Advances in the Genetic Basis of Coronary Artery Disease

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Current Atherosclerosis Reports 2005, **7:**235–241
Current Science Inc. ISSN 1523-3804

Exciting advances have been made recently in genetic studies of coronary artery disease (CAD), myocardial infarction (MI), and ischemic stroke. One disease-causing gene for CAD and MI has been identified as *MEF2A*, which is located on chromosome 15q26.3 and encodes a transcriptional factor with a high level of expression in coronary endothelium. Approximately 1% to 2% of CAD patients may carry an *MEF2A* mutation. Four new susceptibility genes have been identified using genome-wide association studies or genome-wide linkage studies: *LTA* (encoding cytokine lymphotoxin-α) on 6p21.3 for MI; *LGALS2* (encoding galectin-2, an LTA-interacting protein) on 22q12-q13 for MI; *ALOX5AP* (encoding 5-lipoxygenase activating protein involved in synthesizing potent proinflammatory leukotrienes) on 13q12-13 for MI and stroke; and *PDE4D* (encoding phosphodiesterase 4D) on 5q12 for ischemic stroke. These studies identify a new mechanism, the myocyte enhancer factor 2 (MEF2) signaling pathway of vascular endothelium, for the pathogenesis of CAD, and also confirm the role of inflammation in the disease process.

Introduction

Coronary artery disease (CAD) is the leading cause of death in developed countries. The prevalence of CAD is rising rapidly in developing countries due to increased exposure to CAD risk factors, which may lead to an expanding epidemic [1]. CAD is the most common heart disease that is believed to be caused by multiple genetic factors, environmental factors, and interactions among these factors. Identification of these genetic and environmental factors will provide valuable information for prevention and control of CAD. Significant advances have been made recently in identifying disease-causing genes and susceptibility genes for CAD, myocardial infarction (MI), and ischemic stroke. This review highlights the most important discoveries made in the past 2 years.

Genetic Loci for Coronary Artery Disease and Myocardial Infarction

Two different types of linkage analyses have been used to map the chromosomal locations of genes for CAD and MI [2]. In linkage analysis, the goal is to identify at least one polymorphic marker at a specific chromosomal location that co-inherits with the disease, which then suggests that the marker and disease gene are located close to each other, and the location of the marker is taken as the location of the disease gene. The first type of linkage analysis is model-based linkage analysis using large families in which the inheritance pattern of the disease gene in the families is clearly defined. The family or families are generally genotyped with approximately 400 polymorphic markers that span the entire human genome every 10 cM. The genotyping data is then analyzed with a model-based linkage program, yielding a logarithm of the odds (LOD) score for each marker. The LOD score represents the log base 10 of the likelihood ratio favoring linkage. An LOD score of 3.0 or higher for a marker that co-segregates with the disease in a family or families is considered as significant evidence for identification of linkage. We recently studied a large family with 13 patients with CAD (nine of them are also affected with MI) [3••]. The inheritance pattern of CAD and MI in the family is autosomal dominant. Genome-wide genotyping and model-based linkage analysis identified significant linkage of CAD and MI with a polymorphic marker D15S120 on chromosome 15q26.3 with a LOD score of 4.19. The results defined the first genetic locus for autosomal dominant CAD and MI (*adCAD1*) onto chromosome 15q26.3 (Fig. 1A) [3••]. We also studied several other large families with CAD and MI with an autosomal dominant–inheritance pattern and found that they are not linked to *adCAD1*, suggesting that there are other new genetic loci for CAD and MI. Future studies will identify chromosomal locations of other autosomal dominant CAD and MI genes (*adCAD2* and others).

The second type of linkage analysis is model-free analysis using hundreds of small nuclear families with

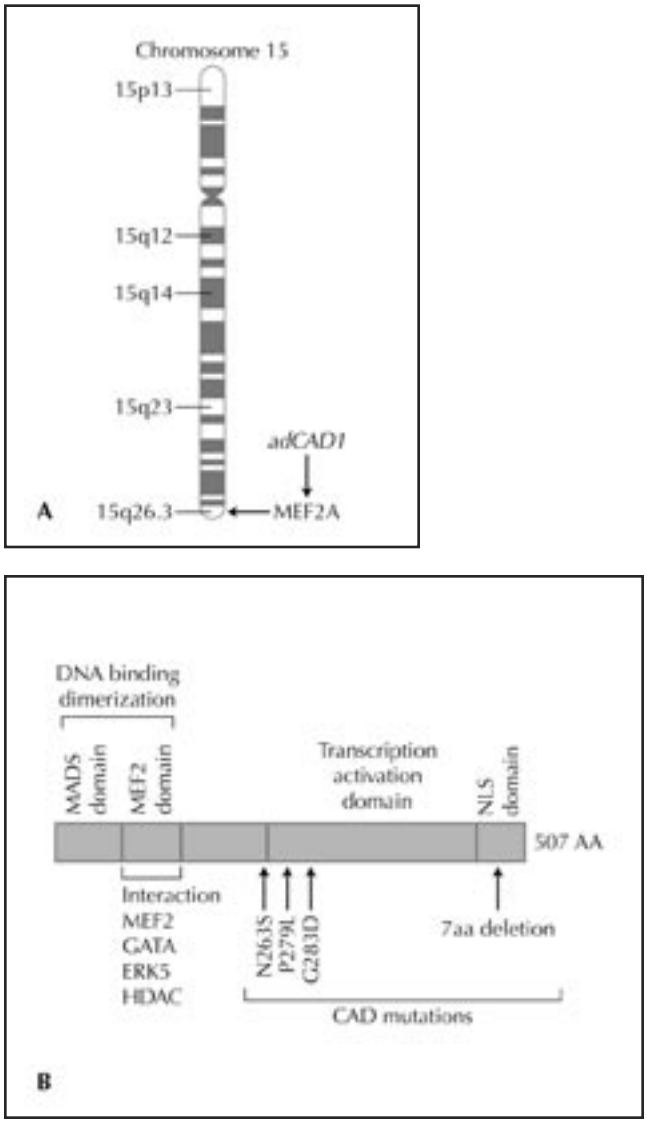

Figure 1. Identification of the first gene, *MEF2A*, for autosomal dominant coronary artery disease (CAD) and myocardial infarction (MI) on chromosome 15q26.3 (*adCAD1*). The locations of mutations that cause CAD and MI are shown. **A,** Ideogram of chromosome 15 showing the location of *adCAD1* and MEF2A. **B,** Domain structure of transcriptional factor MEF2A. (MADS—MCM1, agamous, deficiens, serum response factor; NLS—nuclear localization site.) (*Adapted from* Wang *et al.* [3••] and Bhagavatula *et al.* [16••]; with permission.)

at least two affected siblings in each family. Similar to model-based linkage analysis, genome-wide genotyping is usually performed with about 400 markers providing genome coverage. The amount of marker-allele sharing observed in the sibling pairs with that which would be expected if no linkage is present is then compared. A significant excess of shared alleles by concordant siblings, and a significant dearth of sharing by discordant siblings, is considered as evidence for linkage. *P* values or LOD scores are calculated without assumption of any inheritance model, and an LOD score of 3.6 or a *P* value of 2.2×10^{-5} indicates significant linkage [4]. Two susceptibility loci with significant linkage have been identified for MI on chromosomes 1p34-36 [5•] and 14q [6•], and one susceptibility locus with suggestive linkage was identified for MI on 13q12-13 (Table 1) [7••]. For CAD, four susceptibility loci with significant linkage have been mapped to chromosomes 2q21.1-22 [8•], 3q13 [9•], 16p13-pter [10•], and Xq23-26 [8•], and one locus with suggestive linkage was mapped to 2q36-37.3 [11•]. Similar studies were carried out for stroke, and one susceptibility locus was mapped on chromosome 5q12 (Table 1) [12••]. Many more genetic loci remain to be identified for CAD, MI, and stroke in the future.

Genes Identified for Coronary Artery Disease and Myocardial Infarction

When a genetic locus is mapped, candidate genes that are located in the region and potentially relevant to the disease physiology are selected for identification of pathogenic mutations that cause the disease (disease-causing gene, monogenic trait) or for identification of single nucleotide polymorphisms (SNPs) and SNP haplotypes that are associated with the disease (susceptibility gene, complex trait). We can define disease-causing genes as the genes that are directly responsible for the pathogenesis of disease when mutated. The criteria for finding a disease-causing gene are 1) identification of a mutation that co-segregates with all affected members, but not with normal members in the family used for linkage analysis; 2) identification of other mutations in the same gene in other patients or families; 3) absence of the mutations in 100 to 200 normal control subjects; and 4) identification of functional effects of the mutations on the gene. The examples of disease-causing genes include potassium channel genes *KCNQ1* and *KCNH2* for long QT syndrome [13,14], and cardiac sodium channel gene *SCN5A* for Brugada syndrome [15]. Identification of a disease-causing gene makes genetic testing immediately available for some cases of the disease.

The gene at the *adCAD1* locus on chromosome 15q26.3 has been identified as *MEF2A* encoding a transcriptional factor. A 21-base pair (bp) deletion in exon 11 of *MEF2A* was identified in all 13 patients, but not in normal individuals in the original family used for mapping the *adCAD1* locus. The deletion was not present in normal family members and 119 normal control subjects [3••]. The 21-bp deletion blocked nuclear localization of the MEF2A protein and markedly reduced transcriptional activation activity of MEF2A [3]. The mutant MEF2A can suppress the activity of wild-type MEF2A [3••], suggesting that the 21-bp deletion disrupts the transcriptional activation of MEF2A by a dominant-negative mechanism. Recently, the author's team [16••] identified three new mutations in exon 7 of *MEF2A* in four of 207 independent CAD/MI patients (1.93%). However, we did not identify

Table 1. Susceptibility loci for CAD and MI identified by model-free linkage analysis with sibling pairs

Chromosomal location	Trait	Gene	LOD score (*P* value)	Multiplex families, *n*	Ethnic group
1p34-36	MI	?	11.98 (< 10^{-12})	428	White (USA)
13q12-13	MI + stroke (women)	ALOX5AP	2.86	296	White (Iceland)
14q	MI	?	3.9 (0.00015)	513	White (Germany)
2q21.1-22	CAD	?	3	156	White (Finland)
2q36-37	ACS	?	2.63	61	White (Australia)
3q13	CAD	?	3.3	438	White (USA)
16p13-pter	CAD	?	3.06 (0.00017)	99	Indo-Mauritians (India)
Xq23-26	CAD	?	3.5	156	White (Finland)
5q12	Stroke (ischemic)	PDE4D	4.4 (3.9 x 10^{-6})	179	White (Iceland)

ACS—acute coronary syndrome; CAD—coronary artery disease; LOD—logarithm of the odds; MI—myocardial infarction.

these three mutations or other mutations in 191 control subjects with normal angiograms [16••]. The three new mutations disrupt transcriptional activation of MEF2A by a loss-of-function mechanism. Interestingly, patients with the 21-bp deletion appear to be much more severe with a higher incidence of MI than the carriers with the three loss-of-function mutations. These results establish *MEF2A* as the first disease-causing gene for CAD and MI, and suggest that nearly 2% of the CAD/MI population may carry an *MEF2A* mutation. A genetic testing kit is expected to be available for CAD/MI patients soon, which will identify high-risk individuals who may delay or prevent onset of MI by aggressive lifestyle modifications and/or pharmacologic therapies.

We define susceptibility genes as the genes that increase or decrease the risk of development of disease. The criteria for identification of a susceptibility gene include 1) identification of an SNP (not necessarily nonsynonymous) or an SNP haplotype that shows a significantly higher frequency (increased susceptibility, increased risk) or a lower frequency (decreased susceptibility, protective effect against the disease) in a population of patients than in a population of matched, normal control subjects (designated as a population-based, case-control association study); 2) replication of the earlier association in an independent population or by identification of one of alleles of the SNP or a specific SNP haplotype that is preferentially transmitted to affected individuals in family-based association studies using transmission disequilibrium test analysis; and 3) demonstration of functional effects of the SNP or SNP haplotype on the gene. Susceptibility genes have a predictive value for a population of patients, but have no predictive or diagnostic values for individual patients. Helgadottir *et al*. [7••] studied *ALOX5AP* encoding 5-lipoxygenase activating protein (FLAP) involved in inflammation as a candidate gene at the suggestive MI locus on chromosome 13q12-13 (Table 1). A case-control association study with 779 MI patients and 624 control subjects in Iceland identified a haplotype (HapA) involving four SNPs in *ALOX5AP* that was significantly associated with MI and stroke [7••]. In a British population, a different haplotype (HapB) was significantly associated with MI and stroke [7••]. In a separate study with 450 patients with ischemic stroke and 710 control subjects from Aberdeenshire, Scotland, HapA was significantly associated with ischemic stroke, but no association was detected for HapB [17]. It is important to point out that the original linkage at 13q12-13 was identified for female patients and not for male patients or male and female patients together, but the association of *ALOX5AP* variants with MI and stroke disappeared if the genotyping data were analyzed separately for female patients only [7••]. This suggests that there may be another gene that increases susceptibility to MI in female patients under the 13q12-13 linkage peak.

The *PDE4D* gene encoding phosphodiesterase 4D was suggested to be the susceptibility gene for stroke at the 5q12 locus [18••]. Different haplotypes of *PDE4D* were found to be significantly associated with combined carotid and cardiogenic strokes (the forms of stroke related to atherosclerosis or ischemic stroke). Phosphodiesterase 4D can degrade the second messenger cyclic AMP (cAMP), a key signaling molecule involved in inflammatory responses of vascular cells to oxidized lipids [18••]. The association between PDE4D and ischemic stroke will need to be validated in an independent population.

In addition to genome-wide linkage studies, many susceptibility genes for atherosclerosis, CAD, and MI have been identified using candidate gene–association studies. Simply, a gene with potential involvement in CAD and MI was selected and tested for its association with CAD or MI in a population-based, case-control design. Numerous possible associations have been identified, and examples include the genes for apolipoprotein E (apoE), lipoprotein(a) or apo(a), ApoAI-CIII-AIV, tissue

plasminogen activator , fibrinogen, von Willebrand factor, platelet glycoprotein IIIa, lipoprotein lipase, hepatic lipase, paraoxonase, cholesterol ester hydrolase, factor V, factor VII, angiotensin-converting enzyme, angiotensinogen, thrombospondins 1, 2, and 4, connexin 37, plasminogen activator inhibitor-1, matrix metalloproteinase-3, methylenetetrahydrofolate reductase, inducible NO synthase, and many other genes [19–22]. Some of these associations may turn out to be false-positive associations due to the long-standing problems with case-control association studies, including small sample sizes, selection bias, population admixture, and improper matching between cases and controls.

Susceptibility genes for CAD and MI can also be identified by genome-wide, case-control association studies. Approximately 50,000 to 100,000 SNPs are required to cover the entire human genome, and they can be used to genotype a population of CAD/MI patients and a population of matched control subjects. If the frequency of an SNP allele or genotype is significantly different between the two populations, the SNP is considered to be associated with the risk of CAD and MI (P value of $< 5 \times 10^{-7}$ was proposed to be a cut-off value for achieving significance [23]). Ozaki *et al.* [24••] performed the first genome-wide, case-control association study for MI using 92,788 gene-based SNPs with 94 Japanese patients with MI. Positive SNPs with a nominal significant P value of 0.01 were identified and further studied in 1133 MI cases and 1006 control subjects [24••]. Significant association was identified for three SNPs in the LTA gene (exon 1 10G/A, intron 1 252A/G, exon 3 p.Thr26Asn) ($P = 2.2 \times 10^{-5}$ to 3.3×10^{-6}) [24••]. LTA encodes lymphotoxin-α, a cytokine that mediates immune responses and inflammation. One of the SNPs increased expression of LTA by 1.5-fold (SNP 252A/G in intron 1), and another SNP increased expression of adhesion molecules and cytokines including vascular cellular adhesion molecule-1, intercellular adhesion molecule-1, tumor necrosis factor, interleukin-1A and interleukin-1B, and selectin E, by twofold (p.Thr26Asn in exon 3) [24••]. The results provide very intriguing evidence that LTA variants increase susceptibility to MI. The finding was further supported by a study involving more than 400 parental-proband trios families that showed positive association of SNP p.Thr26Asn with CAD in white Europeans [25]. An independent, case-control association study in a separate Japanese population, however, failed to identify any association between SNP 252A/G in intron 1 or p.Thr26Asn in exon 3 of the *LTA* gene and MI [26]. Negative association was also found with either CAD or MI, or with the risk of restenosis, death, or MI after coronary artery stenting with SNP 252A/G in intron 1 in a German population [27,28]. Many more studies are clearly needed to further test the association of *LTA* variants and MI.

The positive association between *LTA* variants with MI was strongly supported by a recent finding from the same group that an SNP in *LGALS2*, a regulatory gene for *LTA*, is associated with MI [29••]. Ozaki *et al.* [29••] found that the *LGALS2* gene encodes galectin-2, a member of the galactose-binding lectin family, which binds to LTA and regulates the extracellular secretion of LTA. SNP 3279C/T in intron 1 of *LGALS2* was significantly associated with MI ($P = 2.6 \times 10^{-6}$). The minor allele of the SNP has a protective role against MI. The minor allele T reduced expression of *LGALS2* by 50%, which is expected to decrease the extracellular level of LTA, leading to less inflammation and reduced risk to MI. The positive association between *LGALS2* and MI still needs to be replicated in another independent Japanese population or other populations.

MEF2A Signaling Pathway and Coronary Artery Disease and Myocardial Infarction

The myocyte enhancer factor 2 (MEF2) represents the second class of transcriptional factors that regulate expression of many muscle-specific genes. The first class of regulatory proteins for muscle gene expression includes myoD, myogenin, MYF5, and MRF4, and they were identified earlier than MEF2. Four different MEF2 factors, designated as MEF2A to MEF2D, are known and are encoded by different genes. MEF2 was originally identified in 1989 as a protein that binds to a conserved A/T-rich DNA sequence (CTA(A/T)4TAG) within an enhancer upstream of the transcription initiation site of the muscle creatine kinase gene (mck) [30]. The first *MEF2* gene was cloned in 1991 and designated as *MEF2A* [31]. The *MEF2A* gene was also cloned by Yu *et al.* [32] in 1992 and was found to be ubiquitously expressed, with highest levels found in skeletal muscle, heart, and brain. Yu *et al.* [32] also cloned the second member of this family of transcriptional factors, MEF2B. In 1993, the third member, MEF2C, was cloned and found to be expressed mainly in skeletal muscle and brain [33]. The fourth member, MEF2D, was cloned in 1993 [34]. Different from the other three MEF2 factors, MEF2D was expressed in undifferentiated myoblasts.

The four MEF2 transcriptional factors share high homology (about 95% similarity) at the N-terminal MADS (MCM1, Agamous, Deficiens, Serum response factor) domain and MEF2 domain, but the C-terminal regions are more divergent [35]. The MADS domain is required for DNA binding and homo- or heterodimerization (Fig. 1B). The adjacent MEF2 domain influences DNA binding and mediates interactions with transcriptional cofactors, including MyoD, GATAs, NFAT, TH receptor, p300/PCAF, 14-3-3, ERK5, HDACs, MITR, and Cabin (Fig. 1B). The transcriptional activation domain and nuclear localization signals are located at the C-terminal regions of MEF2.

Identification of *MEF2A* mutations in families and patients with CAD and MI clearly links the MEF2

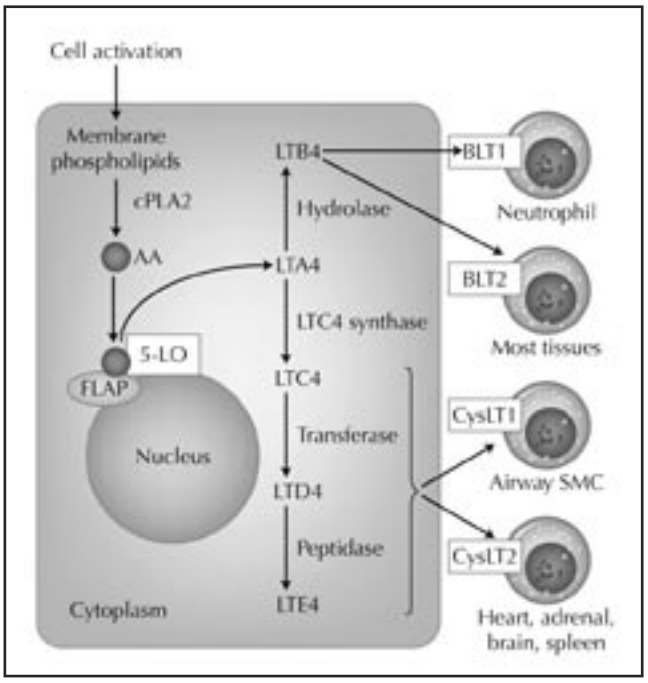

Figure 2. Diagram of the 5-lipoxygenase (5-LO) pathway for biosynthesis of the proinflmamatory leukotrienes (LT) LTA4, LTB4, LTC4, LTD4, and LTE4. (AA—arachidonic acid; BLT1—high-affinity receptor for LTB4; BLT2—low-affinity receptor for LTB4; cPLA2—cytosolic phospholipase A2; CysLT— cysteinyl leukotrienes; FLAP—5-lipoxygenase-activating protein; Hydrolase—LTA4 hydrolase; Transferase—γ-glutamyl transferase; SMC—smooth muscle cell.)

signaling pathway to an important human disease [3••,16••]. Detection of a high level of expression of MEF2A in the endothelium of coronary arteries [3••] suggests that an early step or triggering event in the development of CAD and MI may involve deregulation of specific transcriptional programs in the endothelium, which is expected to cause abnormal development or function of the endothelium. The endothelium is a critical barrier between blood and arteries and plays a protective role against damages of coronary arteries by blood elements such as platelets and monocytes. Defective or malfunctioned endothelium will be more susceptible to inflammation and the formation of an atherosclerotic plaque, which may result in thrombosis, MI, and sudden death [3••].

5-Lipoxygenase Pathway and Coronary Artery Disease and Myocardial Infarction

Lipoxygenases (LOs) are a family of lipid-peroxidizing dioxygenases that play an important role in the biosynthesis of lipids and inflammatory and allergic reactions [36,37]. They incorporate molecular oxygen into unsaturated fatty acids. 5-LO is a nonheme iron enzyme that dioxygenates the fifth location of arachidonic acid (AA), generating leukotrienes (LTs) that are potent inflammatory agents mediating immune responses and tissue homeostasis. The pathway of LT biosynthesis starts with liberation of AA from membrane phospholipids by cytosolic phospholipase A2 and movement of AA to the nuclear membrane, where it binds to 5-LO–activating protein (FLAP) (Fig. 2) [36,37]. FLAP is an 18-kDa membrane protein that interacts with 5-LO and is required for cellular activity of 5-LO. FLAP presents AA to 5-LO to produce 5-hydroperox eicosatetraenoic acid (5-HPETE). 5-HPETE is dehydrated to produce LTA4. LTA4 can be converted into LTB4 by LTA4 hydrolase in neutrophils and monocytes. In eosinophils, mast cells, and basophils, LTA4 is conjugated with reduced glutathione and forms LTC4 by LTC4 synthase, the first of the cysteinyl LTs (CysLTs). LTC4 can be converted into LTD4 and LTE4 by a transferase and peptidase, respectively. LTs bind to their respective receptors on cell surface (G-protein–coupled receptors BLT1 and BLT2 for LTB4; and CysLT1 and CysLT2 receptors for LTC4, LTD4, and LTE4) and facilitate inflammation, immune responses, and host defense against infection [36,37].

The 5-LO pathway has been best known for its association with asthma, but recent evidence suggests that it is also associated with atherosclerosis, CAD, and MI. The first study that implicated the 5-LO pathway in atherosclerotic CAD was identification of the *5-LO* gene as a susceptibility gene for atherosclerosis in mice [38]. A major locus for atherosclerosis resistance in mice was mapped to a chromosome 6 region where the *5-LO* gene is located. Expression of 5-LO was reduced by fivefold in a congenic strain with the atherosclerosis-resistant chromosome 6 region. Deletion of one *5-LO* allele in *5-LO*$^{+/-}$/*LDL*$^{-/-}$ mice decreased lesion size by 95%. These results suggest that decreased *5-LO* expression leads to resistance to atherosclerosis in mice. The second study involved 470 healthy individuals and a polymorphism (tandem Sp1-binding motif GGGCGG) in the promoter of the *5-LO* gene [39]. Carriers with two variant alleles had significantly increased intima-media thickness (a putative indicator of atherosclerosis) when compared with carriers with the common wild-type allele [39]. Paradoxically, the two variant alleles are associated with reduced expression of *5-LO*, which contradicts with the finding from mice that decreased expression of 5-LO was associated with atherosclerosis resistance. Thus, replication of this study in a different population will be important. The third study is the study described in the previous section in which variants of *ALOX5AP* (encoding FLAP) were associated with increased susceptibility to MI and stroke [7••,17].

The implication of the 5-LO or LT pathway in atherosclerosis and MI may have clinical applications. In the study by Dwyer *et al.* [39], dietary intake of marine omega-3 fatty acids was significantly associated with decreased intima-media thickness only in carriers with two variant alleles. In the study by Helgadottir *et al.* [7••] of *ALOX5AP* (FLAP), production of LTB4 by neutrophils

upon stimulation with ionomycin was significantly higher in male carriers with HapA genotype than control subjects. Agents that block LTB production may reduce the risk of MI and stroke in patients with the HapA genotype.

Conclusions

Atherosclerotic CAD has been viewed as a disease of lipid metabolism and inflammation. Recent identification of several new susceptibility genes for atherosclerosis, including *LTA* and *LGALS2* for MI, *ALOX5AP* for MI and stroke, and *PDE4D* for ischemic stroke, further strengthens the view that inflammation plays an important role in the disease process. Lymphotoxin-α (encoded by *LTA*) and its interacting protein galectin-2 (encoded by *LGALS2*) mediate a wide variety of inflammatory, immunostimulatory, and antiviral responses. The 5-LO pathway involving key proteins 5-LO and FLAP (encoded by *ALOX5AP*) is a major source of leukotrienes, a group of potent inflammatory mediators. Phosphodiesterase 4D (encoded by *PDE4D*) degrades cAMP, a key signaling molecule involved in inflammatory responses of vascular cells.

Notably, recent genetic studies of CAD and MI have also identified a new pathogenic pathway (or mechanism) for the pathogenesis of CAD and MI. Identification of *MEF2A* mutations in CAD and MI patients without apparent hypercholesterolemia and detection of a high level of expression of *MEF2A* in coronary endothelium suggest that an early trigger for the pathogenesis of CAD and MI may be dysfunction or abnormal development of the endothelium due to altered transcriptional reprogramming of gene expression caused by genetic mutations.

Identification of new genes for CAD and MI, the disease-causing genes in particular, will have a profound impact on clinical management of CAD and MI. Disease-causing genes, including *MEF2A* and other new disease-causing genes to be identified in the future, can be directly used for developing a genetic testing kit for early, accurate, and presymptomatic diagnosis of CAD and MI. Many individuals with a very high risk of developing CAD and MI can be identified early by genetic testing. Genetic testing is particularly significant for CAD and MI, as the first symptom for many patients may be acute MI and sudden death. Lifestyle modifications and other therapeutic options may be used to delay the onset of the disease or prevent MI in high-risk individuals. Moreover, genes for CAD and MI can be used as targets for developing new drugs for treating atherosclerosis.

The future research in the field of genetics of CAD and MI will focus on 1) identification of new disease-causing genes and susceptibility genes; 2) identification of molecular mechanisms for the pathogenesis of CAD and MI by molecular characterization of newly identified CAD and MI genes; and 3) genotype-phenotype correlation studies and translation of basic findings to clinical applications, including gene-specific therapies for CAD/MI patients.

Acknowledgments

This work was supported by the NIH grants R01 HL73817, R01 HL65630, R01 HL66251, the Chinese Ministry of Science and Technology National High Technology 863 Project No. 2002BA711A07, and an American Heart Association Established Investigator award.

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