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Genetics paired with CT angiography in the setting of atherosclerosis

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ABSTRACT

Coronary artery disease (CAD) continues to be the leading cause of morbidity and mortality globally. Although the etiological mechanisms for CAD have not been fully elucidated, however, most would agree that atherosclerotic plaques progressively narrow the coronary arteries are the earliest manifestations and the principal cause of CAD. The emergence of revolutionary imaging technologies such as cardiac CT angiography, noninvasive computed fractional flow reserve and intravascular ultrasound provided the possibility of detecting and monitoring phenotypes associated with subclinical atherosclerosis. Meanwhile, with the widespread use of high-throughput genotyping pipeline such as next-generation sequencing, combined with big data-driven solutions in bioinformatics, translating the emerging genetic technologies into clinical practice and, therefore, provide valuable insight into the CAD study. In this review, we briefly describe the latest noninvasive cardiac imaging techniques for atherosclerosis-related phenotypes' detection, mainly focusing on the coronary artery calcification, plaque burden and stenosis. Furthermore, we highlight the state-of-the-art genotyping techniques and its application in the field of CAD translational study. Finally, we discuss the clinical relevance of genetics paired with noninvasive imaging in the setting of coronary artery atherosclerosis.

1. Introduction

To date, coronary artery disease (CAD) has become an enormous public health problem in the United States. Approximately 1.1 million American adults have at least one major adverse cardiac event in 2014 [1,2]. Furthermore, by 2030, the prevalence of CAD in the United States is predicted to be 9.5% [2]. Atherosclerosis is a condition in which plaque forms in the arteries, and finally, impaired myocardial blood flow is the primary pathogenic mechanisms of obstructive CAD. Atherosclerosis is a typical complex disease process caused by multiple genetic and environmental factors' interactions. Over the past decade, genetics paired with cardiac imaging tools in the setting of atherosclerosis have made considerable progress. Rather than presenting an exhaustive review of atherosclerosis-related phenotypes for translational study, in this review, we focus on the latest applications of cardiac CT angiography (CTA)-based noninvasive imaging techniques in subclinical atherosclerotic plaque detection (qualitative and quantitative) and the challenge of treatment and prevention of CAD. Besides, we also highlight the state of knowledge of high-throughput genotyping, which has been given a tremendous boost in translational studies of atherosclerosis.

Twenty years ago, even an experienced physician also faced the frustrating situation when a patient has chest pain but with a "normal" Electrocardiogram (EKG) or normal cardiac enzyme readings. Indeed, at that time, few robust imaging tools are available for the morphological information of the coronary arteries, as well as the presence and severity of CAD evaluation in addition to the EKG-based tests. Today, advances in noninvasive cardiac imaging technologies, represented by coronary CTA, have provided unprecedented opportunities for plaque burden composition measurements, quantification and validation, stenosis and risk assessment and even the determinants of stent implantation with faster, more reliable and accurate ones than ever before.

As a matter of fact, early identification and effective monitoring plaque burden progression have a remarkable prognostic value in CAD patients. The coronary artery atherosclerosis phenotypes comprise qualitative and quantitative artery calcification, plaque burden and discriminate degrees of stenosis. To date, CTA can offer a high-resolution, three-dimensional (or 3D) digital imaging and visualization of the coronary arteries and other adjacent structures. Particularly worth mentioning is that the noncalcified atherosclerotic plaque burden can be recognized as an earlier manifestation of atherosclerosis than calcified plaque, previously required significant radiation dose exposure to obtain and now can be accurately assessed by CTA with very low radiation dose exposure without loss of image quality [3–5]. Relying on the innovative hardware–software architecture, today's CTA can accurately assess the vulnerable nonobstructive plaque and the native vessel lesions even in asymptomatic high-risk patients with satisfactory sensitivity and specificity at average 0.29-mSv radiation dose exposure [6,7], which is almost comparable with a chest X-ray [8]. In comparison with traditional angiography, the latest CTA can evaluate asymptomatic patients for CAD screening just taking less than 30 min and coronary artery calcium scan less than 5 min. These features make CTA increasingly

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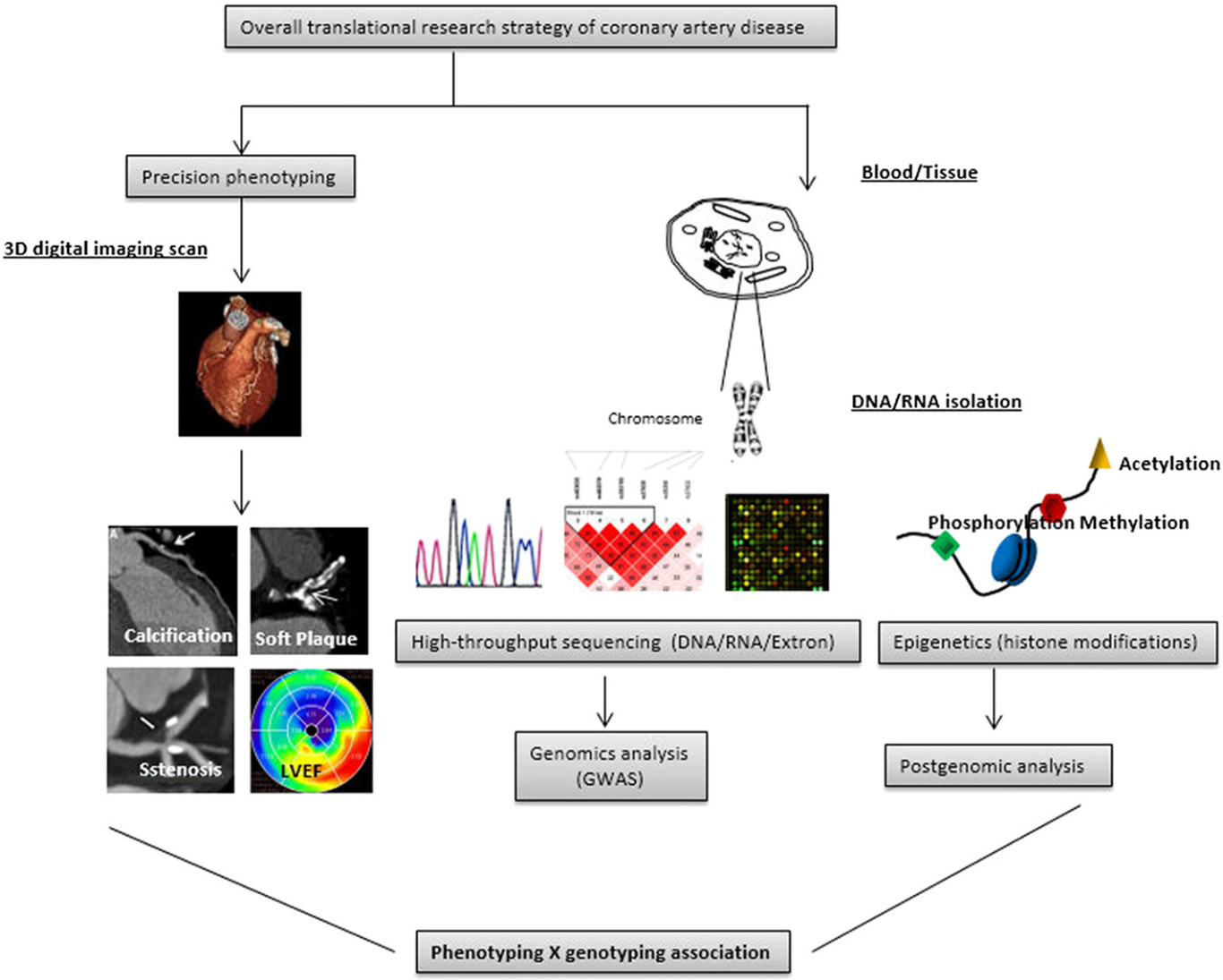

Fig. 1. Flowchart of CAD translational study.

being applied in translational studies of subclinical atherosclerosis and CAD for qualitative, quantitative and semiquantitative determination of phenotypes (Fig. 1).

Although there is a widespread use of CTA for the evaluation of atherosclerotic plaque, in vivo assessment of the progressive narrowing of the coronary arteries is a significant issue as well. In other words, whether the patient's stenosis will trigger a series of clinical syndrome due to the resistance to blood flow is a significant clinical question must be asked and answered. Today, in most of the clinical centers across the United States, the invasive cardiac catheterization in patients with chest pain and a clear history of CAD is preferentially examined after a stress test. However, this pattern remains controversial because a certain amount of patients were finally identified with no significant blockages. Therefore, in 2014, the U.S. Food and Drug Administration cleared Heart Flow fractional flow reserve (FFR) computed tomography software, a novel noninvasive technique to investigate CAD patient's coronary flow dynamics across a stenosis based on computed tomography (CT) scan data [9]. Today, the FFR management in stable CAD patients became Class I and Class IIa guideline recommendations [10,11]. Notably so far, evidence-based data for FFR_{CT} application is limited. Nevertheless, some initial studies have demonstrated that the value for FFR_{CT} in avoiding potential unnecessary angiography procedures [12] affects patient care and economic outcomes [13,14].

Beyond CTA, intravascular ultrasound (IVUS) is another promising in vivo technique for assessing plaque burden [15], lesion severity and lesion characteristics [16] and stent sizing [17]. IVUS can precisely detect arteries and luminal narrowing with an axial resolution of 150 μm, which is critical to stent implantation, as well as calcified stenosis treatment strategy selection. Although it is a well-validated modality compared to histopathology with an accuracy of 90% in detecting all types of plaque morphology [18], however, use of IVUS is limited due to its invasiveness and expense.

2. The dilemma and limitations of microsatellites or expressed sequence tags genetic markers atherosclerosis study

The interactions of genetic and environmental factors is responsible for the pathogenesis and progression of atherosclerosis. With the development and widespread application of noninvasive cardiac imaging techniques, a series of remarkable progress had been making toward unraveling the genetic basis of CAD and atherosclerosis.

Acute plaque rupture is the cause of most adverse CAD events [19]. Predicting the progress and prognosis of CAD by plaque volume and composition (fibrous, fibro-fatty, necrotic core, dense calcium) is a hotspot in the latest genetic research. Before Genome-Wide Association Study (GWAS) era, the candidate gene and linkage association are two

major approaches to study the etiology of CAD and atherosclerosis based on pedigree and twin data. At least three potential factors respond to this dilemma. Firstly, a slew of weak associations was identified, and a considerable amount of genetic variation could not be replicated [20]. Secondly, most studies only continue with a priori accessible biological pathway knowledge; more importantly, an irrelevant assumption often led to a bias toward the identification of novel genes [21]. Thirdly, candidate gene and linkage association approaches are mainly utilized in identifying single gene disorders in families with a particular phenotype, though, occasionally, it also can be applied to multifactorial disease locus mapping under exceptional circumstances.

3. GWAS—nature's complement of high-throughput data-driven strategies

There has been a turning point due to the development and maturity of high-throughput genotyping technologies, such as whole genome sequencing (WGS) and high-density single nucleotide polymorphism (SNP) array. Before these techniques were identified, carrying out a large-scale genetic study among populations was difficult due to lack of high-density genetic markers, as well as study subjects from cohorts or case–controls are unrelated. Research toward linkage disequilibrium and the structure of haplotype blocks in the human genome of the International HapMap Project (HapMap) advanced the science. These latest findings directly linked to the realization of GWASs. Overall, the GWAS is a powerful tool for identifying novel genes or gene regions associated with genetic disorders and complex traits. Unlike the candidate gene or linkage association approach, the GWAS needs not to know pathological pathways or biological knowledge in advance. In GWAS study, if the allele frequencies are significantly higher in cases compared with controls, this locus or variation will be recognized as disease causing or associated with the disease phenotypes. In other words, associated genetic variations can serve as multiple molecular marker bindings to specific regions of the entire human genome where the disorder resides. These advantages coupled with significant reductions in the price of high-throughput genotyping made GWAS become a robust, unbiased genome screening tool for identifying genetic architecture associated with complex disease traits.

4. GWAS–the ultimate solution or not?

The first robustly replicated GWAS result for CAD was published by the Wellcome Trust Case Control Consortium (WTCCC) in 2007 despite two landmark studies published prior [22,23]. These studies link human chromosome 9p21.3 locus with CAD for the first time. After 10 years of unremitting effort, by 2015, a total of 46 genome-wide significant loci for CAD were thus far identified by GWAS [24]. The results covered well-known biological pathways, such as the lipoprotein and cholesterol metabolisms regulating gene LDLR [25], PCSK9 [25], lipoprotein and LDL metabolism gene [26], endothelial function gene denNOS3 and ESR1 [27]; cardiac troponin T (hs-cTnT level regulating gene, such as NCOA2 and TNNT2 [28]) from major cohorts such as Multi-Ethnic Study of Atherosclerosis [29], WTCCC [30], Atherosclerosis Risk in Communities (ARIC) [31], Coronary Artery Risk Development in Young Adults [32], Cardiovascular Health Study, and Framingham Heart Study (FHS) [33]. In addition, some common genes involved in the control of plasma cholesterol and triglyceride levels, such as cholesteryl ester transfer protein (CETP) for high-density lipoproteins (HDL) and Apolipoprotein E (APOE) for low-density lipoprotein (LDL) have also been identified [34]. Table 1 summarizes the significant findings based on large, population-based cohort through GWAS. Notably, despite achievements, various phenotypes of interest so far have failed to be uncovered by GWAS [35]. Despite the high number of genetic loci identified through GWAS, these do not encompass any protein-coding genes, thus failing to explain the heritability patterns of CAD phenotypes [36]. Thus, there is still a disappointment and lack of optimism regarding the robustness of the current GWAS results. Part of the issue was the limitation of phenotypes for CAD. Invasive angiography was rarely performed in large-scale studies, and the numbers of clinical events made genetic studies difficult. Studies evaluated phenotypes mainly from the social and demographic indicators plus few clinical indicators, such as blood pressure, glucose, and lipid levels. Sometimes electrocardiograms and echocardiography were added. The introduction of cardiac CT has opened new opportunities to study a phenotype of CAD in an asymptomatic population. Noninvasive imaging allows the precise phenotyping of atherosclerotic plaques and shows strong prognostic potential to predict coronary events. However, the qualitative and quantitative phenotypes, such as the severity and distribution of calcification, luminal narrowing and plaque type (calcified, noncalcified and partially calcified plaques), the level and range of myocardial ischemia, and coronary in-stent restenosis, became a major threat to control's selection [37] or even led to selection bias due to the misclassification of a case during GWAS [38]. In addition, to obtain robust results, GWAS usually needs large samples size support; however, at this point, the typical clinical trials or translational study have used only a few hundred individuals limiting the ability to study the null hypothesis [39].

5. The emerging technologies—influence cannot be ignored

Taking into account the fast, efficient, and affordable genetic marker generation, we are cautiously optimistic about the genetic testing that will increasingly become an indispensable tool for the study of a wide array of human diseases, including CAD. However, the challenge remains for extracting genetic information to improve the clinical utility in early detection, risk stratification, and prognosis assessment, for example, the priority is how to interpret these vast amounts of genetic data. So, let us take GWAS as an example. The most significant limitation GWAS now faces mainly includes haplotype blocks, or Tag SNPs-based data analysis strategies (cover a limited portion of the genome) plus vast amounts of genetic variation, either rare or common mutations, have been associated with synonymous mutations, even though these loci individual has a genome-wide statistical significance. This situation that leads to GWAS may have to give way to human WGS, which provide a base-by-base, high-resolution view of the entire genome [40,41] once the eventual cost of for an individual's entire genome <US$1000 [42], and the maturity of big data processing through a pipeline platform. Unlike other types of genetic screening approaches, for example, targeted resequencing or exome-sequencing WGS offers a “comprehensive” view of the entire genome. In other words, the WGS will generate not only an enormous number of base-by-base genetic data but also more valuable genomic structural variation, including copy number variations (CNVs) genetic data SNPs and large-scale insertions/deletions.

Indeed, the first human genome sequencing cost almost US$3 billion and took a full 15 years. In contrast, by 2014, over 45 human genome sequences can be finished in a single day, at a cost of approximately US$1000 [43]. It is no exaggeration that the fast and cost-efficient next-generation sequencing (NGS) is becoming the core of high-throughput genotyping technologies in the genomic era. The novel high-throughput gene expression platform, such as SOLiD Next-Generation Sequencing from Applied Biosystems and Array-Based Gene Expression Analysis from Illumina, is developing rapidly. In today's market, NGS is available by three parallel sequencer manufacturers: Illumina Solexa Genome Analyzer, Applied Biosystems SOLiDTM System, and Roche/454 FLX Pyrosequencer. Compared with the Tag SNPs, identified from DNA sequence variants (the foundation of GWAS), the NGS, based on the combination of high-throughput sequencing and integrate sophisticated bioinformatic analyses offered more comprehensive genetic information, including functional SNPs discovery, encoding RNAs, CNVs, chromatin immunoprecipitation assessment, and even epistemic variation detection and protein-binding

Table 1
The major large-scale GWAS for onset of CAD (in chronological order)

Study title	The time of publication	Reference	Major cohort or study population	Phenotypes/Trait	Main findings
Candidate pathway-based genome-wide association studies identify novel associations of genomic variants in the complement system associated with coronary artery disease [13]	2014	PMID: 25249547	Chinese Han population	• CAD	• SNP rs7842 in C3AR1 and rs4400166 in C6 are significantly associated with CAD
Genome-wide association study of coronary and aortic calcification implicates risk loci for coronary artery disease and myocardial infarction [14]	2013	PMID: 23561647	The Dutch and Belgian Lung Cancer Screening Trial (Nederlands Leuvens Longkanker Screenings Onderzoek trial)	• CAD detected by CT-based imaging • Myocardial infarction (MI) • Aortic calcification	• SNP rs4977574 at was significantly associated with CAD/MI • SNP rs3825807 at ADAMTS7 gene and rs12526453 at PHACTR1 gene were significant association with coronary artery calcification
Genome-wide association analysis of incident coronary heart disease in African Americans: a short report [15]	2011	PMID: 21829389	African Americans	Coronary heart disease (CHD)	SNP rs1859023 located at 7q21 near the PFTK1 gene is are significantly associated with CAD
A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease [16]	2011	PMID: 21378988	• Europeans • South Asians	CAD	• LIPA on 10q23, PDGFD on 11q22, ADAMTS7-MORF4L1 on 15q25, a gene rich locus on 7q22 and KIAA1462 on 10p11 whites were significantly associated with incident CAD
Genome-wide association study identifies a new locus for coronary artery disease on chromosome 10p11.23 [17]	2011	PMID: 21088011	• German MI Family Study (GerMIFS) III (KORA) • GerMIFS I and GerMIFS II • PopGen	• CAD MI	• SNP rs3739998 at 10p11.23 (KIAA1462 gene) was significantly associated with CAD/MI
Genetic variants identified in a European genome-wide association study that were found to predict incident coronary heart disease in the atherosclerosis risk in communities study [18]	2010	PMID: 19955471	• Biracial, prospective cohort study (ARIC) • Whites • African Americans	CHD	• rs599839, rs1333049, and rs501120 in whites were significantly associated with incident CHD • rs7250581 in African Americans is significantly associated with incident CHD
Novel genetic variants linked to coronary artery disease by genome-wide association are not associated with carotid artery intima-media thickness or intermediate risk phenotypes [19]	2009	PMID: 18675980	• British Caucasian families	• Onset of CAD • Carotid artery intima-media thickness (CIMT)	• SNPs at Chr 9 (rs1333049, rs7044859, rs496892, rs7865618), Chr6 (rs6922269) and Chr2 (rs2943634) are not associated with CIMT
New susceptibility locus for CAD on chromosome 3q22.3 [20]	2009	PMID: 19198612	• German MI Family Study II (GerMIFS II) • TCCC CAD study • GerMIFS I1 • MI Genetics Consortium	• MI	• locus on 3q22.3 in MRAS gene (rs9818870) was significantly associated with MI • rs2259816 in the HNF1A-C12orf43 region was associated with MI
Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for CAD [21]	2009	PMID: 19198611	• Wellcome Trust Case Control Consortium CAD study • German Myocardial Infarction Family Study I (GerMIFS I)	• CAD	• SLC22A3-LPAL2-LPA gene cluster are significantly associated with CAD
Genome-wide association study for subclinical atherosclerosis in major arterial territories in the NHLBI's Framingham Heart Study [22].	2007	PMID 17903303	FHS	• Ankle–brachial index, • Coronary artery calcification • Abdominal aortic calcification • Carotid intimal medial thickness (IMT)	The five most significant associated SNPs with subclinical atherosclerosis phenotypes • Ankle brachial index (rs7989017,rs7546903,rs6135095, rs1875517,rs6507763) • Common carotid artery IMT(rs1039610,rs1587893,rs28207, rs4814615,rs2470209) • Internal carotid artery IMT (rs933890,rs8075776,rs252984, rs10490889,rs10516308) • Coronary artery calcification (rs10483853,rs10507130, rs9321354,rs220457,rs10505182)
Genomewide association analysis of coronary artery disease [9].	2007	PMID: 17634449	• Wellcome Trust Case Control Consortium • The German MI Family Study	• CAD	SNPs rs599839 (1p13.3), rs17465637 (1q41), rs501120 (10q11.21), and rs17228212 (15q22.33) were significantly associated CAD
A common allele on chromosome 9 associated	2007	PMID: 17478681	• Ottawa Heart Study (OHS-1) • The Copenhagen City Heart	• CHD	rs10757274 and rs2383206 were significantly associated with CHD

Table 1 *(continued)*

Study title	The time of publication	Reference	Major cohort or study population	Phenotypes/Trait	Main findings
with coronary heart disease [10].			Study • The Dallas Heart Study • Ottawa Heart Study population (OHS-3)		
A common variant on chromosome 9p21 affects the risk of myocardial infarction	2007	PMID: 17478679	• deCODE	• MI	Variants located in gene CDKN2A and CDKN2B (chr 9p21.3)were associated with risk of MI
Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls [23].	2007	PMID: 17554300	• Caucasians from England, Scotland and Wales (Great Britain) • British Birth Cohort (58C) UK Blood Services	• CAD	• SNP rs1333049 located 9p21.93–22.12 was significantly associated with CHD • SNPs rs17672135 (1q43),rs383830(5q21), rs6922269(6q25), rs8055236(16q23), rs7250581(19q12) show moderate evidence of association with CAD

region's detections, which directly linking the human disease phenotypes. Notably, these novel technologies offered streamlined sample preparation and data analysis tightly integrated with ready-to-use reports. These innovations offer the possibility of transforming large-scale WGS to the microscale assaying of disease or infected tissue or even peculiar clusters of cells acquired from screening or surgical procedure with a real affordable investment for massive-scale genomic studies.

So far, a limited number of initial CAD translational studies based on NGS strategy were conducted. Shea et al. [44] focused a 240-kb region on Chr9p21 using Illumina platform and validated the results using the 1000 Genome Project. They found that NGS is also robust for rare or low-frequency variants (a minor allele frequency, MAF<5%) detection. In fact, this study is very meaningful because it is the first time that the researchers can identify rare SNPs involved in the risk of cardiovascular disease (CVD) using high-throughput research method. Virtually all previous studies have only focused on the common genetic variants (MAF>5%). In addition, a very recent validation study seeks to test the sensitivity and specificity of NGS in a familial hypercholesterolemia population [45]. The results indicated that polymerase chain reaction (PCR)-based enrichment tech combinations with NGS have potential value in the aspects of the molecular diagnosis of familial hypercholesterolemia. Although little data were presented, NGS is attracting increasingly widespread attention and will add value to CAD translational study. We are cautiously optimistic that the revolutionary genome-sequencing technologies paired with the cutting edge of noninterventional imaging technologies will bring enormous changes to translational studies in human disease.

6. Insight from the clinical trials

There are growing signs of a single genetic account for a relatively minor proportion of CAD phenotypes [46,47]. In most cases, the complex interaction of an individual's genetic architecture and the environmental factors, lifestyle, and behaviors, such as smoking, alcohol intake, physical activity, and a dietary pattern, and even medical intervention contributes to the occurrence and development of CAD. Recent large, well-designed clinical trials tried to increase the power of genetic studies to identify novel genomic loci that are associated with CAD traits and finally to construct comprehensive genome-wide association mapping.

The Genetic Loci and the Burden of Atherosclerotic Lesions study (GLOBAL) is an excellent model to comprehensively and accurately acquire CAD phenotype by noninvasive imaging technologies paired with state-of-the-art genotype. This clinical trial is by far the single largest international, prospective, multicenter panomic study to identify the novel pathway, biomarker, and drug targets for the development and progression of CAD and risk factors such as atherosclerosis [48]. Up to 10,000 patients from 48 institutions in 9 countries and across North America, Australia, and Europe are to be enrolled. The massive data will be analyzed with biological network-driven bioinformatics. No pilot data were presented yet by GLOBAL; however, the precision phenotype assessment by noninvasive CTA, including the quantification of coronary artery calcium (Fig. 2), the qualitative (the presence of any atherosclerotic plaque) and quantitative of overall coronary plaque burden and luminal stenosis (≥50% and≥70% diameter stenosis) (Fig. 3), paired with massive "panomic" data, including WGS, transcriptase sequencing, methylation, metabolisms, unbiased proteomics, and lipidosis, is worth expecting.

Beyond anatomical noninvasive image technologies, several latest clinical trials using genetic paired functional noninvasive image technologies are worth mentioning. The COMPASS study (Coronary Obstruction Detection by Molecular Personalized Gene Expression) is a prospective study aims to evaluate the ability of Corus® CAD, a blood-based gene expression test to accurate identify obstructive CAD in a population of symptomatic nondiabetic patients referred for stress testing with myocardial perfusion imaging (MPI). In this study [49], the investigators integrate multivariable including age, gender, and gene expression to develop a novel gene expression evaluation system, which was named gene expression score (GES). GES ranges from 1 to 40 that may align the possibility of the presence of obstructive CAD [50]. Based on super high sensitivity (89%) and negative predictive value (96%) plus patients with a low GES (≤15) presenting a very low incidence of revascularization and adverse cardiac events over 1 year, the authors conclude that first-line use Corus® CAD may avoid unnecessary invasive cardiac tests for obstructive CAD identification compared to the traditional algorithm of stress MPI.

In 2012, the first large outcome-based randomized trial—the The Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) was launched to evaluate whether the anatomical testing with CTA as a first-line test versus functional testing, including exercise electrocardiogram, stress echocardiography, and stress magnetic resonance imaging that can improve clinical outcomes in stable, asymptomatic low-risk or intermediate-risk low-risk individuals, discern patients with possible CAD [51]. The study expects to enroll 10,000 symptomatic-referred patients under noninvasive diagnostic test for CAD from approximately 200 clinical centers in North America. The PROMISE study shortened follow-up from 4 years to one (due to budget restraints) and thus became significantly underpowered to show any differences between functional and anatomic testing for CAD. However, it is worthwhile to note that, CardioDx, Inc., as a principal partner of the study and a pioneer of cardiac genomic diagnostics, applied NGS technologies in this study to identify the expression of genes [52].

As demonstrated in the other study, using the same GES strategy for CAD study is the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) study. The PREDICT is a prospective, multicenter, observational study aimed to establish a diagnostic blood assay by real-time PCR to quantify the genes' expression of atherosclerotic CAD and stenosis measured by CTA [50,53]. Unlike other similar trails, in this study, Alexandra Lansky et al. pooled high-throughput gene expression data to validate a previously developed novel gene expression evaluation system—GES [54] to predict the likelihood of obstructive CAD. Numerous validation studies

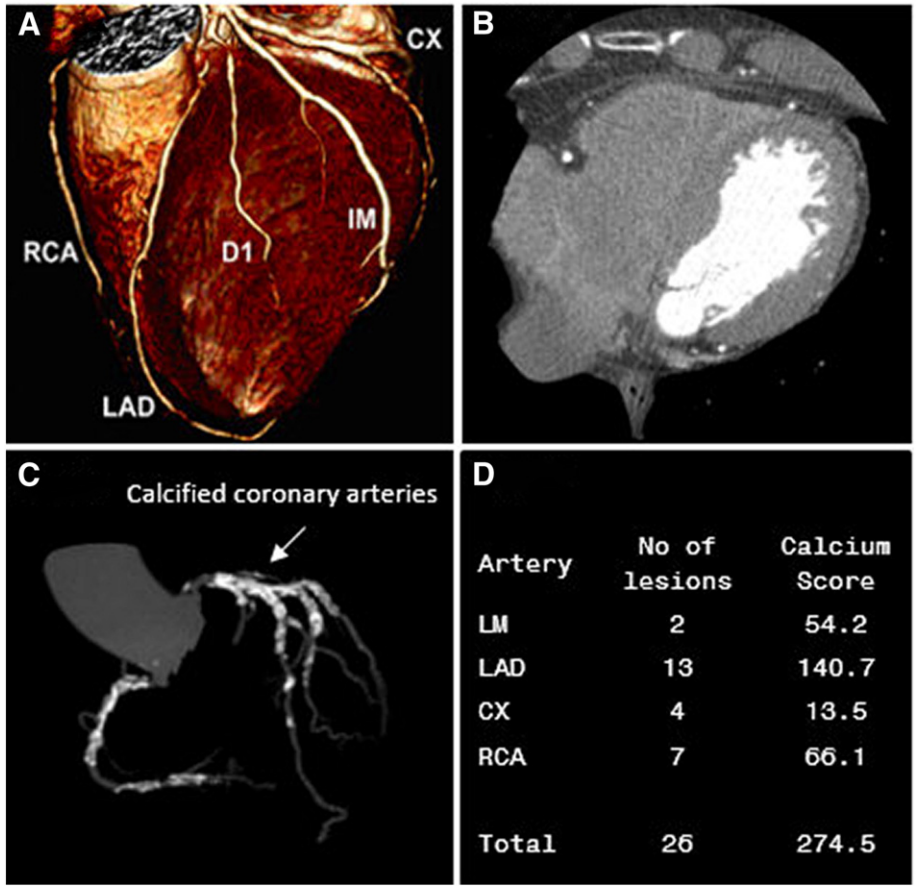

Fig. 2. Calcium Score Measurement (AHA segments classification based). (a) (top left) shows coronary arteries from CTA; (b–c) calcified coronary arteries; (d) calcium score measurement.

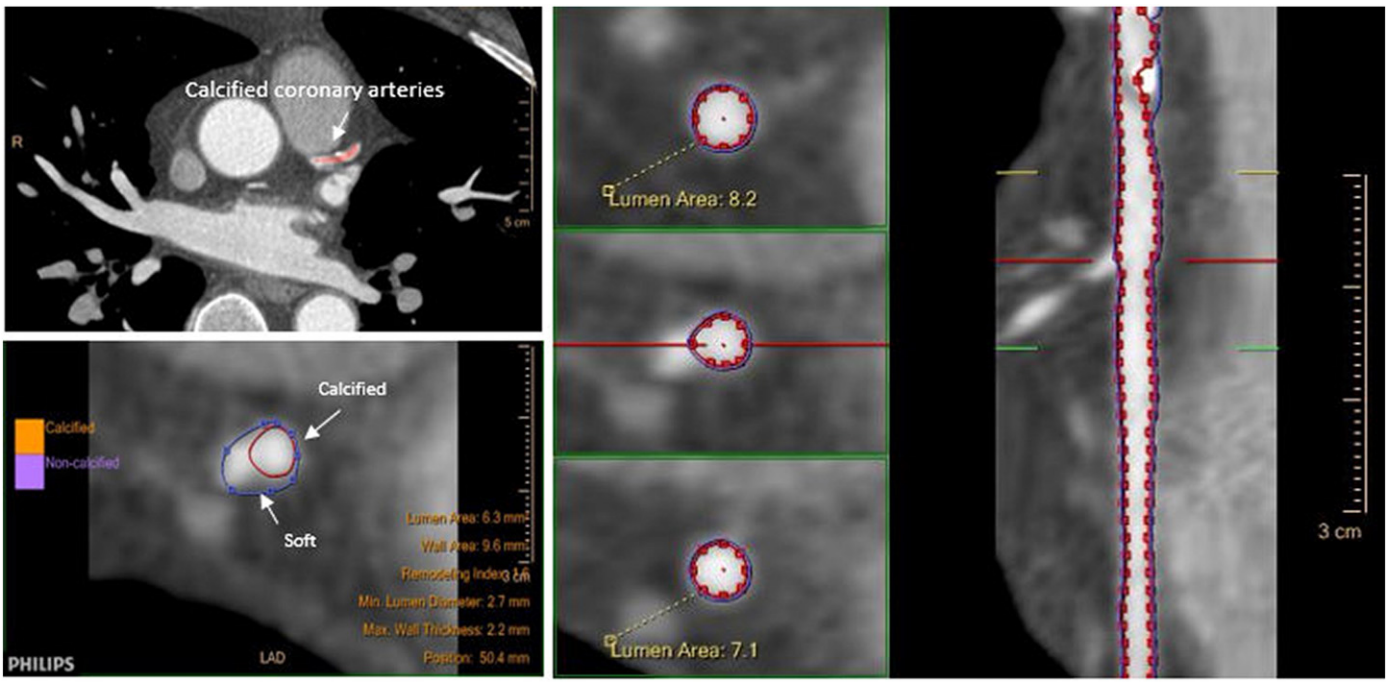

Fig. 3. Automated volumetric measurement of plaque burden and lumen area evaluation. Automated volumetric measurement of plaque burden and lumen area evaluation. Orange: Calcium plaque burden >150 HU, Purple: Noncalcium plaque burden (soft plaque — 100–30 HU; fibrous plaque: 30–150 HU).

include the association between GES and atherosclerotic plaque burden, as well as coronary luminal stenosis status (blockages or narrowing) also be performed in nearly the same period. In these validation studies, a representative cross-sectional epidemiological study from Voros et al. used clinical trial data from both PREDICT and COMPASS [55] to validate if the GES, a novel gene expression evaluation system, predicts plaque burden as measured by quantitative cardiac CTA [50]. They got an excellent dose–response relationship between GES and plaque burden and luminal stenosis. The sensitivity of low GES score and high GES scores to predict coronary artery calcification is 0.71 and 0.97, respectively. In addition, both the low GES score and high GES score had an excellent sensitivity (0.90) and specificity (0.87) for the prediction of 70% coronary luminal stenosis.

The assessment of tissue characteristics, lesion morphology, and hemodynamics by angiography with FFR, IVUS, virtual histology, and noninvasive computed tomography in atherosclerotic plaques (ATLANTA) study aims to evaluate the correlation between coronary plaque composition and peripheral gene expression [56]. Joshi et al. quantified coronary plaque volume, composition (mainly dense calcium, fibrous tissue, fibro-fatty tissue, necrotic core) and finally a peripheral blood GES was calculated. Preliminary data suggest that the increased GES was significantly associated with higher plaque volume. Beyond that, more GES also was significantly associated with a more vulnerable necrotic core plaque and dense calcium. This result suggested GES's value in qualitatively predicting obstructive coronary disease or quantitatively related plaque volume and vulnerable plaque dense calcium phenotype [57].

The AtheroRemo–IVUS (Inflammation and Vascular Wall Remodeling in Atherosclerosis–Intravascular Ultrasound Study) is a prospective, observational study, which aims to investigate the potential mechanisms behind the development of vulnerable plaques and to identify novel genetic markers of atherosclerotic plaque during its different developmental stages among patients who underwent coronary catheterization for diagnosis or exclusion of acute coronary syndrome and stable angina pectoris [58]. AtheroRemo evaluated for the first time the potential association between coronary artery histology obtained by IVUS and genetic as well as lipidomic markers. So far, the AtheroRemo revealed a few novel genomic markers of CAD [58] and novel circulating biomarkers associated with coronary plaque characteristics and CAD outcome [59]. We look forward to seeing more valuable results, such as microRNA profiling and gene expression related to the development of vulnerable plaques.

In addition, a pioneering study regarding the genetic influence on plaque characteristics was reported. Based on twin study data from Italy and the United States, Taranaki AD, et al. found that twins can inherit coronary plaque characteristics [60]. Pál Maurovich-Horvat reported a similar result. They performed coronary CTA study based on 208 twin subjects (62 MZ and 42 DZ) from the ongoing Burden of Atherosclerotic Plaques in Twins study. At European Congress of Radiology (ECR) 2015 meetings in Vienna, they report that the calcified plaque burden has a stronger heritability than a total plaque burden. It is worthy of mentioning that another interesting and significant conclusion obtained from this study is, compared with coronary calcium, a strongly heritable trait; the nonspecific plaque burden can be easily affected by the lifestyle and environment. In any case, this consequence is good news, particularly for intermediate and high-risk population intervention.

7. Integrating the gene expression profiling into genetic variation strategy—a potential countermeasure?

In the past two decades, of course, significant progress has been made in identifying the genetic causes of human disease, particularly with the help of microarray technology and its derivatives. High-throughput gene expression has been gradually used in CAD translational studies. Notably, most of the foregoing analysis, even GWAS based on DNA microarray-based static genetic profiling, such as Tag SNPs (polymorphisms) failed to show gene or genomic regulatory networks [61]. However, owing to the dynamic nature of the transcription and gene expression throughout the pathological course of the disorders, integrating dynamic gene expression profiling or transcriptional profiling into genetic variation represents a novel strategy, has a unique advantage, and may offer a valuable insight into genome architecture in unraveling potential signaling pathways and therapeutic targets of human complex disease. To date, high-throughput, whole-genome based genotyping platform such as Illumina's HumanHT-12 Gene Expression BeadChip can target more than 48,000 transcripts easily. These technological advances enabled researchers to evaluate the association of phenotypes of interest with both gene expression and genetic variations across the genome efficiently [62]. Initial studies have shown significant potential in analyzing CVD traits [63,64]. Hägg S et al. used the Stockholm Atherosclerosis Gene Expression cohort to determine whether environmental exposures will affect the arterial lipid accumulation and inflammation, thereby affecting the development of CAD. Functionally associated genes, rather than individual genes, will be meaningful for CAD development. The transcriptional profiles of multiple tissues, including liver, skeletal muscle, visceral fat, unaffected arterial wall, and atherosclerosis gathered from CAD patients' bypass surgery. The authors conclude that functionally associated genes cluster in the transendothelial migration of the leukocytes pathways, referred to as the atherosclerosis module, essential for atherosclerosis and CAD development [65]. Taurino C et al. analyzed biological pathway miRNA expression, including oxidative phosphorylation and mitochondrial function, and finally, they confirmed the value of gene expression profiling as a promising tool for discovery of CAD-related genes and biologic pathways. Although so far, limited pilot data are available, we can foresee the use of integration strategy to identify biological pathways that may provide insights into novel targets for the intervention of CAD and adverse events' prevention.

8. Concluding comments

We have summarized the current knowledge and technologies about genetics paired with CTA in the setting of atherosclerosis. With the help of technological innovations in the laboratory, physicians can quickly and accurately assess the entire human genome for genetic variations that may be partly responsible for the primary abnormalities and pathogenesis of CAD. In addition, increased knowledge of the genome and its application in translational studies of CAD will improve risk prediction and facilitate the development of novel therapies for a high-risk population. Although considerable challenges exist, advances such as high-throughput genotype platforms, including whole-exome sequencing and exon chip, screen protein coding or binding regions across the genome to make individualized medicine a reality [66]. More importantly, advances in NGS technologies provided the possibility of collecting thousands of study subjects' genotypes at a practical price point. These innovations are necessary for big data-driven cutting-edge bioinformatics products, which are emerging fields in CAD translational study. In other words, integrating NGS, whole genome exon chip, gene-expression score system and paired with anatomical and functional noninvasive image techniques is a natural complement to CAD translational study.

9. The challenge of deal with the individual's specific or abnormal genetic test results

Genetics paired with noninvasive cardiac imaging may offer at least two advantages to address the individual's specific or abnormal genetic test results. Firstly, the recommendations for follow-up period to individuals with a very high inherited risk for coronary artery atherosclerosis (personalized medicine model). Monitoring the progression of coronary calcification is an example: initial data have demonstrated

the association between Renin-Angiotensin System (RAS) gene polymorphisms [67], matrix Gla protein gene [68], and the progression of subclinical atherosclerosis in specific populations. Currently, 2–5 years of follow-up interval are considered acceptable depending on the number and severity of risk factors. However, once the signs or symptoms of coronary artery atherosclerosis appear, appropriately shortening the monitoring time interval is necessary, in particular, for individuals with inherited genetic variation. Secondly, the requirements for comprehensive interventions strategy for CAD events prevention. With the in-depth understanding of the mechanisms of epigenetic modifications, such as DNA methylation, microRNA, and histone, modification may interfere with diet, lifestyle, and environmental exposure interaction [69]. Thus, this is a reasonable assumption that comprehensive interventions for counteracting epigenetic modification, such as heart-healthy lifestyle changes, diet, exercise, medicines, and so on can be expected to bring significant benefits to the individuals at increased hereditary risk, although no clinical data are available so far.

In summary, we have briefly reviewed the advantages and limitations of the current state-of-the-art genetic research in the field of atherosclerosis. We also highlight major achievements for noninvasive imaging approaches to facilitate earlier detection, diagnosis, and prognostic evaluation of coronary artery calcification and plaque burden. CAD is a clinical disorder that encompasses a heterogeneous group and controlled by multiple genetic loci, pathways, and environmental factors. Identification of multiple contributions requires a coordinated, multidisciplinary approach, and the integration of resources includes investigators, physicians, laboratory staff, genetic statistician, and even sociologists who have an interest in genetics. The task is challenging, but it is certainly worth spending the effort and finances. The information we discussed in this review article is highly clinically relevant to cardiovascular translational study. We expect that this information will not only play an active role in filling clinical gaps in knowledge but also spark inspiration for future research.

Disclosure statement

No.

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