Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease

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Background Cardiovascular risk models remain incomplete. Small-molecule metabolites may reflect underlying disease and, as such, serve as novel biomarkers of cardiovascular risk.

Methods We studied 2,023 consecutive patients undergoing cardiac catheterization. Mass spectrometry profiling of 69 metabolites and lipid assessments were performed in fasting plasma. Principal component analysis reduced metabolites to a smaller number of uncorrelated factors. Independent relationships between factors and time-to-clinical events were assessed using Cox modeling. Clinical and metabolomic models were compared using log-likelihood and reclassification analyses.

Results At median follow-up of 3.1 years, there were 232 deaths and 294 death/myocardial infarction (MI) events. Five of 13 metabolite factors were independently associated with mortality: factor 1 (medium-chain acylcarnitines: hazard ratio [HR] 1.12 [95% CI, 1.04-1.21], *P* = .005), factor 2 (short-chain dicarboxylacylcarnitines: HR 1.17 [1.05-1.31], *P* = .005), factor 3 (long-chain dicarboxylacylcarnitines: HR 1.14 [1.05-1.25], *P* = .002); factor 6 (branched-chain amino acids: HR 0.86 [0.75-0.99], *P* = .03), and factor 12 (fatty acids: HR 1.19 [1.06-1.35], *P* = .004). Three factors independently predicted death/MI: factor 2 (HR 1.11 [1.01-1.23], *P* = .04), factor 3 (HR 1.13 [1.04-1.22], *P* = .005), and factor 12 (HR 1.18 [1.05-1.32], *P* = .004). For mortality, 27% of intermediate-risk patients were correctly reclassified (net reclassification improvement 8.8%, integrated discrimination index 0.017); for death/MI model, 11% were correctly reclassified (net reclassification improvement 3.9%, integrated discrimination index 0.012).

Conclusions Metabolic profiles predict cardiovascular events independently of standard predictors. (Am Heart J 2012;163:844-850.e1.)

Despite advances in diagnosis and treatment, cardiovascular disease is expected to remain the leading cause of death and disability in the United States and worldwide.[1,2] Effectively counseling patients about risks and directing advanced therapies to patients who are most likely to benefit from them will require advances in risk stratification. High-throughput molecular profiling techniques show potential for identifying biomarkers for better risk classification and for advancing our mechanistic understanding of the pathophysiology of cardiovascular disease. Metabolomics is the study of small-molecule metabolites that are by-products of cellular metabolism. As an emerging discipline for molecular profiling, metabolomics may increase understanding of human diseases and clinical risk because changes in metabolite levels provide a real-time estimate of disease state and reflect the integrated effects of genomic, transcriptomic, and proteomic variations.

The primary goal of the Measurement to Understand the Reclassification of Disease of Cabarrus and Kannapolis Cardiovascular Study (MURDOCK CV) is to assess the use of molecular profiles integrated with clinical data to form "clinomic" profiles for improved risk classification for clinical cardiovascular events. In this component of the MURDOCK CV study, we hypothesized that baseline metabolomic profiles would predict incident cardiovascular events in patients referred for evaluation of suspected coronary artery disease.

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Methods

Study population

Details of the MURDOCK CV study design have been published.[3] Briefly, consecutive patients undergoing cardiac catheterization at Duke University Medical Center and enrolled in the CATHGEN biorepository between 2001 and 2007 who met the eligibility criteria were identified (N = 6,466). Metabolomic profiling was performed on 2,023 of these patients consecutively enrolled from 2004 to 2007. We used fasting plasma collected in EDTA tubes via the arterial sheath at the time of cardiac catheterization, chilled it to 4°C, centrifuged it within 30 minutes, separated it into aliquots, and froze it at −80°C. Demographics, medical history, angiographic data, and longitudinal follow-up were collected through the Duke Databank for Cardiovascular Disease, which, since 1969, has archived this information on all Duke patients undergoing cardiac procedures. Follow-up included determination of mortality, myocardial infarction (MI), and coronary revascularization procedures; vital status was further confirmed through the National Death Index and the Social Security Death Index.

Both the CATHGEN biorepository and the MURDOCK CV study were approved by the Duke University Institutional Review Board. Before collection of blood samples, all study participants provided written informed consent.

Laboratory methods

Using a targeted mass spectrometry–based approach,[4] we quantitatively determined the levels of 45 acylcarnitines and 15 amino acids. Proteins were first removed by precipitation with methanol; aliquoted supernatants were dried and esterified with hot acidic methanol (acylcarnitines) or *n*-butanol (amino acids).[4,5] For analysis, we used tandem mass spectrometry with a Quattro Micro instrument (Waters Corp, Milford, MA). Quantification of "targeted" intermediary metabolites was facilitated by adding mixtures of known quantities of stable-isotope internal standards.

Five conventional analytes (total low-density lipoprotein and high-density lipoprotein cholesterol, triglycerides, and glucose), ketones (total and β-Hydroxybutyrate), and total free fatty acids (online Appendix Supplementary Table I) were assayed on a Beckman-Coulter DxC600 clinical chemistry analyzer with reagents from Beckman (Brea, CA) for conventional metabolites and from Wako (Richmond, VA) for free fatty acids and ketones. Methodology and coefficients of variation for each assay have been reported.[6,7] The laboratory (Sarah W. Stedman Nutrition and Metabolism Center Metabolomics/Biomarker Core Laboratory, Duke University, Durham, NC) was blinded to event status, and samples were randomly distributed without knowledge of event status.

Statistical methods

Given colinearity of metabolites residing in overlapping pathways, principal component analysis was used to reduce the large number of correlated metabolites to a smaller number of uncorrelated factors. Metabolites with >25% of values as "0" (ie, below the lower limits of quantification) were not analyzed (C6 and C7-DC acylcarnitines). Varimax rotation was used to produce identifiable factors; factors with an eigenvalue of ≥1.0 were retained based on the common Kaiser criterion.[8] Metabolites with a factor load of ≥0.4 are reported as composing a given factor. Scoring coefficients were constructed and used to calculate factor scores for each patient (weighted sum of the standardized metabolites within that factor, weighted on the factor loading for each metabolite).

Two primary end points were specified a priori: death (all-cause mortality any time after index catheterization) and death or MI (all-cause mortality or subsequent MI after index catheterization). *Myocardial infarction* was defined as cardiac biomarker elevation above the upper limit of normal in the appropriate clinical setting (chest pain, cardiac arrest, syncope, or other symptoms suggestive of cardiac ischemia) consistent with the universal definition of MI.[9]

We examined the univariable and multivariable associations of principal component analysis–derived metabolomic factors with death and death or MI by adding these factors to Cox proportional hazards clinical models for each end point. Models were constrained to retain all clinical covariates when metabolomic factors were added, even if they were no longer statistically significant. Multivariable models included all metabolomic factors significant in univariable analyses. Models were further adjusted for preprocedural heparin use, given the known effects of heparin on lipoprotein lipase activation[10] and our own data showing that nonesterified fatty acids and their by-products increase after heparin administration.[11] Clinical variables included in these models were as follows: modified Charlson index (excluding coronary artery disease, diabetes, and renal disease assessed individually), age, red cell distribution width, diabetes, weight, heart rate, sex, white blood cell count, chest pain frequency, corrected QT interval, ejection fraction, diastolic and systolic blood pressure, hemoglobin level, blood urea nitrogen, Duke Index (0-100 scale of coronary disease severity), smoking, left ventricular hypertrophy, creatinine, atrial fibrillation/flutter, sodium, heart failure severity, and left bundle-branch block. For the end point of death or MI, covariates included these same clinical variables except for sodium and left ventricular hypertrophy.

We examined the discriminative capability of the clinical models and the clinical models inclusive of metabolomic factors using receiver operating characteristic curves and c-indices. As a global assessment of model strength, we also compared changes in the log likelihoods of models with and without metabolomic factors. For reclassification analyses, we defined 4 categories of 5-year risk for death and death or MI (low [<5%], moderate [5%-10%], high [1%-20%], and very high [>20%]). Patients were assigned to categories according to their 5-year risk for these events predicted by the previously outlined clinical covariates. Patients were assessed for reclassification within these categories according to their predicted 5-year risks of death or death or MI as determined by the clinical model inclusive of metabolomic factors. Actual Kaplan-Meier event rates within each stratum were evaluated and used to assess correct or incorrect reclassification based on the clinical model inclusive of metabolomic factors. Overall measures of reclassification (net reclassification improvement and the integrated discrimination index, a measure not requiring categories of risk[12]) were calculated. In addition, similar reclassification analyses were performed comparing the metabolomic model with the Global Registry of Acute Coronary Events (GRACE) risk score (a measurement shown to predict mortality at 6 months in patients with acute coronary syndromes[13]).