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Plasma Phospholipids and Sphingolipids Identify Stent Restenosis After Percutaneous Coronary Intervention

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ABSTRACT

OBJECTIVES The aim of this study was to evaluate the diagnostic utility of plasma metabolomic biomarkers for in-stent restenosis (ISR).

BACKGROUND ISR remains an issue for patients after percutaneous coronary intervention. Identification of biomarkers to predict ISR could be invaluable for patient care.

METHODS Next-generation metabolomic profiling was performed in the discovery phase from the plasma of 400 patients undergoing percutaneous coronary intervention. In the validation phase, targeted analysis was conducted using stable isotope dilution–multiple reaction monitoring mass spectrometry in another independent group of 500 participants.

RESULTS A set of 6 plasma metabolites was discovered and validated for the diagnosis of ISR as early as 1 month after percutaneous coronary intervention. This biomarker panel classified patients with ISR and control subjects with sensitivity of 91% and specificity of 90% in the discovery phase. The diagnostic accuracy in the independent validation phase was 90% (95% confidence interval: 87% to 100%). The defined 6 metabolites all belong to sphingolipid and phospholipid metabolism, including phosphatidylcholine diacyl C36:0, phosphatidylcholine diacyl C34:2, phosphatidylinositol diacyl C36:4, phosphatidic acid C34:1, ceramide, and sphingomyelin diacyl 18:1/20:1. These biomarkers play essential roles in cell signaling that regulates the proliferation and migration of vascular smooth muscle cells.

CONCLUSIONS Next-generation metabolomics demonstrates powerful diagnostic value in estimating ISR-related metabolic disturbance. The defined plasma biomarkers provide better early diagnostic value compared with conventional imaging techniques. (J Am Coll Cardiol Intv 2017;■:■–■) © 2017 by the American College of Cardiology Foundation.

With the rapid advancement of interventional cardiology over the past decade, percutaneous coronary intervention (PCI) has become the treatment of choice for atherosclerotic coronary artery disease revascularization. The major limitation of PCI is in-stent restenosis (ISR), which is characterized by intimal hyperplasia, smooth muscle cell proliferation, and vascular renarrowing (1).

The rate of ISR with bare-metal stents ranges from 20% to 30% (2) and with drug-eluting stents from 5% to 10% (3). Noninvasive blood biomarkers for the

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ABBREVIATIONS AND ACRONYMS

ISR = in-stent restenosis

IVUS = intravascular ultrasound

MRM = multiple reaction monitoring

PCI = percutaneous coronary intervention

QCA = quantitative coronary analysis

S1P = sphingosine-1-phosphate

VIP = variance in projection

VSMC = vascular smooth muscle cell

identification of restenosis-prone patients could potentially alter decision making regarding treating patients with multiple stents or offering more prolonged antiplatelet therapy to patients at higher risk for ISR.

Although the molecular etiology of ISR remains unclear, previous studies have identified several associations between changes in cellular metabolism and the occurrence of ISR (4,5). This suggests a potential clinical application of an ISR-specific metabolome for diagnostic purposes. Metabolomics identifies a large number of metabolites in the biological systems and their changes associated with pathophysiological conditions. A few metabolic biomarkers have been identified for heart failure, myocardial infarction, and incident coronary heart disease (6–8).

However, data regarding metabolic biomarkers of ISR are limited. Previously, on the basis of analysis of serum samples using gas chromatography-mass spectrometry, Hasokawa et al. (9) showed that among 83 metabolites analyzed, 8 were significantly different between minor and major restenosis groups, including amino acids and sugars. However, the study targeted only a limited number of metabolites, and no validation step for the selected markers was conducted. With the development of analytic techniques, next-generation metabolomics based on liquid chromatography coupled with high-resolution mass spectrometry is more robust and sensitive, allowing the accurate detection of hundreds of metabolites simultaneously, which facilitates the discovery of novel biomarkers for early diagnosis and prevention of coronary heart diseases.

In the present study, we performed comprehensive metabolomic profiling of plasma samples from patients who had undergone PCI and developed ISR using our next-generation metabolomics platform and identified a panel of plasma metabolite markers with potential for the early prediction of ISR. In addition, we performed an independent blinded cross-validation using a separate group of 500 patients with PCI. To improve the clinical applicability of the selected biomarkers, we also quantified the absolute concentrations of these metabolites in the plasma of both control subjects and patients with ISR using stable isotope dilution-multiple reaction monitoring (MRM) mass spectrometry.

METHODS

PATIENTS AND STUDY DESIGN. The study protocol was approved by the Institutional Review Board of the Beijing Anzhen Hospital ethics committee (AZHEC2012-0516). Verbal and written consent was obtained from all subjects. The discovery phase included patients admitted for PCI to Beijing Anzhen Hospital between January 2013 and November 2013, and each patient received 1 second-generation rapamycin-eluting stent (Firebird 2, Microport, Shanghai, China). Patients were scheduled for 1- and 6-month follow-up after stent implantation. At 1 month, blood samples were drawn from each patient into lithium-heparin tubes, and plasma was obtained by centrifugation for 10 min at 1,000 g using a refrigerated centrifuge. The plasma samples were then transferred to clean tubes and stored at −80°C for further use. At 6-month follow-up, coronary angiography was performed using standard Judkins techniques. Quantitative coronary analysis (QCA; Pie Medical Imaging, Maastricht, the Netherlands) was performed by 2 experienced interventional cardiologists to evaluate restenosis, defined as angiographic stenosis ≥50%. Intraobserver variability and interobserver variability of QCA were determined. Intravascular ultrasound (IVUS; Philips Volcano, San Diego, California) was applied to verify the accuracy of the angiographic classification of ISR. Correlation analysis of QCA measurements and IVUS calculation was conducted. IVUS was also performed to guide the selection of matched control subjects. Patients without restenotic lesions were used as the control group. Participants were excluded for the presence of other major disorders, including active inflammation, renal diseases, cancer, diabetes, and liver cirrhosis. Among 400 patients enrolled in the discovery stage, 36 patients with ISR and 38 age- and sex-matched patients without ISR were used for untargeted metabolomic analysis to search for biomarkers. The remaining 326 patients were used for targeted analysis. The study flow is depicted in **Figure 1**, and baseline patient characteristics for untargeted analysis are listed in **Table 1**. Detailed procedures are described in the Online Appendix.

The validation phase of the study was composed of another independent set of patients admitted to Anzhen Hospital, affiliated with Capital Medical University, for PCI between May 2014 and June 2015. The inclusion criteria and study protocols were identical to those of the discovery phase. A total of 500 patients were enrolled in the validation stage. The experimental design flow for the validation cohort is depicted in Online Figure 1, and patient characteristics are described in Online Table 1.

UNTARGETED METABOLOMIC PROFILING. Details of untargeted metabolomic analysis are provided in the Online Appendix. Briefly, metabolites in the plasma