) have shown that single nucleotide polymorphisms in chromosome 9p21.3 locus and other loci are associated with CAD or ischemic stroke. In this review, I have summarized the genetics of CAD and ischemic stroke and have identified susceptibility genes and loci implicated in these conditions based on the results of GWASs. In addition, I have reviewed GWASs highlighting the association of polymorphisms in the chromosome 9p21.3 locus or other loci with CAD or ischemic stroke. The results of these studies may provide insights into the functions of the implicated genes in the development of CAD and ischemic stroke.

1. Coronary artery disease

Coronary artery disease (CAD) is an important clinical problem because it is associated with a high mortality rate. In 2012, the total number of individuals affected by CAD and myocardial infarction (MI) was 15.5 and 7.6 million, respectively, in the United States. In 2011, the annual incidence of new and recurrent cases of MI and fatal CAD was 935,000, with an annual mortality of 375,295 cases [1].

The main causal and treatable risk factors of CAD include hypertension, diabetes mellitus (DM), dyslipidemia, chronic kidney disease (CKD), and smoking. In addition, recent studies have highlighted the importance of genetic factors and their interactions with environmental factors in the development of CAD [2–5]. The common forms of CAD are multifactorial and are caused by functional alterations in many genes, each having a relatively small effect and working alone or in combination with other modifier genes, environmental factors, or both.

Despite recent advances in therapy for acute coronary syndrome, such as use of drug-eluting stents [6], CAD remains the leading cause of death in the United States. Disease prevention is an important strategy for reducing the overall burden of CAD, and identification of biomarkers of disease risk can help in risk prediction and use of accurate interventions to reduce the risk of cardiovascular events.

1.1. Familial aggregation of CAD

Twin and family studies have established that CAD aggregates in families, with a family history of early-onset CAD being a risk factor for the disease. Familial clustering of CAD might be explained in part by heritable quantitative variations in the known risk factors of CAD. However, evidence suggests that family history increases the

* Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan. Tel.: +81 59 231 5387; fax: +81 59 231 5388.
E-mail address: yamada@gene.mie-u.ac.jp.

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risk of CAD independently of the known risk factors [7,8]. High-risk families account for a substantial proportion of early-onset CAD cases in the general population. One study reported that families with a history of early-onset CAD represented only 14% of the general population but accounted for 72% cases of early-onset CAD (men aged <55 years and women aged <65 years) and 48% cases of CAD among all ages [9]. History of early-onset CAD in a first-degree relative approximately doubles the risk of CAD; however, the reported relative risk is in the range of 1.3–11.3 [7,10]. The highest relative risk of CAD-associated death is seen in monozygotic twins when 1 twin dies of early-onset CAD [7]. Furthermore, the risk of CAD is higher with a sibling history of MI than with a parental history of early-onset CAD [11]. A family risk score for CAD evaluates the ratio of observed CAD events to the expected events in an individual's first-degree relatives after adjusting for age and sex after the first event. A higher family risk score indicates a higher risk of CAD.

1.2. Genome-wide association studies on CAD or MI

Recent genome-wide association studies (GWASs) and meta-analyses on CAD or MI [12–20] have implicated chromosome 9p21.3 locus and various other loci and genes in increasing the susceptibility to CAD or MI, mainly in Caucasian populations. A meta-analysis of GWASs identified 45 loci associated with CAD or MI at a genome-wide significance level [20]. The results of this meta-analysis are summarized in Table 1. In the following sections, I have reviewed studies on the chromosome 9p21.3 locus and 7 genes, namely, *LTA*, *BTN2A1*, *PHACTR1*, *LPL*, *PSRC1*, *FLT1*, and *CNNM2*, that are of particular interest in the genetics of CAD.

1.2.1. Chromosome 9p21.3 and CDKN2B antisense RNA 1 gene

Independent GWASs involving single nucleotide polymorphism (SNP) microarrays identified 5 SNPs in the chromosome 9p21.3 locus that were associated with CAD or MI in several white populations [12–15]. McPherson et al. [12] identified 2 susceptibility SNPs rs10757274 and rs2383206 located within 20 kb of each other in the chromosome 9p21.3 locus that were associated with CAD in a Canadian population and 5 other white populations. Helgadottir et al. [13] described an association between MI and 2 SNPs, rs2383207 and rs10757278, located in the same locus in an Icelandic population and in 4 white populations. A GWAS identified an SNP rs1333049 in the same genetic locus in 1926 cases of CAD and 3000 controls from a British population [14] and this finding was replicated in a German population [15]. Later studies showed that this locus was also associated with ischemic stroke [21,22]. Interestingly, independent, population-based, case-control studies also identified several SNPs in the chromosome 9p21.3 locus that were significantly associated with type 2 DM in white populations from England [23], Finland [24], and Sweden [25]. The rs10757278 polymorphism in this locus was associated with abdominal aortic aneurysm and intracranial aneurysm in addition to MI [26]. Schunkert et al. [27] genotyped the rs1333049 (C → G) polymorphism in the chromosome 9p21.3 locus in 7 case-control studies involving 4645 patients with MI or CAD and 5177 controls. The *C* allele of this SNP was uniformly associated with MI or CAD in each study, with pooled analysis showing odds ratio per copy of this risk allele to be 1.29. A meta-analysis of the rs1333049 polymorphism in 12,004 cases and 28,949 controls provided further evidence of the association of this SNP with MI or CAD, with an odds ratio of 1.24 per risk allele.

Although the studies on the association of SNPs in the chromosome 9p21.3 locus with CAD and MI provide important information on the molecular genetics of these diseases, the mechanisms underlying their pathogenesis are yet to be ascertained. The chromosome 9p21.3 locus has 2 flanking recombination hot spots and contains 2 genes encoding cyclin-dependent kinase inhibitors (*CDKN2A* and *CDKN2B*). These 2 genes play an important role in the regulation of cell cycle and belong to a family of genes implicated in the pathogenesis of atherosclerosis because their products inhibit cell growth by inducing transforming growth factor beta 1. However, SNPs that are most strongly associated with MI or CAD lie considerably upstream of these genes, with the nearest SNP being located 10-kb upstream of *CDKN2B*. Therefore, other explanations should be considered for the association of the chromosome 9p21.3 locus with MI or CAD (Fig. 1) [27].

A high-risk CAD haplotype in the chromosome 9p21.3 locus (T [rs10116277]–T [rs6475606]–G [rs10738607]–T [rs10757272]–G [rs10757274]–G [rs4977574]–G [rs2891168]–G [rs1333042]–G [rs2383206]–G [rs2383207]–C [rs1333045]–G [rs10757278]–C [rs1333048]–C [rs1333049]) overlaps the CDKN2B antisense RNA 1 gene (*CDKN2B-AS1*) encoding a large antisense non-protein-coding RNA, which was identified by deletion analysis of an extended French family with hereditary melanoma–neural system tumors. Reverse transcription-polymerase chain reaction has shown that *CDKN2B-AS1* is expressed in atheromatous human vessels (in specimens from patients with abdominal aortic aneurysm or carotid endarterectomy) whose cell type profile is similar to that of atherosclerotic coronary arteries. *CDKN2B-AS1* is also expressed in vascular endothelial cells, monocyte-derived macrophages, and coronary smooth muscle cells, which contribute to atherosclerosis [28].

A conserved non-coding sequence containing a risk allele in the chromosome 9p21.3 locus showed significantly higher enhancer activity than a control conserved non-coding sequence. The observed difference in the regulatory activity of the conserved non-coding sequence was attributed to the rs1333045 (C → T) polymorphism that was in strong linkage disequilibrium with other SNPs in the previously defined chromosome 9p21.3 risk locus. These findings indicate that the risk allele in the chromosome 9p21.3 locus alters the activity of the regulatory sequence, which in turn may change the expression level of *CDKN2B-AS1* or other genes relevant to atherosclerosis [29]. Furthermore, SNPs in the chromosome 9p21.3 locus may act as molecular switches that result in reciprocal changes in the expression levels of short and long *CDKN2B-AS1* transcripts. The risk allele may modify the expression of cell cycle regulatory genes by reducing the expression of the long *CDKN2B-AS1* transcript or by increasing the expression of the short *CDKN2B-AS1* transcripts, thereby promoting the proliferation of arterial smooth muscle cells or other cells relevant to atherosclerosis [29]. *CDKN2B-AS1* transcript also interacts with polycomb repressive complex 1 and 2, leading to the epigenetic silencing of other genes in this cluster and alteration of the expression of several genes related to cell proliferation [29].

These observations suggest that a conserved non-coding sequence in the chromosome 9p21.3 locus has enhancer activity and that the risk allele of the rs1333045 polymorphism within the conserved non-coding sequence increases the transcriptional activity in vascular smooth muscle cells. Thus, allelic variants in the chromosome 9p21.3 locus may increase predisposition to CAD or MI by altering the expression levels of short and long *CDKN2B-AS1* transcripts, thus affecting various cell proliferation pathways [29].

Analysis of gene expression profiles of peripheral blood mononuclear cells showed that levels of *EU741058* and *NR_003529* transcripts of *CDKN2B-AS1* were increased in the carriers of the risk haplotype while the levels of *DQ485454* transcript remained unaffected, suggesting a differential expression of *CDKN2B-AS1* in the chromosome 9p21.3 locus. Similar results were obtained for gene expression profiles of whole blood and atherosclerotic plaque tissue. Expression of *CDKN2B-AS1* transcripts (*EU741058* and