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REVIEW

Molecular genetics of coronary artery disease

Kouichi Ozaki[1] and Toshihiro Tanaka[1,2,3]

Coronary artery disease (CAD) including myocardial infarction (MI) is a common disease and among the leading cause of death in the world. The onset of CAD depends on complex interactions of environmental and genetic factors. To clarify the genetic architecture of MI, we started a genome-wide association study (GWAS) using nearly 100 000 gene-based single-nucleotide polymorphisms (SNPs) from 2000, and identified *LTA* associated with the increased risk of MI in Japanese population. To our knowledge, this is the first study identified a genetic factor for common disease by GWAS in the worldwide. Through examining the LTA cascade by combination of molecular biological and genetic analyses, we have identified additional MI susceptible genes, *LGALS2*, *PSMA6* and *BRAP*, so far. Nowadays a lot of large-scale GWAS have identified numerous genetic risk factors for common diseases. In CAD, 51 loci with GWAS significance ($P < 5 \times 10^{-8}$) have collectively identified by recent large-scale GWAS mainly in Caucasian descent. In this review, we discuss recent advances in molecular genetics for CAD.
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INTRODUCTION

Coronary artery disease (CAD) including myocardial infarction (MI) has been the major cause of mortality and morbidity among late-onset diseases in many industrialized countries with a Western lifestyle.[1,2] MI often occurs without any preceding clinical signs and is followed by severe complications, especially ventricular fibrillation and cardiac rupture, which might result in sudden death. Although recent advances in treatment and diagnosis have greatly improved quality of life for patients after MI, its morbidity is still high. MI is a disease of the vessel that feeds the cardiac muscle called the coronary artery. Irreversible damage to cardiac muscle is occurred by abrupt occlusion of the coronary artery. Plaque rupture with thrombosis is a well-established critical factor in the pathogenesis of MI.[3,4] Although leaving open the question for the detailed mechanisms of plaque rupture, inflammation is thought to have a critical role in its pathology.[5] Epidemiologic studies reveal that coronary risk factors include dyslipidemia, hypertension, smoking, type 2 diabetes mellitus, obesity and inflammation. Although each risk factor seems to be under genetic control, a positive family history is an independent predictor implying a genetic contribution to CAD, and estimated the genetic heritability to account for 40–50%.[6,7] Common genetic variants including single-nucleotide polymorphisms (SNPs) are believed to contribute to genetic risk of diseases.[8–10] In this context, in 2000 we started genome-wide association studies (GWASs) of this disorder using nearly 100 000 gene-based SNPs (http://snp.ims.u-tokyo.ac.jp/)[11] by high-throughput multiplex PCR-invader assay system[12] in Japanese population and identified several genes conferring risk of MI, including *LTA*.[13–15] Although the functions of these susceptible genes in MI pathogenesis are under investigation, these findings showed the potent power of GWAS, which is hypothesis free, to identify unexpected anchors to further understand the disease. Through examining the LTA (Lymphotoxin - α) cascade by combination of biological and genetic analyses, we have identified additional MI susceptible genes[16–18] so far. On the other hand, improvement of infrastructure for genomic diversity such as haplotype/linkage disequilibrium structure (http://hapmap.org/)[19] as well as genotyping and statistical technique permit more large-scale/comprehensive analysis to identify genetic backgrounds of common diseases. To date, numerous large-scale GWASs and meta-analyses for CAD have conducted mainly in Europe and the US, and totally identified 51 loci with GWAS significance.[20–38] Although these loci have modest effect with the increased relative risk from 1.05 to 1.92 and estimate the heritability accounts for $<10\%$,[6,7,37] these results provide new insight and several important biological pathways for CAD. In this review, we focus on genetic association results for CAD and their biological role for the pathogenesis.

THE FIRST GWAS IN A JAPANESE POPULATION

In 2000, we started a GWAS with 94 MI patients[13,39,40] and 658 controls in Japanese population using a high-throughput multiplex PCR-invader assay method[12] with ~100 000 gene-based SNPs[11] as a first step in comprehensive association study. To our knowledge, this is the first GWAS with SNP identified a disease susceptible gene in the worldwide. Through this GWAS, one SNP in the *LTA*, encoding an inflammatory cytokine lymphotoxin-α, on chromosome 6p21.3 was identified as a candidate susceptibility locus for MI in Japanese

[1]Laboratory for Cardiovascular Diseases, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [2]Department of Human Genetics and Disease Diversity, Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences, Tokyo, Japan and [3]Bioresource Research Center, Tokyo Medical and Dental University, Tokyo, Japan
Correspondence: Dr K Ozaki, Laboratory for Cardiovascular Diseases, RIKEN Center for Integrative Medical Sciences, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama 230-0045, Japan.
E-mail: ozakikk@riken.jp
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population. Following linkage disequilibrium, haplotype mapping and further functional analyses revealed that two functional SNPs (rs909253; *LTA* intron 1 252 A>G and rs1041981; exon 3 804C>A) were in complete linkage disequilibrium in this locus and conferred risk of MI. Among white Europeans (in the Precocious Coronary Artery Disease (PROCARDIS) study), a transmission disequilibrium test analysis of 447 trio families with CAD demonstrated that the *LTA* 804C allele (26 N-LTA) was excessively transmitted to affected offspring ($\chi^2 = 8.44$, $P = 0.002$, recessive association model).[41]

LGALS2, ENCODING GALECTIN-2 THAT INTERACT WITH LTA, AND MI

After identifying *LTA* as a novel genetic risk factor for MI, we searched for proteins that interact with LTA to better understand its role in the pathogenesis of this disease.[16,39,40] Using both the *Escherichia coli* two-hybrid system and a phage display method, we identified a protein, galectin-2, as a binding partner of LTA. Because galectin-2 was shown to bind to LTA, we examined whether variations on *LGALS2* (encoding galectin-2) were also associated with susceptibility to MI, and found one SNP (rs7291467; 3279C>T) in intron 1 of *LGALS2*; this substitution represses the level of galectin-2 expression and shows a significant association with MI. Resent meta-analysis also demonstrated the association between rs7291467 SNP and MI.[42] This genetic substitution seemed to affect the transcriptional level of galectin-2, which led to altered secretion of LTA, thereby affecting the degree of inflammation. We also found that galectin-2 binds to tubulins, which are important components of microtubules, suggesting a role in intracellular trafficking (Figure 1). It is likely that LTA is another molecule that uses the microtubule cytoskeleton network for translocation, and galectin-2 mediates LTA trafficking through binding to microtubules although the precise role of galectin-2 in this trafficking machinery complex has yet to be elucidated.

PSMA6, ENCODING AN INTERCELLULAR LTA SIGNALING MOLECULE, AND MI

Because interaction of LTA with its receptor strongly activates nuclear[17] factor κB (NFκB) by proteasomal degradation of its inhibitory partner, I kappa B (IκB) protein,[43] we hypothesized that the variation(s) in the genes encoding proteasomal proteins could confer risk of MI. The 20S proteasome, which is composed of 7 α- and 10 β-subunits, is the core particle for 26S proteasome system.[44] We performed association study using selected representative SNPs (tagSNP) and found that one SNP (rs1048990) in the 5′-untranslated region (UTR) of exon 1 (5′UTR –8C>G) of *PSMA6*, encoding proteasome subunit, alpha type, 6 was significantly associated with MI. This association was robustly replicated with nearly same effect size to Japanese in a large Chinese cohort and a meta-analysis.[45] The SNP, located within 5′UTR of exon 1 in this gene, enhanced the transcriptional level of *PSMA6.* Moreover, suppression of *PSMA6* expression level using siRNA in cultured coronary vascular endothelial cells as well as T-lymphocyte cell line reduced activation of NFκB, a central mediator of inflammation,[46] by stabilizing phosphorylated IκB. Thus, the levels of PSMA6 protein influence the degree of inflammation, indicating that the *PSMA6* variant is a genetic risk factor for MI in Asian population.

BRAP, ENCODING A GALECTIN-2-BINDING PROTEIN, AND MI

To facilitate understanding the molecular pathways that underlies the risk of MI, we systematically searched binding partners for galectin-2 and identified BRAP, BRCA1-associated protein, as a binding partner of galectin-2. Resulting we found the strong association for two SNPs, rs3782886, in exon 5 (90 A>G, R241R), and rs11066001, of *BRAP* with increased risk of MI ($P < 10^{-20}$, odds ratio = ~1.5).[18] These associations were successfully replicated with both additional Japanese and Taiwanese cohorts. Interestingly, this allele was observed in neither CEPH (Centre d'Etude du Polymorphisme Humain) individuals nor Yoruba individuals (http://www.hapmap.org),[19] indicating that these SNPs are likely to be present only in Asian populations. No association of the SNP and conventional risk factors including age was

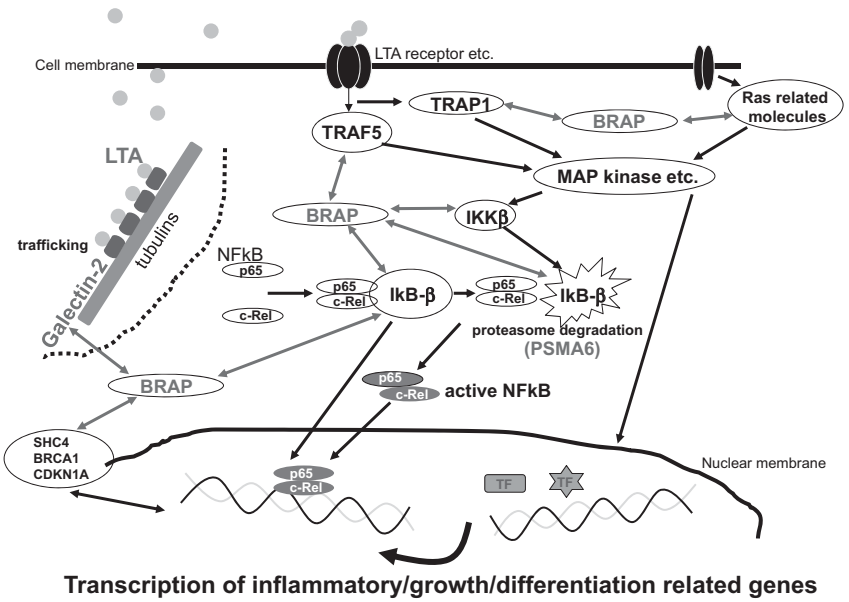

Figure 1 Possible BRAP-inflammatory cascade for MI pathogenesis. Red arrows indicate direct interaction for BRAP. MI, myocardial infarction; TF, transcription factor. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

observed, indicating significant SNP in *BRAP* is an independent risk factor of MI.

The two SNPs in *BRAP* showing very strong associations did not cause amino-acid substitutions, thus we examined whether these SNPs would affect *BRAP* expression by luciferase assay. The intron3 270 A nonrisk allele showed approximately half of the transcriptional activity of the 270G risk allele, which could be explained less binding of potential transcriptional repressor to the risk allele observed in gel-shift experiments, and thus have a role in the pathology of MI.

BRAP and galectin-2 proteins colocalized in the cytoplasm and nucleus in cultured human coronary artery smooth muscle cells and in the smooth muscle cells and macrophages in atherosclerotic plaques and observed in the majority of polymorphic smooth muscle cells and activated macrophages. BRAP was initially identified as a binding partner of BRCA1 through interaction of its signal peptide,[47] and is also known to be an E3 ubiquitin ligase that associates with Ras and MAP kinase signaling through regulation of the scaffolding activity of kinase suppressor of ras.[48] The MAP kinase signaling has critical physiological function implicating cell survival regulation, growth, differentiation, transformation and production of proinflammatory factors.[49,50] Galectin-2 also binds lymphotoxin-α and is implicated in the inflammation (Figure 1). Knocking down experiment for *BRAP* showed an inhibition of NFκB activation in human coronary artery endothelial cells,[19] indicating that expression changes of *BRAP* affect the expression levels of the NFκB-dependent inflammatory molecules. Furthermore, we found that the BRAP protein interacts several molecules related to inflammation and cell proliferation, such as major components of I kappa kinase (IKK)-signalosome (Figure 1).[51] Taken together, these results implicated that a higher *BRAP* expression level from risk allele may enhance the degree of inflammation through activation of NFκB–I kappa kinase (IKK)-signalosome proteins, thereby playing a critical role in the pathogenesis of MI. In Figure 1, we show possible implication of BRAP cascade and inflammatory molecules in the pathogenesis of MI. Further investigation of BRAP-inflammatory pathway will provide useful information for the development of novel therapy using pharmaceutical approaches.

FIFTY ONE CAD LOCI IDENTIFIED BY LARGE-SCALE GWAS

The significant progress of genotyping platform for large samples and its statistical methods (for example, imputation and haplotype tagging) allows more comprehensive genetic approach for common diseases. Table 1 shows 51 identified loci with genome-wide significance ($P<5\times10^{-8}$) by large-scale GWASs mainly in Western countries. The effect size for each variant, which estimated with odds ratios, are very small, and 'missing heritability' still lies similar to other common diseases.[52] In the 51 CAD loci, only 15 loci associated with the conventional risk factors (Table 1; 12 with lipid traits and 3 with blood pressure), indicating that the considerable unknown mechanisms underlie the CAD pathogenesis, which remain to be clarified.

In 2007, several GWAS with several thousands of samples and a large number of SNP (~1 000 000) discovered the association of variants on chromosome 9p21.3 and CAD[20–22] that was robustly replicated in other race[33,36,38,53–57] excluding African ancestry[22,58,59] so far.[60] The 9p21.3 risk ratio increase at early age of CAD onset with small effect,[61] but seems to be an independent of other conventional risk factors for CAD.[7,60] This locus also associated with the risk of other diseases includes type 2 diabetes,[20,62–64] abdominal aortic and intracranial aneurysms,[65] dementia, Alzheimer's disease,[66] clinical/subclinical phenotype for CAD[67–70] and cancers,[71,72] suggesting pleiotropic effect of associations for the locus with the disease phenotypes. The 9p21.3 risk variants for CAD located in 3′ region of *CDKN2B-AS1*, a long noncoding RNA, near the genes *CDKN2A* and *B*, encoding cyclin-dependent kinase inhibitor proteins. Functional analyses of 9p21.3 revealed that the higher mRNA expression of *CDKN2B-AS1* was associated with the CAD risk allele of 9p21.3; however, expression of *CDKN2A/B* mRNA was inversely associated.[73–75] An expression quantitative trait locus (eQTL) analysis revealed a statistical association between *CDKN2B* expression and the 9p21.3 SNP in adipose tissue.[31,76] By means of putative enhancer identification for the 9p21.3 CAD locus and subsequent chromatin conformation capture to detect long-range chromosome interaction, Harismendy *et al.*[77] revealed that the enhancer interval physically interacts with the gene loci *CDKN2A/B, MTAP* and further interval downstream of *IFNA21*, encoding interferon, alpha 21, in vascular endothelial cells. However, other studies with several cells including aortic smooth muscle and endothelial cells to follow-up the above findings do not support interferon-mediated inflammatory effect for 9p21.3 variant[78,79] indicating unknown mechanisms for the 9p21.3 risk variant remain to be still elucidated.

In the GWAS between CAD patients with MI and those without MI, Reilly *et al.*[30] identified significant evidence for a protective role to several SNPs tagging the O allele in ABO blood group at chromosome 9p34.2 with MI. This locus was replicated in a Japanese population with MI[38] but not in those with CAD.[36] *ABO* encodes proteins (transferase A, alpha 1-3-*N*-acetylgalactosaminyltransferase; transferase B, alpha 1-3-galactosyltransferase) that transfers carbohydrate to von Willebrand factor (vWF), implicated to the blood group system. The O allele encodes a protein without any enzyme activity by a deletion of guanine-258 near the *N*-terminus of the protein and thus does not modify the vWF structure, which thought to facilitate the proteolysis of vWF and resulting lower circulating vWF and factor VIII. ABO blood group was also associated with low-density lipoprotein (LDL) cholesterol, type 2 diabetes and inflammatory adhesion molecules and angiotensin-converting enzyme activity.[80–84] Together, these results indicated that ABO proteins may have multiple functions related to thrombosis and/or plaque rupture that confer risk of MI. Although the clarification of detailed mechanism implicated to MI for ABO and clinical studies are needed, individuals with blood group A, B or AB might receive possible therapies such as treatment of antiplatelet agent in the future.[60,85]

In the molecules identified GWAS discovery, *PCSK9* encoding a calcium-dependent serine endoprotease belong to proprotein convertase subtilisin/kexin (PCSK) enzyme family that cleaves latent precursor proteins into biologically active products. PCSK9 is a molecule initially identified gain of function mutations in the gene for two families with hypercholesterolemia,[86] and is a therapeutic target to reduce LDL cholesterol that clinical trials are currently under investigation.[87–91] PCSK9 protein interacts with hepatic LDL cholesterol receptor and inactivate the receptor by its degradation.[92,93] Recent phase 3 clinical studies demonstrated that a fully human monoclonal antibody against the PCSK9, which inhibits interaction with the LDL receptor, dramatically reduce circulating LDL cholesterol level in humans without significant side effect.[89–91] Another molecule identified GWAS, *FURIN*, also encoding a member of PCSK family, and highly expressed in human atherosclerotic plaques,[94] suggesting FURIN protein might also be a drug target for CAD/atherosclerosis, although further functional significance implicated to CAD/atherosclerosis remains to be elucidated. These are good examples that the genetic risk factors significantly contribute to the disease

pathogenesis and give potential druggable molecules for the treatment of CAD, suggesting potent power of GWAS that provides novel unexpected insight and knowledge for the future evidence-based medicine.

In the 51 loci, 6 were identified by GWAS with Asian population. We have identified susceptible loci for MI near the genes *IRX1*, *BRAP-ALDH2*, *PLCL2* and *AP3D1-DOT1L-SF3A2* on chromosome 5p15.3, 3q24.3, 12q24 and 19p13.3, respectively, in a Japanese

Table 1 Loci associated to the risk of CAD with genome-wide significance identified by large-scale GWAS

SNP ID	*Chromosome*	*Nearest gene*	*Possible CAD-related function*	*Risk/nonrisk allele*	*RAF*	*OR*	*Ref.*	*Year*
rs599839[a]	1p13.3	*SORT1*	LDL metabolism	A/G	0.77	1.29	23	2007
rs11206510[a]	1p32.3	*PCSK9*	LDL metabolism	T/C	0.81	1.15	25	2009
rs17465637	1q41	*MIA3*	Inhibition of inflammatory cell proliferation	C/A	0.71	1.2	23	2007
rs17114036	1p32.2	*PPAP2B*	Lipid synthesis	A/G	0.91	1.17	31	2011
rs4845625	1q21	*IL6R*	Inflammation	T/C	0.47	1.06	37	2013
rs6725887	2q33.1	*WDR12*	—	C/T	0.14	1.17	37	2009
rs515135[a]	2p24-p23	*APOB*	Cholesterol metabolism	G/A	0.83	1.07	37	2013
rs2252641	2q22.3	*ZEB2-AC074093.1*	—	G/A	0.46	1.06	37	2013
rs1561198	2p11.2	*VAMP5-VAMP8-GGCX*	—	A/G	0.45	1.06	37	2013
rs6544713[a]	2p21	*ABCG5-ABCG8*	Cholesterol metabolism	T/C	0.3	1.06	37	2013
rs9818870	3q22.3	*MRAS*	Cell proliferation, adhesion	T/C	0.15	1.15	24	2009
rs4618210	3p24.3	*PLCL2*	Inflammation	G/A	0.42	1.1	38	2014
rs273909[b]	4q31.1-q31.2	*GUCYIA3*	Cell differentiation, chemotaxis	G/A	0.81	1.08	37	2013
rs1878406	4q31.22	*EDNRA*	Vasoconstriction, inflammation	T/C	0.15	1.1	37	2013
rs11748327	5p15.3	*IRX1*	—	C/T	0.76	1.25	33	2011
rs273909	5q31.1	*SLC22A4-SLC22A5*	—	C/T	0.14	1.07	37	2013
rs12526453	6p24	*PHACTR1*	—	C/G	0.65	1.12	25	2009
rs3798220[a]	6q25.3	*LPA*	Lipid metabolism	C/T	0.02	1.92	27,28	2009
rs17609940[a]	6p21.31	*ANKS1A*	—	G/C	0.75	1.07	31	2011
rs12190287	6q23.2	*TCF21*	—	C/G	0.62	1.08	31	2011
rs6903956	6p24.1	*C6orf105*	—	A/G	0.07	1.65	32	2011
rs6929846	6p22.1	*BTN2A1*	—	T/C	0.06	1.51	34	2011
rs10947789	6p21	*KCNK5*	—	T/C	0.76	1.07	37	2013
rs4252120	6q26	*PLG*	Inflammation	T/C	0.73	1.07	37	2013
rs10953541	7q22.3	*BCAP29*	—	C/T	0.8	1.07	29	2011
rs11556924	7q32.2	*ZC3HC1*	—	C/T	0.62	1.09	31	2011
rs2023938	7p21.1	*HDAC9*	Hematopoiesis	G/A	0.1	1.08	37	2013
rs264[a]	8p22	*LPL*	Lipid synthesis	G/A	0.86	1.11	37	2013
rs2954029[a]	8q24.13	*TRIB1*	Lipid metabolism	A/T	0.55	1.06	37	2013
rs1333049	9p21.3	*CDKN2A, B/ANRIL/IFNW1/IFNA21*	Cell proliferation, inflammation	C/G	0.47	1.47	20–22	2007
rs579459[a]	9q34.2	*ABO*	Thrombogenesis	C/T	0.21	1.33	30,31	2011
rs501120	10q11.1	*CXCL12*	Inflammation, lipid metabolism	T/C	0.87	1.17	25	2009
rs1412444	10q23.2-q23.3	*LIPA*	Lipid related	T/C	0.42	1.09	29	2011
rs2505083	10p11.23	*KIAA1462*	Endothelial cell function	C/T	0.38	1.07	29	2011
rs12413409[b]	10q24.32	*CYP17A1, CNNM2, NT5C2*	Lipid synthesis	G/A	0.89	1.12	31	2011
rs974819	11q22.3	*PDGFD*	Inflammation, lipid synthesis	T/C	0.32	1.07	29	2011
rs964184[a]	11q23.3	*ZNF259, APOA5-A4-C3-A1*	LDL metabolism	G/C	0.13	1.13	31	2011
rs3184504[a,b]	12q24	*SH2B3*	—	T/C	0.38	1.13	26	2009
rs671	12q24	*BRAP-ALDH2*	Inflammation	A/G	0.28	1.43	33,36,38	2012
rs4773144	13q34	*COL4A1, COL4A2*	Plaque destabilization	G/A	0.44	1.07	31	2011
rs9319428	13q12	*FLT1*	Angiogenesis, inflammation	A/G	0.32	1.06	36	2013
rs2895811	14q32.2	*HHIPL1*	—	C/T	0.43	1.07	31	2011
rs3825807	15q25.1	*ADAMTS7*	Smooth muscle cell activation	A/G	0.57	1.19	29–31	2011
rs17514846[b]	15q26.1	*FURIN-FES*	Cholesterol metabolism	A/C	0.44	1.07	37	2013
rs216172	17p13.3	*SMG6, SRR*	—	C/G	0.37	1.07	31	2011
rs12936587	17p11.2	*RASD1, SMCR3, PEMT*	—	G/A	0.56	1.07	31	2011
rs46522	17q21.32	*UBE2Z, GIP, ATP5G1, SNF8*	Insulin resistance (*GIP*)	T/C	0.53	1.06	31	2011
rs1122608[a]	19p13	*LDLR*	LDL metabolism	G/T	0.75	1.15	25	2009
rs3803915	19p13.3	*AP3D1-DOT1L-SF3A2*	—	C/A	0.19	1.12	38	2014
rs2075650[a]	19p13.32	*APOE-APOC1*	LDL metabolism	G/A	0.14	1.14	35	2011
rs9982601	21q22	*SLC5A3-MRPS6-KCNE2*	—	T/C	0.13	1.2	25	2009

Abbreviations: CAD, coronary artery disease; GWAS, genome-wide association study; ID, identifier; LDL, low-density lipoprotein; OR, odds ratio; RAF, risk allele frequency; Ref.; references; SNP, single-nucleotide polymorphism; —, unknown function for CAD.
[a]Associated with lipid traits.
[b]Associated with blood pressure.

population.[33,38] In other Japanese GWAS, Yamada *et al.*[34] identified a functional SNP in *BTN2A1* on chromosome 6p22.1 and Takeuchi *et al.*[36] reported a locus at *BRAP-ALDH2* as CAD risk with GWAS significance. In Han Chinese population, Wang *et al.*[32] identified a locus at 6p24.1 significantly associated with the increase risk of CAD by GWAS. However, these loci were failed the association in the Caucasian GWASs. This ethnic difference may be explained by the variance among ethnicity in allelic frequencies and sample numbers, which influences the study power and also the risk ratio. Others might include the ethnic divergence in the accurate linkage disequilibrium distribution, possibility of undiscovered hidden variations for European decent, leaving open the question of the disease association in other ethnicities for these loci.

The broad putative functions of near genes for the CAD loci are listed in Table 1. These functions can roughly be divided into three groups implicating inflammation, lipid metabolism and unknown function. Recent large-scale association study by the CARDIoGRAMplusC4D Consortium identifies 15 novel loci for CAD.[37] They also conducted network analysis using 233 genes mapped in the Ingenuity Knowledge Base (INGENUITY, Redwood City, CA, USA) from the top 222 SNPs defined by the false discovery rate (FDR) analysis in their GWAS.[37] They resulting identified the four most canonical pathways, atherosclerosis signaling, liver/retinoid X receptor activation, farnesoid/retinoid X receptor activation and acute phase response signaling, consisting of molecules related to lipid metabolism and inflammation[37] genetically involved in the CAD pathogenesis. The detailed functional role for these molecules/pathways implicated atherosclerosis, thrombosis and plaque rupture for CAD remains yet to be clarified by numerous experiments; these data provide useful information of the novel molecular targets for further biological and pharmacological investigation.

IDENTIFICATION OF RARE VARIANTS FOR CAD BY NEXT-GENERATION SEQUENCER

Recent great advances of high-throughput DNA sequencing technologies, called next-generation sequencer, and its informatics tools to analyze large sequence data set permit comprehensive search for rare pathogenic variants (mutations) in whole genome or protein-coding region of genome (exome) with large individuals. For CAD, the Exome Sequencing Project performed an exome sequencing of 18 666 genes in about 4000 individuals from European and African ancestry and conducted an association for the rare variants with plasma triglyceride levels. They identified several loss-of-function mutations in *APOC3*, encoding apolipoprotein C. The mutation carriers were 39% lower in triglyceride levels than those of noncarriers and were 40% lower in the risk of CAD than those of noncarriers.[95] Another exome sequencing with ~5000 subjects of each early-onset MI and control identified the rare coding variants in two genes, *APOA5* and *LDLR*, respectively, that are associated with the increased risk of MI at exome-wide significance.[96] The increased risk ratios for MI in these carriers were at 4.2-fold for *LDLR* and 2.2-fold for *APOA5*, respectively. Other exome study of large MI family showed that the dysfunctional mutations in nitric oxide signaling genes, *GUCY1A3* and *CCT7*, conferred increased risk of MI.[97] *In vitro* and *in vivo* functional analyses suggested accelerated thrombosis formation by impaired nitric oxide signaling through reducing expression and enzymatic activity of the mutated encoding proteins. Although contribution of these variants in the CAD heritability is relatively small ($< \sim 1\%$), these results will provide new insights into early detection of asymptomatic patients and/or biological, physiological and pharmaceutical search for novel medicine of CAD.

CONCLUSION

Our initial hypothesis-free GWAS ultimately led to identification of a possible MI pathology by mediating an inflammatory cascade includes IKK-signalosome and BRAP encoded by gene that robustly associated with the increased risk of MI in Asian population. Resent pathway analysis by CARDIoGRAMplusC4D Consortium also suggests that the inflammatory cascade including NFκB signaling has potential pivotal role in the CAD pathogenesis.[37] Possibly, a common final pathway that emerges as inflammation is present in the pathogenesis. These findings came from hypothesis-free, genome-wide, large-scale studies, indicating the potent power of such studies to identify a pathogenetic anchor of common diseases.

Numerous GWAS identified considerable numbers of unexpected genes for CAD susceptibility and provided novel clues in the future preventive medicine for the genetic tests and novel therapeutic targets for CAD. However, each genetic factor has only small effect size, and the combination of all variants does not explain much of the heritability estimated the previous epidemiological studies. Several exome-sequencing studies for CAD discovered important genetic variants and provided variable information in the field, however missing parts of heritability are not still filled. Although possible contribution of another type of heritability, called epigenetics, cannot be excluded, it is reasonable to assume that the additional rare genetic variants with relatively large effect for CAD are likely to reside in promoter and enhancer elements including histone-modification regions, DNase hypersensitivity and methylation sites that regulate the gene transcription. Genomic sequencing as target for these regulatory elements with large individuals and appropriate informatics tools will clarify this issue in the near future. Most of the genetic variants identified through GWAS are independent of conventional risk factors and do not explain easily its molecular function mediating the susceptibility, thus unraveling the underling molecular basis of CAD by these genetic factors will be focused on the next era.

CAD attributable to atherosclerosis is a leading cause of death in many countries. Although further investigations are needed to assess the potential clinical utility of current SNP loci owing to their modest effect sizes, we believe that the knowledge of genetic factors contributing to its pathogenesis provides a useful clue for development of diagnostic methods, treatments and preventive measures through combinations of genetic risk variants and clinical information (for diagnostic methods) and clarification of the molecular mechanism in the pathogenesis of causative genes (for therapeutics) for this common but serious disorder.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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