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Understanding the genetics of coronary artery disease through the lens of non-invasive imaging

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Summary

Coronary artery disease is a common disease with a known heritable component that has spurred interest in genetic research for decades, resulting in a handful of candidate genes and an appreciation for the complexity of its genetic contributions. Recent advances in sequencing technologies have made large scale association studies possible adding to our current understanding of the genetics of coronary artery disease. Sifting through the statistical noise, however, requires selecting effective phenotypic markers. New imaging technologies have improved our ability to detect subclinical atherosclerosis in a safe and reproducible manner in large numbers of patients. We propose here that advances in imaging technology have generated improved phenotypic markers for genetic association studies of coronary artery disease.

Keywords

Coronary artery disease; non-invasive cardiovascular imaging; complex genetic analysis; atherosclerosis; carotid intima-media thickness; coronary artery calcium; PET; MRI; CCTA; phenotype

Introduction

Coronary artery disease (CAD) arises from long-term atherosclerotic changes in the cardiac vasculature that progress into obstructive lesions and interfere with adequate perfusion of the heart. Progress in clinical management of atherosclerosis has lead to a recent decline in mortality rates associated with CAD, yet it remains the most common cause of death worldwide.[1–4] CAD accounts for over one third of all adults deaths; over half of these cases are virtually asymptomatic until the first fatal presentation of acute myocardial infarction or sudden cardiac death.[5,6] The remaining patients with CAD endure chronic

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symptoms that serve as one of the leading disability adjusted-life year factors projected to 2020.[7] Within the US alone, it is estimated that one-half of all middle-aged men and one-third of all middle-aged women will develop some clinical manifestations of CAD.[1,2]

Patients with CAD often report family histories of premature cardiovascular disease. The Framingham Offspring Study affirmed that individuals with at least one parent with premature cardiovascular disease (onset <55 yo in men, <65 yo in women) had a significantly increased risk of suffering a cardiovascular event in comparison to individuals with no such family history (age adjusted OR men=2.6, women OR=2.3).[4] In fact, one study suggested that the heritability of CAD is as high as 63%. [8] The known heritable component of CAD has spurred interest in genetic research for decades, but limitations in gene analysis techniques until very recently have restricted findings to a few candidate genes and an appreciation for the complexity of genetic contributions at play in CAD. Indeed, the non-Mendelian contributions of multiple genes towards the CAD phenotype have eluded classical methods of candidate gene analysis such as linkage analysis and candidate gene studies.[9–11]

The past decade has seen extraordinary advancements in genetic analysis techniques that have revolutionized research approaches for diseases with complex heritability. The completion of the human genome project in 2001 marked the end of a long effort to catalog all human genes.[12,13] Subsequently, the identification of positions of single nucleotide polymorphisms (SNPs) as part of the HapMap Project [14], and later as part of the 1000 genomes project [21], allowed for characterization of common SNP variations (present at an allele frequency of 5% or greater) in multiple human populations.

The completion of these databases led to the rise of genome-wide association (GWA) studies — large case-control studies in which the association of these SNPs and common complex traits are tested. GWA studies test the common variant common disease hypothesis by identifying specific loci associated with the occurrence of a common disease trait. As a testament to the power of this technique, the first large GWA studies for CAD were published in 2007, from the Ottawa Heart Study,[15] deCODE Genetics,[16] and the Wellcome Trust Case-Control Consortium [17] demonstrating an association between a region in chromosome 9p21 and coronary artery disease. GWA studies have continued to be used to successfully identify a multitude of SNPs associated with diseases of complex heritability.[18–20] Yet, despite the demonstrated efficacy of the GWA approach, there are limitations to this technique, including the inability to identify rare variants associated with complex diseases, and a difficulty in validating functional and/or mechanistic implications SNPs located in non-coding regions of the genome.

Common variants as examined by GWA studies only explain a small fraction of heritable risk for complex disease posing the question as to whether rare variants might be able to contribute towards this unexplained heritability [24, 25]. The identification of rare variants in large cohorts of patients in a cost-effective manner was made a reality by the development of massive parallel sequencing techniques that vastly improved original capillary electrophoresis based Sanger methods of sequencing.[12,21,22] Using these techniques Ng and colleagues were able to perform targeted capture and massive parallel sequencing of the exomes, protein coding regions in the genome, of twelve humans giving rise to whole exome sequencing.[23] When applied to large cohorts of patients, whole exome sequencing has the potential to shed light on the pathogenesis of complex diseases by identifying rare variants with large effect sizes, thereby addressing some of the weakness of GWA studies. [21,22] Furthermore, the increasing output and decreasing cost of recent sequencing technologies make whole genome sequencing a real possibility in the not too distant future. [24,25]

Since CAD directed GWA studies began four years ago, many polymorphisms have been associated with hard endpoints of CAD, such as MI,[26,27] premature MI,[28–32] and stroke (locus 4q25).[33] Sifting through the statistical noise, however, requires selecting effective phenotypic markers. Hard endpoint markers for CAD, such as MI or SCD, are the result of chronic and complex disease process with differing potential causes and contributory risk factors. This heterogeneity makes it difficult to tease apart mechanistic contributions from the various loci identified and decrease the statistical power of large-scale genetic association studies.

These clinical events are generally preceded by the development of sub-clinical atherosclerosis characterized by thickening of the arterial wall due to accumulation of cholesterol rich material. New imaging technologies have made possible the precise measurement of subclinical atherosclerosis in large patient populations promising to enhance the ability of genetic association studies to elucidate the mechanisms at play in CAD. Notably, prior assessment of the extent of coronary involvement in CAD required invasive imaging or substantial radiation risk with serial imaging. Due to the risk of invasive catheterization, only patients with advanced disease have routinely been characterized. In comparison, “black-blood” magnetic resonance imaging can detect atherosclerotic thickening of the coronary arteries prior to both calcium formation and arterial narrowing. [34] Such tests can be performed without radiation or injection of iodine-based dyes.

Furthermore, these techniques are also able to characterize different components within atherosclerotic plaques, making it possible to seek genetic determinants of plaque composition. Specifically, improvements in multi-slice coronary computed tomography angiography (CCTA) have allowed this technique to become increasingly used for non-invasive characterization of plaque composition, particularly that of non-calcified plaque. Patients found to have plaques with evidence of positive remodeling with low-attenuation on CCTA have been found to be at increased risk for developing acute coronary syndrome. [35,36] Furthermore, plaques demonstrating spotty calcification have also been associated with an increased risk of acute coronary syndrome.[37] Head to head comparison of CCTA with gold-standard intravascular ultrasound (IVUS) revealed that lipid-rich/low attenuation plaques, which are deemed to be at a higher risk to cause acute coronary syndrome than their fibrous counterparts, can be identified by CCTA with sensitivity of 95% and specificity of 80% when using a cut-off of 5.5% pixels with an attenuation of ≥ 30 Hounsfield Units (HU). [38]

Current Progress of Genetic Studies Using Widely Available Non-Invasive Imaging Techniques

The newest imaging techniques share several properties that make them attractive phenotyping tools. They boast high spatial resolution and lower radiation making it feasible to study subclinical CAD in large cohorts of patients. Coronary Artery Calcium Score (CAC) and Carotid Intima Media Thickness (CIMT) are two imaging techniques that have been widely used in genetic studies of subclinical atherosclerosis.

Coronary Artery Calcium (CAC)

Calcification is a common early feature of atheroma that typically indicates presence of plaque when found in the coronary arteries.[39,40] It is strongly correlated with atherosclerosis, confirmed by histopathological analysis [41,42] as well as in vitro intravascular ultrasound.[43,44] Combining CAC scoring with FRS results in a high reclassification rate in the intermediate risk FRS cohort, demonstrating the benefit of imaging of subclinical coronary atherosclerosis.[45,46] Lastly, the risks of atherosclerosis

and risk prediction extend beyond CAD; the Rotterdam coronary calcification study demonstrated a significantly graded association between coronary artery calcification and stroke. The results suggest that coronary calcification as detected by CT may be useful to identify individuals at high risk of stroke as well as CAD.[47]

Several genes linked to arterial calcifications in humans have been identified by means of candidate gene analysis. Pfohl and colleagues used this approach to identify an association between the insertion/deletion polymorphism of the angiotensin I-converting enzyme gene (ACE), which had been previously associated with myocardial infarction, and coronary artery calcification as measured by intravascular ultrasound.[48]

The ApoE gene, which codes for a protein involved in catabolism of triglyceride rich lipoproteins, has also been implicated in CAD pathogenesis following candidate gene analysis with CAC phenotyping.[49] Kardia and fellow researchers show that when established CAD risk factors are taken into account, the ApoE genotype influences the probability of having CAC. E-selectin, a protein involved in leukocyte adhesion postulated to play a role in the inflammatory plaque formation,[50,51] has also been demonstrated to have significant association with CAC in women younger than 50 years of age Epidemiology of Coronary Artery Calcification Study.[52]

Other genes implicated through candidate gene analysis include the matrix metalloproteinase 3 (MMP-3) gene. In the Helsinki Sudden Death study, the MMP-3 genotype was found to be significantly associated with the amount of coronary artery calcium found at autopsy.[53] CAC has also been associated with polymorphisms in the gene encoding the matrix G1a protein (MGP), known to bind calcium ions with a proposed inhibitory function in biomineralization within vasculature.[54–56] Candidate genes also include pro-inflammatory gene products, like chemokine receptor 2 (CCR2), that are involved in chemotactic recruitment of monocytes as part of response to inflammatory stimuli. CCR2 has been linked with decreased coronary artery calcification after adjustment for coronary risk factors and ethnic differences; polymorphisms are seen more frequently in African-Americans than Caucasian populations, with less atherosclerosis noted in individuals with the polymorphism.[57,58]

The Rotterdam Coronary Calcification Study represents one of the few published GWA pertaining to CAC. In contrast to its candidate gene counterpart, this study failed to show an association between the insertion/deletion (I/D) polymorphism of the angiotensin I-converting enzyme gene (ACE) and coronary calcification in the general population.[59] A subsequent GWA analysis of the Rotterdam Coronary Calcification study demonstrated no significant associations between the ACE I/D polymorphism and coronary calcification in strata of cardiovascular risk factors.[59] Though this result could be related to issues of power, it is interesting that the GWA analysis does not replicate the candidate gene analysis within the same cohort. Another GWA study involving CAC was the genome-wide multipoint mode-of-inheritance-free linkage analysis on affected sibling pairs performed by Lange and colleagues, which identified two specific chromosomal loci, 6p21.3 with a maximum logarithm of the odds (LOD) score of 2.22; and 10q21.3, with maximum LOD score of 3.24, that may harbor genes associated with arterial calcification.[60]

Carotid Intima Medial Thickness (CIMT)

The superficial position of the carotid arteries makes them a target of localized, non-invasive imaging with ultrasound. CIMT has commonly been used as a measure of subclinical atherosclerosis to assess cardiovascular disease risk.[61] In fact, increases in CIMT have been shown to be associated with increased risk of cardiovascular events.[62,63] Though focal plaque is often a clinical concern, CIMT is more strongly heritable than carotid plaque

after adjustment for risk factors. The NHLBI Family Heart Study implemented variance components analysis to decompose total variance within the CIMT phenotype into genetic, random, and measureable covariates.[64] Heritability of carotid plaque, defined as a ratio of additive genetic variance component to the residual phenotypic variance contributors, demonstrated 0.52 heritability for carotid plaque without covariate adjustment and 0.13 after adjustment for age, sex, field center, and major vascular risk factors.[65]

A comprehensive meta-analysis of CIMT candidate gene studies by Paternoster,[66] suggested only ApoE polymorphisms possess convincing association with CIMT, with E4 demonstrating increased average CIMT and E2 with decreased CIMT. Furthermore, in the Women Ischemia Syndrome Evaluation Study (WISE), Chen et al demonstrated a significant association between CAD severity as determined by invasive angiography and the E4 polymorphism.[67] Taken together with the association between ApoE and CAC described by Kardia and colleagues,[49] these results lend further credence to the role of ApoE in atherosclerosis formation. Linkage studies by quantitative trait loci have additionally identified a region on chromosome 12 associated with atherosclerosis candidate gene SCARB1, a high density lipoprotein (HDL) receptor, reported with a maximum LOD score of 4.1.[68]

In comparison, GWAS using CIMT phenotypes have highlighted a 2q33-35 region with significant linkage (3.08) including NOSTRIN, IGFBP2 and IGFBP5 genes—none of which have yet been independently tested for association with CIMT.[69] Furthermore, GWAS have been performed within specific populations. HIV positive individuals have been characterized with accelerated atherosclerosis, likely from a combination of disease as well as medication. A GWA study within a cohort of HIV positive men identified 2 functional SNPs in ryanodine receptor 4 (RYR3) polymorphisms on chromosome 15. RYR3 is found in arterial endothelial cells and demonstrates tight linkage disequilibrium with R^2 of 0.97, thus significantly associated with common CIMT well above the Bonferroni correction.[70] A separate GWAS done on Caribbean Hispanic families with significantly heritable though modest CAD disease manifestations revealed flanking polymorphisms for the SOX6 gene, which plays a pleiotropic effect on obesity and osteoporosis, similar findings have yet to be published, given the relatively new genetic study methods.[71]

Perhaps the most comprehensive CIMT GWAS study has been recently published by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) investigators.[72] In this seminal study, genetic and phenotypic data collected from over 40,000 patients demonstrated significant associations between SNPs in or near genes involved in cellular signaling, lipid metabolism and blood pressure homeostasis and CIMT and carotid plaque. Furthermore, two regions were associated with CAD in the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis (CARDIoGRAM) consortium.[26] This study demonstrates the potential of using subclinical atherosclerosis phenotyping techniques in large scale genetic association studies to shed light on the mechanisms at play in atherosclerosis.

The Future of Genetics and Imaging: Emerging Imaging Technologies

Although genetic association studies using currently available imaging techniques have already greatly contributed to our understanding of CAD, the current studies do not fully explain the heritability of this disease. Thus it is very likely that improved vascular imaging of both coronary and carotid arteries may improve the power of these studies to elucidate the genetic basis of atherosclerosis. Such tools include coronary computed tomography angiography (CCTA), coronary and carotid magnetic resonance imaging (MRI) and positron emission tomography (PET).

CCTA

The coronary arteries have been traditionally difficult to image but new advances in CCTA technologies have largely overcome this challenge. Studies using 16 to 320 slice multi-detector CT (MDCT) have been able to detect plaques of potential clinical importance. Such plaques tend to be large (2–17 mm in length, averaging close to 8 mm) with sizeable necrotic cores. Large plaques are often found in the mid and proximal coronary arteries that are readily accessible by CCTA (spatial resolution ~0.5 mm). 95% of high risk plaques are confined to proximal and middle segments of coronary arteries, and most of these lesions are accompanied by vessel remodeling.

Shortly after the introduction of the 64 slice MDCT in 2005, two multicenter trials (ACCURACY and Core64) demonstrated overall plaque detection sensitivity of 95–97% and specificity of 89–90%. [73] Both studies demonstrated slightly improved sensitivity for non-calcified plaque detection from 78% to 93% compared to their 16 slice predecessors. [73–75] Additionally, the Core64 multicenter trial demonstrated less than ideal NPV and PPV around 83% and 91% respectively. [76] When compared with intravascular ultrasound, agreement between 64-slice MDCT and IVUS was greater in the proximal and middle segments, [77] indicating that concordance was likely a function of plaque size and composition. Quantification of plaques revealed systematic underestimation of non-calcified plaque and overestimation of calcified plaque, the latter likely secondary to calcium blooming. [78,79]

The newest 320 slice MDCT boasts increased temporal resolution and z-direction coverage, satisfying technical prerequisites for coronary atherosclerotic plaque imaging. [80–86] Improving spatial and temporal resolution is making qualitative and quantitative assessments of plaque size, composition, and remodeling progressively more feasible. [75,87–90]

In the face of these advancements, two main challenges remain. The first challenge involves radiation exposure, which has prevented serial imaging with CCTA in large-scale clinical trials. Improving temporal resolution with 320 slices has led to a decrease in radiation. Further measures have also been taken to reduce radiation exposure during CCTA, most notably through the design of retrospective EKG-dependent tube current modulation. [91] In addition, advanced iterative reconstruction techniques and improved radiation detectors allow further reduction in radiation dose. Radiation doses 15–20 mSv or more with 16 slice MDCT were once common; it is now possible to lower radiation dose to approximately 1 mSv. [92] By comparison, invasive angiography of the coronary arteries is associated with radiation doses of approximately 7 mSv. [93] For reference, background radiation exposure is approximately 3.1 mSv per year. [93] Radiation workers (e.g. physicians, technologists) have allowable exposures up to 50 mSv per year.

The second challenge is technical: CCTA still provides poor definition of the outer vessel boundary. Presently the poor definition of vessel boundaries with CCTA has resulted in high intra-observer variability of 20 to 37%. [75,94] Manufacturers have attempted to address this issue by creating automated segmentation tools unaffected by display settings, which have improved this variability to below 20%. [73,94] Nevertheless, these programs still require frequent manual adjustment and need further refinement before they can become fully automated and standardized. In addition to standardization, full use of CCTA would require a semi-quantitative means of assessing plaque volume, as using this as a continuous variable would be statistically preferable in genetic association studies. Current software used to measure plaque volume has not been validated and the reproducibility of this technique has not yet been established.

While good agreement has been established between MDCT and IVUS, the high variability in plaque quantification that occurs with different plaque compositions and sizes, as well as qualitative analysis with histopathologic confirmation, must both be addressed. Future optimization of MDCT based coronary imaging should be directed towards either refinement of automated segmentation, or to establishing standard assessment criteria/settings. Despite these limitations, plaque characterization with CCTA at present is such that this technique can be reliably used for large patient cohorts in clinical trial settings.

Coronary MRI

As different plaque characteristics have been associated with different levels of thrombotic risk, Magnetic Resonance Imaging (MRI) is an attractive imaging technology as it has the capacity to distinguish between various soft tissues. The focus of MR has largely been based on imaging of carotid vessels; high-intensity signal in carotid plaques is predictive of coronary events.[95] While initial results are promising, imaging the carotids with MRI is a method that requires much streamlining as there are various factors that contribute to suboptimal imaging. First, vessels with small but complicated plaque structures requires high resolution, high signal to noise ratio (SNR), and good lumen to wall contrast for plaque visualization. Furthermore, calcification, lipid rich necrotic cores, and hemorrhage co-exist within one plaque and require multiple contrast weightings or contrast agent injection for quantification. Effective use of MRI in plaque imaging protocols would necessitate optimization of these issues.

In spite of these limitations, MRI is indeed a strong imaging technology that will likely play a larger role in phenotyping atherosclerosis in future genetic studies due to its myriad benefits: it confers no radiation risk and is therefore suitable for serial imaging, is strongly validated against histologic findings for both identifying major imaging biomarkers of atherosclerosis. In terms of quantification, it yields accurate[96] and reproducible morphologic measurements,[97,98] with variability of 5 to 10 percent.[99] From a qualitative standpoint, it has already been widely accepted as a tool for plaque component identification [100,101] both ex vivo and in vivo.[96,102] With proper window selection, multicontrast MRI enables identification of all major components of plaque, including thrombi [103,104], fibrous cap,[103,105] lipid rich necrotic core,[96] and hemorrhage.[106] Hemorrhage may also be imaged using heavily T1 gradient echo-based sequences, as blood has a short relaxation time. [103,107] Using AHA 8 category classification, Cai and colleagues demonstrated that MRI can reliably classify plaques into the AHA 8 categories [108] with reproducible results ($\kappa = 0.73$).[109]

Contrast enhanced MRI contributes to the imaging potential by further improving detection of fibrous cap from lipid-rich necrotic core. Additionally, contrast-kinetic modeling MRI can be used as a measure of neovascularization[110] and inflammation.[111] Finally, diffusion MRI [112] and 3D ultra-short TE MRI [113] have been shown to help identification of lipid rich necrotic core and calcification respect in ex vivo specimens. The ability of MRI to delineate so many plaque components allows for more refined phenotyping (for example, for mechanistic players in the process of atherosclerosis-driven neovascularization) that will help reduce the statistical noise that comes with GWAS and whole-exome sequencing.

Like carotid MRI, coronary artery MRI is an emerging research technology without radiation exposure. Technical challenges limit the spatial resolution of MRI of the heart to approximately 1.5 mm due to coronary artery motion and central location of the coronary arteries. Nevertheless, plaque identification [114] and coronary artery narrowing[115] are readily identified with this modality. Limitations of MRI for large scale studies include high

cost (approximately double that of CCTA) and availability that is limited to specialized centers.

PET and SPECT

Positron emission tomography has been used to study myocardial perfusion in patients with ACE and ApoE polymorphisms. The ApoE4 gene was found to have the strongest association followed by ACED and Apog-219 T polymorphisms.[32] The combined structural and metabolic components secure a place in future of cardiovascular imaging; [116] this wealth of information, however, comes at the cost of higher levels of radiation (> 10 mSv), and lower availability.[93] The spatial resolution of PET is inherently low (4–8 mm), so that radiotracer activity in small coronary or even carotid arteries requires an additional imaging method, usually CT, to show the location of the abnormality. Nevertheless, the potential of identifying molecular markers in conjunction with anatomic changes makes PET a potentially powerful imaging tool.

Like PET, single-positron emission computed tomography (SPECT) operates through non-invasive targeted radiotracers to localize biological processes.[117] Hybrid scanners, such as PET/CT, SPECT/CT, and recently MRI/PET co-localize targeted biological processes to their precise anatomic locations. Hybrid scanners are now widely available, and they have been successfully used to visualize unstable atherosclerotic plaque by targeting FDG and peripheral benzodiazepine receptors.[118–120] The hybrid scanners have also been used to study infarct-induced changes in myocardial metabolism (FDG), [118] sympathetic neuroreceptor function (^{123}I -MIBG),[121,122] angiogenesis (^{111}In -VEGF and ^{111}In -RP748), [123,124] and myocardial remodeling (^{111}In -RP782).[125] Though these techniques are still in their clinical inception, they hold great promise to assist discovery and validation of novel cardiac biomarkers through their simultaneous acquisition of mechanistic insight with spatiotemporal orientation.

In conclusion, the rapid progress in genetic sequencing technology and recent advances in non-invasive cardiovascular imaging technology have opened new methods of studying the complex genetic interactions behind chronic, common diseases like atherosclerosis. With these advances, we are truly poised to pursue a greater understanding of this deadly disease at a mechanistic level that may alter screening methods, identify major prognostic factors, and inform treatment.

Expert Commentary/Five year view

Advanced imaging technology, especially MRI, CT and PET scanning, is highly suited to noninvasive evaluation of large patient populations. MRI in particular is free of radiation and has low associated risks. These tests vastly improve the ability to define the precise phenotype of a patient compared to nonspecific serum biomarkers. Whereas a patient may have biomarkers that *predict* they *may* eventually get atherosclerosis, the imaging tests actually *define* atherosclerosis presence and its extent. When combined with genetic testing, imaging provides a highly accurate phenotype and is potentially applicable to large patient populations. Over the next 5 years, we expect that large scale imaging studies will increasingly be developed to study key clinical questions. The data from such trials will ideally be combined with genetic analysis to determine the gene-phenotype interactions that result in the cardiovascular disease.

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Key Issues

- Coronary artery disease (CAD) is the result of chronic changes in the cardiac vasculature that progress into obstructive lesions that interfere with adequate perfusion of the heart.
- Despite reductions in CAD mortality due to recent advances in medical management, CAD is still the leading cause of death worldwide, accounting for 1/3 of all mortality in patients over 35 years of age. Over half of these cases are asymptomatic until first presentation, which is often dramatic and sometimes deadly.
- CAD is known to run in families. The Framingham Offspring Study found that individuals with at least one parent with premature cardiovascular disease (onset <55 yo in men, <65 yo in women) had a significantly increased risk of suffering a CV event in comparison to individuals with no such family history (age-adjusted OR men=2.6, for women, OR=2.3)
- The completion of the Human Genome Project in 2001, followed by the HapMap Project and later by the 1000 Genomes Project, led to a cataloging of all common polymorphisms in the human genome. The creation of these catalogs made it possible to do genome-wide association (GWA) studies in which a large number of common gene variants are screened to find statistically significant associations with select phenotypes.
- Though the GWA approach has contributed greatly to our understanding of the genetics of CAD, it is often difficult to prove a functional significance in the variants outside of gene-encoding areas identified by this technique. Furthermore, GWA studies are unable to reveal unknown variants and omit rare gene variants from their screen due to limitations in the haplotype catalog.
- Advances in sequencing technologies have made possible whole exome sequencing, in which all of the protein coding regions in a genome are sequenced. Whole exome sequencing can be applied to large cohorts of patients making it possible to identify all gene variants attributable to a defined phenotype of interest.
- Non-invasive imaging methods to detect subclinical atherosclerosis hold great potential in providing effective phenotypes for large scale genetic association studies.
- Current progress in coronary computed tomographic angiography (CCTA) has demonstrated improved temporal resolution with the 320 slice multi-detector scanner, capable of imaging the entire myocardium in one heartbeat. Concurrent advancements have been made to reduce radiation exposure: with the combination of iterative reconstruction and EKG-dependent tube current modulation, it is possible to lower radiation dose to approximately 1 mSv. For comparison, the annual background radiation exposure for a US citizen is just over 3 mSv.
- Magnetic resonance imaging (MRI) of the carotids has been capable of visualizing all structural components of carotid plaques with strong histologic confirmation. While coronary visualization is still in its inception, plaque identification and coronary artery stenoses are both readily identifiable with this modality. MRI carries the additional advantage of no radiation exposure, making it an attractive technique for serial imaging.

- Positron emission tomography (PET) offers simultaneous structural and metabolic (18-FDG), at the price of higher doses of radiation (14.1 mSv) and higher cost. Recently, MRI has been combined with PET imagers in a hybrid scanner, which will reduce the radiation exposure by more than half the original dose. The hybrid scanners will also allow for imaging with new molecular markers of structural remodeling (collagen) and inflammation (benzodiazepine receptors).