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Comprehensive Metabolomic Characterization of Coronary Artery Diseases

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ABSTRACT

BACKGROUND Pathogenesis and diagnostic biomarkers for diseases can be discovered by metabolomic profiling of human fluids. If the various types of coronary artery disease (CAD) can be accurately characterized by metabolomics, effective treatment may be targeted without using unnecessary therapies and resources.

OBJECTIVES The authors studied disturbed metabolic pathways to assess the diagnostic value of metabolomics-based biomarkers in different types of CAD.

METHODS A cohort of 2,324 patients from 4 independent centers was studied. Patients underwent coronary angiography for suspected CAD. Groups were divided as follows: normal coronary artery (NCA), nonobstructive coronary atherosclerosis (NOCA), stable angina (SA), unstable angina (UA), and acute myocardial infarction (AMI). Plasma metabolomic profiles were determined by liquid chromatography–quadrupole time-of-flight mass spectrometry and were analyzed by multivariate statistics.

RESULTS We made 12 cross-comparisons to and within CAD to characterize metabolic disturbances. We focused on comparisons of NOCA versus NCA, SA versus NOCA, UA versus SA, and AMI versus UA. Other comparisons were made, including SA versus NCA, UA versus NCA, AMI versus NCA, UA versus NOCA, AMI versus NOCA, AMI versus SA, significant CAD (SA/UA/AMI) versus nonsignificant CAD (NCA/NOCA), and acute coronary syndrome (UA/AMI) versus SA. A total of 89 differential metabolites were identified. The altered metabolic pathways included reduced phospholipid catabolism, increased amino acid metabolism, increased short-chain acylcarnitines, decrease in tricarboxylic acid cycle, and less biosynthesis of primary bile acid. For differential diagnosis, 12 panels of specific metabolomics-based biomarkers provided areas under the curve of 0.938 to 0.996 in the discovery phase (n = 1,086), predictive values of 89.2% to 96.0% in the test phase (n = 933), and 85.3% to 96.4% in the 3-center external sets (n = 305).

CONCLUSIONS Plasma metabolomics are powerful for characterizing metabolic disturbances. Differences in small-molecule metabolites may reflect underlying CAD and serve as biomarkers for CAD progression.
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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

AUC = area under the curve

CAD = coronary artery disease

NCA = normal coronary artery

NOCA = nonobstructive coronary atherosclerosis

OPLS-DA = orthogonal projection to latent structure-discriminant analysis

ROC = receiver-operating characteristic

SA = stable angina

UA = unstable angina

VIP = variable importance in the projection

Coronary artery disease (CAD) remains a leading cause of mortality worldwide. According to the Global Burden of Disease Study 2013, CAD was responsible for an estimated 8.14 million deaths (16.8%) globally that year (1). On the basis of clinical symptoms, extent of arterial blockage, and myocardial injury, CAD is divided into different categories: nonobstructive coronary atherosclerosis (NOCA), stable angina pectoris (SA), unstable angina pectoris (UA), and acute myocardial infarction (AMI) (2). UA and AMI are also referred to as acute coronary syndrome (ACS).

If the molecular mechanisms of CAD could be deciphered, its incidence and associated mortality might be reduced. Multiple, complex molecular events characterize the progression of CAD. Atherosclerosis, a common cause of angina and AMI, is a slow and complicated process (3). What exactly causes plaque, how plaque develops over time, and why plaque dislodges to form a clot are largely unknown. Experimental models of atherogenesis provide information about the molecular mechanisms of plaque growth. Nevertheless, the transition from coronary stability to instability is less well understood because animal models of this progression are unavailable (4).

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With early screening and differential diagnosis of CAD, optimal patient-specific therapies can be initiated. The current clinical diagnosis differentiates between the types of CAD on the basis of symptoms, electrocardiogram (ECG), cardiac markers, stress testing, and coronary angiography (5-7). Among these methods, invasive coronary angiography is the diagnostic "gold standard" (8), but its specialized technology and high cost limit it to a select population (5). On the one hand, a sizable portion of individuals who underwent invasive angiography had been shown to have normal coronary arteries (9). On the other hand, episodes of myocardial ischemia or infarction are possible after atypical symptoms in some patients with CAD, especially in patients who are elderly or have diabetes (10).

Abnormal metabolism also characterizes CAD. Metabolic alterations in the heart result in changes in the metabolome of biofluids (11). Metabolites could clarify pathogenesis for potential therapeutic targets. A combination of multiple small-molecule metabolites may offer excellent diagnostic values (12). Metabolomics, a rapidly expanding field in systems biology, measures metabolic alterations in response to disease progression (12). Plasma, a frequently considered pool of metabolites, has been a source for metabolic profiling (13). Liquid chromatography mass spectrometry provides the most compatible technique for sensitive detection of small-molecule metabolites with robust reliability and reproducibility (14,15). This work describes a comprehensive metabolomic evaluation for identifying types of CAD.

METHODS

PATIENTS AND STUDY DESIGN. Patients enrolled from center 1 (the Affiliated Wujin Hospital of Jiangsu University, Changzhou, China) between August 2009 and December 2015 formed the discovery and test phases. Subjects recruited at 3 other centers constituted the external validation phase between January 2014 and December 2015 (center 2, the First People's Hospital, Nantong, China; center 3, Northern Jiangsu People's Hospital, Yangzhou, China; and center 4, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Inclusion criteria were symptoms of chest pain, cardiovascular risk factors, ischemic changes in ECG, or elevated myocardial enzymes. Coronary angiography was required to confirm the diagnosis. We excluded patients with aortic dissection, pulmonary embolism, malignant tumor, autoimmune disorders, severe infectious diseases, trauma, a recent surgical procedure, severe heart failure with left ventricular ejection fraction <20%, liver dysfunction (alanine aminotransferase level >135 U/l), severe renal dysfunction (creatinine >3.0 mg/dl), or blood-borne infectious diseases, including human immunodeficiency virus/acquired immunodeficiency syndrome, hepatitis B, and hepatitis C. We also excluded patients with myocarditis, pericarditis, and Takotsubo cardiomyopathy. Informed consent was obtained from all patients. This study was performed under the guidance of the Helsinki Declaration and was approved by all centers.

Plasma samples were collected before the coronary angiography surgery and were immediately frozen at −80°C for metabolomic analyses. Acetonitrile was chosen as the optimal extraction solvent over methanol, ethanol, methanol/ethanol (1:1), and methanol/acetonitrile/acetone (1:1:1). To ensure data quality for metabolic profiling, pooled quality control samples were prepared by mixing equal amounts of plasma (10 μl) from 82 patients with normal coronary arteries and 125 patients with CAD. Detailed sample preparation methods are in the Online Appendix.

DEFINITION OF CORONARY ARTERY TYPES. The diagnosis was made on the basis of symptoms,