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CHAPTER 15

Metabolomics, Proteomics, and Genomics: An Introduction to a Clinician

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INTRODUCTION

Cardiovascular disease (CVD) remains to be a major global public-health challenge and the leading cause of mortality globally.[1] A key factor in the fight against CVD is to enhance our understanding of its pathophysiological processes. High-throughput omics technologies have revolutionized CVD research. The omics cascade starts from genomics (e.g., genes), followed by transcriptomics (e.g., RNA transcripts), proteomics (e.g., proteins), and finally the ultimate downstream product, metabolomics (e.g., metabolites) (Fig. 15.1). The advent of omics, including genotyping arrays, proteomics, and metabolomics, alone and in combination, offers unique opportunities to advance the knowledge of molecular mechanisms for CVD and the needs for clinics (Fig. 15.2).

Genomics of CVD studies the impact of DNA variation on CVD and its risk factors. Over the past decades, remarkable progress has been made in identifying and functionally characterizing genetic variants that are associated with multiple CVD conditions.[2,3] Proteomics, which studies systematic profiling of proteins, provides opportunities for unbiased discovery of novel markers to improve disease diagnostic or predictive accuracy. Proteins in the circulatory system mirror an individual's physiology. The recent high-throughput proteomics technology allows rapid identification of clinically relevant biomarkers and has been applied in studies of CVD, aging, and other diseases.[4–9] Metabolomics systematically studies small-molecule metabolites found in biologic samples such as cells, biofluids, tissues, or organisms. These small-molecule metabolites are thought to represent intermediates that profile biological status closely related to phenotypes.[10] Therefore the metabolome may provide a more accurate estimation of a disease status than that provided by genome or proteome, making metabolomics a powerful tool for revealing pathologic or etiologic pathways to complex diseases such as CVD and for monitoring treatment efficacy.

In this chapter, we first summarize the recent advances of genomics on CVD and then focus on various methodological and technological aspects related to proteomics and metabolomic profiling. We also present the integrated omics studies carried out to date on CVD, discussing the potential links that integrate metabolic and genetic studies of some common CVD, including blood pressure/hypertension, coronary heart disease, stroke, and heart failure (HF).

GENOMICS AND CVD

CVD encompasses a range of conditions, including hypertension, coronary heart disease, stroke, and HF, most of which are heritable. Enormous effort has been invested in understanding the relationship between genetic variants responsible for this heritability, including candidate gene, genome-wide association, whole-exome sequencing, and most recent whole-genome sequencing (WGS) approaches.

Candidate gene approach focuses on prespecified genes of interest, such as the causal genes for Mendelian disease. A few Mendelian disease genes have been shown to be associated with CVD,[11–21] including *LDLR* and *APOB* for severe hypercholesterolemia[12,13]; *PCSK9* for familial hypobetalipoproteinemia;[14] and *TNNT2* for hypertrophic cardiomyopathy.[20]

In contrast to candidate gene approach, genome-wide association study (GWAS) scans the entire genome for common single nucleotide polymorphisms (SNPs). SNP is a genetic variation in a single nucleotide which occurs at a specific position in the genome. In most GWASs, bi-allelic SNPs (i.e., two alleles) at appreciable degree within a population are analyzed (e.g., minor

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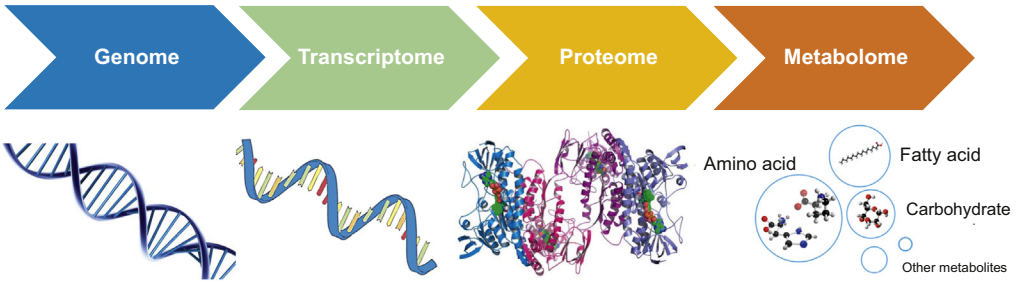

FIG. 15.1 The omics cascade

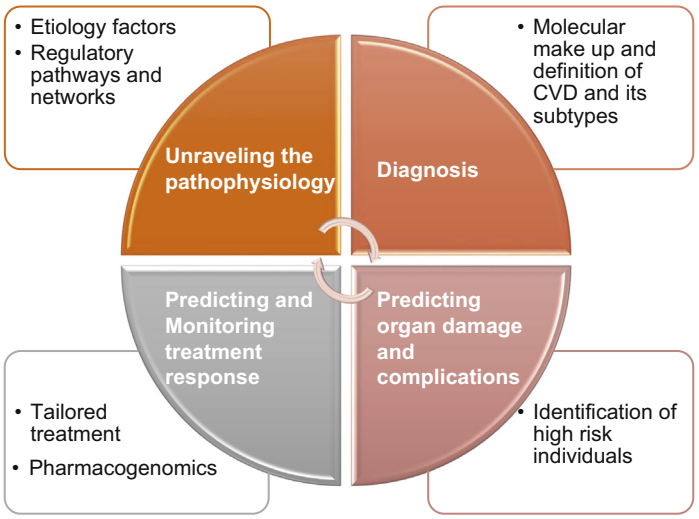

FIG. 15.2 Applications of omics technologies in cardiovascular disease research and clinical needs. *CVD*, cardiovascular disease.

allele frequency > 1%). Early GWASs identified multiple common SNPs for CVD; however, the proportion of variance explained by those SNPs was small, and the causal variants were not clear.[22] The accumulated experience and relative lack of success of initial efforts to identify novel causal variants lead to the formation of collaborative consortia on multiple CVDs to promote novel findings. Table 15.1 provides information on CVD international consortia and their hallmark GWAS work in revealing the common SNPs of specific disease.

With the emerging whole-exome sequencing technology, additional low-frequency and rare variants have been identified on CVD, including blood pressure/hypertension,[23] myocardial infarction,[24] and stroke.[25,26] Whole-exome sequencing is a genomic technique to sequence all protein-coding genes in a genome, which generates detailed catalogs of genetic variation in the protein-coding regions (i.e., both common and low-frequency/rare SNPs). For common complex traits (i.e., lipid levels), studies have demonstrated that low-frequency/rare variants tend to have more deleterious effects, which may be valuable for clinical studies to unravel protective null alleles that can serve as targets for pharmaceutical intervention. For example, targeted sequencing identified loss-of-function (LoF) variants in *PCSK9* that occur in about 3% of the population.[14] Such variants are associated with a low LDL cholesterol level. It has been reported that Black carriers of one of two mutations in *PCSK9* (Y142X and C679X) and White carriers of *PCSK9* R46LL allele have reduced susceptibilities to myocardial infarction,[14,27] implicating *PCSK9* as an attractive therapeutic target. Recent large randomized control trials have

TABLE 15.1
International Consortiums on Cardiovascular Diseases

Consortium	Full Name	Website	Key Paper[a]
ICBP	International Consortium for Blood Pressure	https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000585.v2.p1	Ehret G. B., 2016[112]
CARDIoGRAMplusC4D	Coronary ARtery DIsease Genome-wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics Consortium	http://www.cardiogramplusc4d.org/	Nikpay, M., 2015[113]
ISGC	International Stroke Genetics Consortium	http://www.strokegenetics.org/	Neurology Working Group of the CHARGE Consortium, 2016[114]
HERMES	**HE**art Failu**R**e **M**olecular **E**pidemiology for Therapeutic Target**S**	http://www.hermesconsortium.org/	Ongoing

[a]Most recent published hallmark paper: first author, year, and reference number.

shown that using PCSK9 inhibitor on a background of statin therapy can lower LDL cholesterol levels and further reduce the risk of cardiovascular events.[28]

Most recently, WGS, which allows for a comprehensive view of the sequence of the human genome, has been implemented in CVD research. Morrison et al.[29] applied integrated methodologic steps to interrogate WGS data to characterize the genetic architecture of heart- and blood-related traits. Aggregate tests of low frequency and rare variation identified multiple motifs that were associated with two CVD risk factors, namely, lipoprotein(a) and cardiac troponin T levels, and demonstrated the use of WGS data for characterizing the genetic architecture of complex traits.

PROTEOMICS AND CVD

Proteomic Profiling: Approaches and Technological Platform

The earliest approaches for protein detection were electrophoresis and liquid chromatography. They were widely used in plasma proteomic measures before mass spectrometry (MS); however, the resolution of these two methods is limited to the most abundant proteins.[30] MS is a powerful tool for systematic detection of the full set of proteins present in body fluid, e.g., plasma.[31,32] However, MS-based plasma proteomic profiling is challenging for a few reasons, including difficulty to capture high abundance proteins and lack of reproducible, robust, and high-throughput proteomic workflows.[30] Instead, multiplexed affinity methods have been increasingly applied for plasma proteomics.[33] Affinity-based or targeted assays were developed based on antibodies to target specific proteins[34] and currently are the gold standard for clinical protein analysis. Examples of important immunoassays for CVD research include troponins and natriuretic peptides.[35] Affinity-based immunoassays overcome the limitations for detection of low-abundance proteins and high sample throughput. However, they cannot discover proteins that are not targeted by the assay and can potentially be influenced by coding DNA variants on epitope structures and affinity of reagents. Recently, single-stranded DNA aptamers have been developed as alternative affinity reagents to antibodies to overcome the limitations of immunoassays.[36] Single-stranded DNA aptamers are nucleotides of ~50 base pairs in length which are selected for their ability to bind target proteins or peptides with high specificity and affinity.[37]

The Proteomics Biomarkers for CVD

Although there is a long history for proteomics research, studies in human beings are still limited. For CVD, most proteomic studies to date have been based on experimental models.[38–40] With the advent of commercially available aptamer microarrays, population cohort studies are emerging to explore proteomic profiling of CVD. We highlight the major findings in the following sections and expect that a wave of studies will produce multiple candidate proteins for further testing, as well as genomics influence on human proteome, in the near future.

One of the first proteomics studies on CVD using an aptamer assay was published for risk prediction. Ganz et al. derived and validated a nine-protein score to predict 4-year probability risk of myocardial infarction, stroke, HF, and all-cause death among patients with CHD, using large-scale analysis of circulating proteins.[8] Assessment of circulating biomarkers to predict adverse CVD events among at-risk patients is clinically important. The nine proteins, including troponin I, matrix metalloproteinase-12, and angiopoietin-2, were combined into a score that was reproducibly associated with an increased risk of adverse events. The authors reported that the performance of this risk score was better than the Framingham Risk Score, but still, it only achieved modest discrimination (area under the receiver operating characteristic curve [AUC] at 0.70 compared with 0.64 for a clinical score) highlighting the complexities of clinical risk prediction.

Another landmark study published at about the same time used aptamer-based proteomic platform to identify early protein biomarkers of myocardial injury. Ngo et al.[41] reported that 217 proteins were significantly changed in the peripheral vein blood among patients who underwent alcohol septal ablation for hypertrophic cardiomyopathy, a model of planned myocardial injury in which each patient serves as his/her own biological control.[42] Seventy-nine out of 217 proteins were validated in an independent cohort, including Dickkopf-related protein 4 (a WNT pathway inhibitor) and cripto (a growth factor important in cardiac development). Out of 217, 156 significant proteins were associated with Framingham Risk Score, including aminoacylase 1 and trigger factor 2. The authors also developed a novel workflow integrating DNA-based immunoaffinity with MS to analytically validate aptamer specificity. The scalability of this approach was examined by Jacob et al. using an expanded proteomic platform to investigate a broader range of human proteins for myocardial injury.[43] Despite the promising results, further work is warranted to characterize the clinical relevance of these proteomic markers.

A recent study explored plasma proteome in relationship with the survival of pulmonary arterial hypertension (PAH). Rhodes et al.[44] reported that 20 proteins differentiated survivors and nonsurvivors in patients with idiopathic or heritable PAH. Nine proteins, including interleukin-1 receptor-like 1 (IL1R1/ST2), tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), plasminogen, apolipoprotein-E (ApoE), erythropoietin, complement factor H and factor D, and insulin-like growth factor–binding protein-1, were independent of plasma N-terminal pro-brain natriuretic peptide (NT-pro-BNP), a classic biomarker correlating PAH survival. The functions of these proteins relate to, but are not limited to, myocardial stress, inflammation, pulmonary vascular cellular dysfunction, and structural dysregulation. A cutoff-based score using the panel of nine proteins improved AUC from 0.83 (for REVEAL risk score[45]) to 0.91 and reclassification indices without detriment to calibration. Identification of circulating proteins among patients with PAH, independent of existing clinical assessments, might have a use in clinical management and the evaluation of new therapies.

Future Directions

The emerging proteomics technologies open for an unbiased discovery of novel biomarkers, biomarker profiles, and therapeutic targets. The initial studies largely focused on high-risk individuals with existing CVD conditions. There is a need for accurate cardiovascular risk prediction in the general population. By applying proteomics to large population cohorts, ongoing efforts aim to discover novel cardiovascular biomarkers and highlight potential pathways. Furthermore, genomics findings on proteomic markers are largely focused on well-know proteins, such as troponins, NT-pro-BNP, and lipoprotein particles. Deep integration of genomics and proteomics in the future may provide additional novel cardiovascular biomarkers and characterize the genetic and environmental determinants of protein profiles.

METABOLOMICS AND CVD

Metabolomic Profiling: Approaches and Technological Platform

The human metabolome includes thousands of small-molecule metabolites,[46] but the total number of possibly detectable metabolites is unknown, and the entire metabolome has yet to be fully covered. There are two major distinct technological approaches, "untargeted" and "targeted", for metabolite measurements.[47,48] The scope of these two types of analysis is different, and they both have advantages and disadvantages. The targeted metabolomic approaches enable the absolute quantification of metabolites in the sample. However, it does not enable the discovery of unknown compounds, as it means to measure an a priori defined group of chemically characterized metabolites (e.g., lipids). In contrast, the untargeted metabolomic approaches aim to analyze all the measurable analytes in a sample including unknown chemicals. There are concerns about the semiquantitative nature of the untargeted approach

(i.e., lack of absolute quantification), but it has notable advantages for detecting and semiquantifying (relative quantification) as many metabolites as possible in a biological sample. Therefore untargeted approaches are especially useful for finding novel mechanisms or biomarkers, whereas targeted approaches are great tools for follow-up pathway analyses because of a higher degree of sensitivity and easy identification of compounds. Several review papers have described and contrasted these platforms and approaches.[49,50]

At present, there are two major instrument platforms for measuring metabolite levels in biological samples, namely nuclear magnetic resonance (NMR) and chromatography and MS-based metabolic profiling.[51–53] Both techniques enable high-throughput profiling of large numbers of metabolites simultaneously within a sample, whereas each has unique analytical strengths and weaknesses. NMR spectroscopy technique identifies metabolites by chemical shifts in resonance frequency and provides detailed information about solution-state molecular structures based on atom-centered nuclear interactions.[54] NMR spectroscopy has several advantages, including that it is robust, requires minimal sample preparation, costs low per measurement, has high reproducibility, and has the nondiscriminating and nondestructive nature of the technique.[55,56] However, NMR spectroscopy has a limited sensitivity and can only detect metabolites at medium-to-high levels of abundance although NMR spectroscopy is also quantitative.[47] Alternatively, MS-based metabolomics identifies metabolites based on their mass to charge ratio (m/z) and provides highly selective and sensitive quantitative analyses.[57] Although samples can be infused directly into the mass spectrometer, the more common procedure is initially separating metabolites using chromatography (gas chromatography, liquid chromatography, or ultra-performance liquid chromatography) to facilitate further analyte identification and quantification.[58] Techniques based on different platforms (such as gas chromatography–MS and liquid chromatography-MS [LC-MS]) are better at detecting specific metabolites, making the integration of these techniques desirable to comprehensively study the metabolome. Moreover, such parallel utilization of these serial platforms provides a high sensitivity in identifying and analyzing metabolite components with concentrations as low as femtomolar range.[59]

The Metabolomic Biomarkers for CVD

Large-scale metabolomic profiling, for instance "metabolome-wide" studies, may identify metabolic changes occurring in the process of organ damage even before the appearance of disease and thereby may lead to early identification of individuals at high risk of developing disease including CVD given that a very interesting aspect of metabolomics research is to search for metabolites that could be used as clinical biomarkers of CVD. In addition, the identification of metabolomic risk profiles has the potential to improve risk stratification and explain risk disparities of CVD, for example, the substantial sex and race differences in the burden of CVD.[60] It is generally accepted that metabolite levels are the reflections of gene functional activities and environmental exposures,[61,62] making metabolome an ideal intermediate to better understand the pathophysiology and biological pathways involved in the genesis of clinical CVD events. Moreover, different clinical responses to therapy may result in different metabolite profiles, and hence metabolomic profiling could be used to identify response to therapy and improve the precision of medical management of CVD. In the past few years, various epidemiology studies using metabolomics have successfully linked metabolite levels to the etiology and progression of CVD and its risk factors in multiple ethnicities.[63–71]

Hypertension

Hypertension is an important worldwide public-health challenge[72] as it is a leading risk factor for cardiovascular diseases [73] and overall mortality.[74,75] Animal studies using hypertensive rats have pointed to metabolites, such as succinate and free fatty acids and their role in blood pressure regulation.[76,77] Recent advances in metabolomic techniques enable large-scale human studies, which showed promise in identifying metabolites that are causally linked to the pathogenesis of hypertension.[66,68] In a population of normotensive Blacks who were followed up for over 10 years, a 1-SD difference in serum metabolite 4-hydroxyhippurate (a product of gut microbial fermentation) was associated with 17% higher risk of developing hypertension.[66] Within the same study, the authors also identified a sex steroid pattern that was significantly associated with 72% higher risk (highest vs. lowest quintile) of developing hypertension. One recent study identified the association between hexadecanedioate (a dicarboxylic acid) levels with hypertension and mortality in human and demonstrated that oral hexadecanedioate intake increased blood pressure as well as vascular response to noradrenaline in Wistar-Kyoto rats.[68]

One of the most important potentials of metabolomics research in hypertension may be the monitoring of treatment responses. It was demonstrated

that changes in metabolomic profiles in response to hydrochlorothiazide treatment differed between ethnicities and were able to predict treatment success.[78] In addition, the recent development in assays for comprehensive monitoring of drug metabolites in urine has already been introduced into clinical practice to assess adherence to therapy.[79] The most recent study carried out to search for metabolic biomarkers of antihypertensive drug responsiveness compared metabolic profiles of four different antihypertensive drugs and, in turn, provided supportive evidence that linked fatty acid metabolism to human hypertension.[80]

Coronary heart disease

Tang et al. has demonstrated that circulating trimethylamine-N-oxide (TMAO) is a significant predictor for atherosclerosis, with the gut microbiome being a critical factor regulating this process.[81] A previous study has observed higher circulating levels of choline, betaine, and TMAO in individuals suffering CVD events using untargeted MS-based metabolomics approaches.[71] The catabolism of dietary betaine and choline by intestinal microbes leads to TMAO production. TMAO may promote the progression of atherosclerosis through interfering with reverse cholesterol transport, which subsequently increases the risk of cardiovascular events.[81]

Metabolomic profiling also has identified markers predicting incident CHD events, such as myocardial infarction, unstable angina, and CAD.[63,64,67,69,70] For example, Ganna et al. conducted an untargeted LC-MS analysis and successfully identified three metabolites protectively associated with lower CHD risk (lysophosphatidylcholine 18:1 and 18:2, and sphingomyelin 28:1), and 1 metabolite (monoglyceride 18:2) associated with higher CHD risk in meta-analyses of the discovery and validation samples.[67] However, in the follow-up analysis using Mendelian randomization, only a weak positive causal effect was observed for the association between monoglyceride 18:2 and CHD. In another prospective study that combined CHD and ischemic stroke using CVD as their main outcome, the authors analyzed 68 targeted metabolites including lipids, amino acids, and others in a discovery cohort and subsequently two separate replication cohorts.[70] In meta-analyses of these three cohorts, five metabolites were identified: (1) phenylalanine and (2) monounsaturated fatty acid were associated with higher risk of CVD; and (3) polyunsaturated fatty acids, (4) ω-6 fatty acid, and (5) docosahexaenoic acid were associated with lower risk of CVD.

Stroke

The study of metabolites related with stroke is becoming increasingly more important as a way to broaden our knowledge of the pathological changes that occur in cerebrovascular disease. Because many studies included stroke as one of the CVD event and studied them together as we discussed previously,[64,70,71] the number of metabolomic studies primarily focusing on linking metabolites to stroke is limited. In a Korean population, an NMR-based metabolomic approach was used to identify potential biomarkers of stroke in patients with cerebral infarction, which characterized metabolic pathways of cerebral infarction by anaerobic glycolysis, folic acid deficiency, and hyperhomocysteinemia.[82] Metabolites in relation to the progression and treatment of ischemic stroke, the most common type of stroke, have also been studied.[83,84] For example, in a study of transient ischemic attack patients, novel biomarkers of stroke recurrence was identified and replicated using metabolomic analysis, which improves the predictive power of conventional predictors such as diabetes scale and large-artery atherosclerosis.[84]

Heart failure

Despite the variety of pathophysiological factors contributing to the development and progression in HF, a long-standing concept is that the failing heart has impaired oxidative phosphorylation, depressed oxygen consumption, and compromised ATP production.[85] As a result of the profound changes that occur in energy metabolism during HF, many studies using metabolomic analyses have reported that metabolomics profiles changed among HF patients or differed across patients with different severities of HF.[65,86–89]

As previously reviewed elsewhere, the regulation of myocardial fatty acid β-oxidation and the alterations in fatty acid β-oxidation can contribute to HF.[90] Metabolomic studies have observed that multiple fatty acids altered in the blood and/or urine of HF patients, especially changes in acylcarnitine profiles.[91,92] Hunter et al.[91] identified that circulating long-chain acylcarnitines were increased in HF patients compared with non-HF controls and were greater in HF with reduced ejection fraction than in HF with preserved ejection fraction (HFpEF). Another study that used metabolomic profiling in a subset of HF patients showed that circulating C16 and C18:1 acylcarnitines were increased in patients with end-stage HF and associated with increased risk for mortality and hospitalization of HF.[92] Most recently, a prognostic metabolite profile (PMP) was derived and validated based on quantification of acylcarnitines along with amino acids and organic

TABLE 15.2
Published Review Papers on Metabolomics and Cardiovascular Diseases

First Author	Year	Journal	References
Griffin, J. L.	2011	Nature Reviews Cardiology	115
Rhee, E. P.	2012	Clinical Chemistry	49
Shah, S. H.	2012	Circulation	59
Dona, A. C.	2016	European Journal of Preventive Cardiology	116
Hunter, W. G.	2016	Current Heart Failure Reports	117
Ussher, J. R.	2016	Journal of the American College of Cardiology	118
Ruiz-Canela, M.	2017	Journal of the American Heart Association	94

acids in HF patients. Thirteen metabolite PMPs were derived where low PMPs were related to survival incremental beyond conventional predictors.[93] In addition, there are other metabolites alteration (such as amino acids and ketone bodies) that have been observed in the development of HF.[86–88] All suggest that metabolomic profiling has significant diagnostic and prognostic value for managing HF.

A recently published systematic review summarized 12 articles that have prospectively assessed the association between circulating metabolomics profile and risk of CVD events[94] and showed remarkable heterogeneous results and approaches, suggesting that the standardization of metabolomics profiling platforms, data analysis approaches, and study design is critical. A list of major published review papers on metabolomics and cardiovascular diseases was summarized in Table 15.2.

Future Directions

Metabolomic profiles provide significant insights into biological and pathophysiological pathways that may be altered during the progression of CVD. However, metabolomic profiling performed on blood or urine samples cannot inform us with organ-specific pathophysiological processes. For complex diseases such as CVD, molecular changes that occur within the heart may provide more precise information than the changes across all the organs. Therefore, future assessment of metabolism at the organ levels may supplement the measurements of changes in metabolites in blood or urine samples. Nevertheless, metabolomic signatures in blood or urine can often lead to hypothesis generation, and such hypothesis can be further verified using experimental animal study and/or causal instrument, which ultimately will enhance our understanding of disease pathophysiology.

GENOMIC-METABOLOMIC FINDINGS ON CVD

Metabolomics has been integrated with other 'omic' technologies, such as genomics, to identify novel biological pathways and understand hidden disease mechanisms. For example, linking metabolomics to CVD and identifying relevant genetic loci for CVD-related metabolites opens the possibilities of novel biomarker discovery and hypothesis testing in understanding the etiological pathways to CVD. In addition, metabolomics may serve as a bridge that enables the discovery of formerly undetected associations between genes, metabolic pathways, and disease. In this section, we first highlighted the recent genetic findings for human metabolome, and then reviewed the integration of genomic-metabolomic findings on CVD.

GWAS Findings and Post-GWAS Era

In the past few years, numerous studies have used both traditional GWAS and whole-exome/genome sequencing analyses to map genetic variations on human plasma, serum, and urine metabolites, highlighting the influence of genetic variations on human metabolome among multiple ethnicities.[95–108] Hundreds of common genetic variants on multiple human metabolites have been reported,[97–102] and a Web-based tool, Metabolomics GWAS server, is available to facilitate access to the results (http://mips.helmholtz-muenchen.de/proj/GWAS/gwas/). Of note, this resource was based on results from European ancestry population only.[97,100]

Recent studies focusing on rare and low-frequency variants with marked functional consequences demonstrated a large cumulative effect on metabolite levels. Studies have reported additional variants that modulate metabolite levels independently of the GWAS hits using exome arrays and a targeted analytical approach

for exome sequence.[103–105] Most recently, three studies have assessed the impact of rare and low-frequency variants captured by WGS on human metabolome,[106–108] including both European and African ancestries.

Integrated Genomic-Metabolomic Findings

Ever since the first GWAS with metabolomics study was conducted by Gieger et al.,[96] hundreds of genetic loci have been showed to be associated with one or multiple metabolites so far, and many of them can be further linked to clinically relevant factors of developing CVD. An early example of integrating genomics and metabolomics to promote additional findings for CVD is the story of *FADS1*. Using metabolites as the intermediate phenotypes, *FADS1* was identified as a risk locus for multiple phospholipids, key components of serum lipids.[96] At that time, the association of *FADS1* variants and lipid levels was not strong enough to reach genome-wide significance. Two years later, with significantly increased sample sizes, a GWAS on lipid parameters confirmed the prediction of *FADS1* being a lipid risk locus.[109]

In addition to common variants identified by GWASs, sequencing analyses on rare/low-frequency functional variants and metabolite levels combined with follow-up CVD events can help establish a paradigm for defining the pathophysiology of disease. For example, in a whole-exome sequencing study of African-American population, Yu et al. identified a LoF variant in *SLCO1B1* that was associated with increased levels, a metabolite, which for the first time, was reported for its relationship with HF risk.[105] Hexadecanedioate, a long-chain dicarboxylic acid, was also reported to be significantly associated with increased blood pressure and mortality.[68,105] The aforementioned genetics and metabolomics evidences together implicated a potential pathway for HF.

The integration of genetics and metabolomics can be particularly useful in the context of a focused pathway analysis. We discussed earlier how TMAO linked host metabolism with microbiome metabolism and its association with atherosclerosis and risk of cardiovascular events. Follow-up animal studies have now demonstrated that the *FMO3* gene contributes to variations of TMAO levels in mice. Specifically, there was a relationship between *FMO3* gene expression and plasma TMAO levels, and the mice that expressed *FMO3*, in turn, had increased susceptibility to atherosclerosis.[108]

The integrated approach can also be helpful in monitoring the drug response. In the subsection of metabolomics and hypertension, we highlighted that metabolite profiles that change in response to treatment with hydrochlorothiazide were able to predict treatment success.[78] The authors integrated their metabolomics findings with genomic data and thus identified genetic markers that predicted response to hydrochlorothiazide.[110]

CONCLUSION

Single biomarkers are not sufficient to interpret or characterize complex biological phenomena such as CVD, and new multiomics approaches recognize the importance of characterizing and integrating the interrelation—genomic, proteomic, and metabolomic "fingerprint" of disease and preclinical disease states. In the emerging "systems biology" area, a more comprehensive approach integrating not only genomics and metabolomics but also epigenetics and transcriptomics together provided an accurate picture of biological pathways. A recent system-based study analyzing gene variations, metabolic quantitative trait loci, expression quantitative trait loci, and metabolomics together identified a novel pathway of endoplasmic reticulum (ER) stress in CVD pathogenesis.[111] This ER stress pathway, represented by elevated levels of circulating short-chain dicarboxylacylcarnitine metabolites, would not have been identified from a single platform approach. In the future the integration of data from multiple techniques could help identify pathways that are involved in disease progression, improve early diagnosis, and personalize treatment plan. We expect that comprehensive network linking genetic susceptibility to proteomic and metabolomics changes of CVD will be established.

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