Online Appendix: Vaidya et al.  

Independent Metabolic Syndrome Variants Predict New Onset Coronary Artery Disease

Detailed Methods

Population

The study was approved by the Johns Hopkins Institutional Review Board. Subjects gave informed consent prior to participation. The sample was identified from the GeneSTAR Study (Genetic Study of Atherosclerosis Risk), a family-based study of apparently healthy white and African American siblings (<60 years of age) of index cases admitted to 10 Baltimore area hospitals with CAD events at <60 years of age (1991 to 2000). Index case (N= 522, each defining a separate family) events included myocardial infarction (39.3%), unstable angina with coronary artery bypass surgery (1.2%), unstable angina with percutaneous coronary interventions (58.4%), or stable angina with at least one ≥50% lesion in one or more vessels demonstrated by coronary angiography at the time of a hospital admission (1.2%).

Screening and Metabolic Measures

All eligible apparently healthy siblings (N= 987) underwent a cardiovascular history and physical examination by a cardiologist at baseline. Demographic questionnaire data including self-identified race/ethnicity were collected. Blood pressure was measured at rest using the American Heart Association Guidelines (1). Hypertension was defined as the average of four blood pressure measurements ≥140/90 mmHg and/or the use of an antihypertensive medication. Height, weight, and waist circumference was measured using standardized methods described by NHANES (2). Serum glucose, total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured in the Johns Hopkins Clinical Chemistry Laboratory after subjects had fasted overnight. Low density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula (3). Fasting serum insulin was measured using immunoassay (Coat-A-Count Immunoassay, 1991-1997; or Immulite 2000 Analyzer, 1998-2000). Diabetes was defined by a fasting glucose level ≥6.93 mmol/L and/or taking a hypoglycemic agent or insulin.

Determination of New Onset Coronary Artery Disease Events

Siblings were contacted by telephone at five year intervals from 1992-2008 and completed a standardized health status and cardiovascular disease event questionnaire administered by trained interviewers. Medical records were obtained for all suspected CAD-related events or any reported procedures related to medical evaluation for coronary disease or chest pain or equivalent symptoms. Records were reviewed by two cardiologists, and a cardiovascular epidemiologist. CAD events were classified using standardized criteria from the Framingham Heart Study (4; 5). New onset CAD events included unstable and stable angina pectoris with documented luminal stenosis of at least 50% in one or more vessels with and without revascularization, myocardial infarction, or sudden cardiac death. An external adjudication committee consisting of one non-study cardiologist from Johns Hopkins and a cardiologist from another institution reviewed any event for which discordant classification occurred among the study reviewers.

Measurement of Metabolic Syndrome Variables

The metabolic syndrome variable list commenced with those used in ATP-III including, (a) waist circumference, (b) serum HDL-cholesterol, (c) serum triglycerides, (d) blood pressure, and (e) serum glucose levels. Because the other definitions of metabolic syndrome (6) and the World Health Organization (WHO) metabolic syndrome definition (7) include insulin resistance, we also included fasting insulin. To address insulin sensitivity, we used the Quantitative Check Index of Insulin Sensitivity (QUICKI = 1/[log(fasting insulin) + log(fasting glucose)])(8) This is a normally distributed variable monotonically related to the widely used Homeostatic Model Assessment of Insulin Resistance (HOMA-IR = fasting insulin×fasting glucose)/22.5) (9), which is not normally distributed.
Statistical methods:

Principal Components Analysis

The primary purpose was to identify the natural structure within the selected metabolic variable set, not to reduce the number of variables. Principal components (PC) were estimated from eigenvector decomposition of the correlation matrix. To control for within-family correlations that may bias PC estimation, we used Jong and Kotz’s (10) demonstration of the equivalence of maximizing variance along orthogonal eigenvectors and minimizing mean squared residual error in multiple linear regression. We used linear mixed model regression to estimate the family-cluster specific mean constituent variable offset as a random effect, and subtracted the cluster specific mean value of the variable before performing PC analysis. Age- and sex-adjusted residuals (z-scores) of these familial effect-corrected variables were used to estimate PCs.

The underlying pattern underlying the PC axes were interpreted based on factor loadings with absolute magnitude >0.4 (11-13). The percent of ATP-III-defined metabolic syndrome prevalence by PC-score quartile was also determined.

New Onset Coronary Artery Disease Events

The distributions of demographic and risk factor variables were examined by the presence or absence of a new onset CAD event during the follow-up period. Survival analyses were performed with age of event onset as the underlying time scale (left-truncated and right-censored distribution of age at onset), with individuals entering risk sets at the age of study entry, and exiting risk sets at CAD event or censoring. After examining Kaplan-Meier analyses by quartile of each PC score, to determine whether any graded relationship with CAD existed, the PC variants were dichotomized at the median score.

Cox proportional hazard models were used to examine new onset CAD, adjusting for sex, race, current smoking, LDL cholesterol, and blood pressure medication use. Familial clustering was corrected by estimating the standard errors by bootstrap analysis using families as sampling units.

Model Fit Analysis

The fit of the Cox proportional hazard models using different models with continuous and dichotomized PC scores, and also the ATP III-defined MetS was assessed using the corrected Akaike Information criterion (14). A difference between models of 5 AICc units was considered to be considerably different model fit, while a difference of 10 units was considered to be extremely different model fit (15).

Sensitivity Analyses

Models for CAD incidence which excluded persons with diabetes were assessed. The use of antihypertensive medications at baseline theoretically normalizes blood pressure and may reverse or eliminate its correlation with other metabolic variables.
Supplementary Results

Sample Characteristics

Table A1 shows baseline sample characteristics by new onset CAD event status.

| Table A1: Baseline sample characteristics by incident coronary artery disease (CAD) event status |
|---------------------------------------------------------------|
| **No CAD event (n=881)** | **CAD event (n=106)** | **p** |
| Age (years) | 46.4±7.0 | 49.2±5.9 | <0.001 |
| Follow-up time (years) | 10.2±3.6 | 6.3±3.6 | * |
| Males (%) | 37.9% | 61.3% | <0.001 |
| Black race | 60.3% | 34.9% | <0.001 |
| Current smoking | 31.1% | 40.6% | 0.046 |
| Systolic blood pressure (mmHg) | 133.0±15.8 | 139.7±15.6 | <0.001 |
| Diastolic blood pressure (mmHg) | 84.9±10.2 | 87.4±9.4 | 0.019 |
| Antihypertensive agents (%) | 24.9% | 31.1% | 0.16 |
| Fasting serum glucose (mg/dL) | 5.51±1.99 | 5.80±2.37 | 0.18 |
| Fasting serum insulin (μU/mL) | 9.7 [6.0 to 14.1] | 11.9 [7.1 to 18.2] | 0.008 |
| QUICKI (QUICKI units) | 0.15±0.02 | 0.14±0.02 | 0.011 |
| Hypoglycemic agents (including insulin use) (%) | 8.1% | 8.5% | 0.89 |
| Waist circumference (cm) | 95.6±15.7 | 100.2±14.7 | 0.005 |
| BMI (kg/m²) | 29.8±6.4 | 29.9±5.5 | 0.86 |
| Total cholesterol (mmol/L) | 5.73±1.25 | 6.36±1.39 | <0.001 |
| LDL-cholesterol** (mmol/L) | 3.90±1.13 (n=858) | 4.26±1.10 (n=103) | <0.001 |
| HDL-cholesterol (mmol/L) | 1.37±0.44 | 1.20±0.33 | <0.001 |
| Triglycerides (mmol/L) | 1.21 [0.86 to 1.80] | 1.48 [1.07 to 2.28] | 0.002 |

n=987, 522 families, mean±standard deviation, or median [25th to 75th percentile] for skewed distributions, QUICKI - (Quantitative Check Index of Insulin Sensitivity). *p-value not calculated because the follow-up time is till event if there was CAD event, but till administrative censoring due to end of follow-up among those without event, **calculated LDL-C using the Friedewald formula (3) excludes persons with triglyceride levels exceed 4.52 mmol/L
Principal Components Analysis of Metabolic Variables
Table A2 shows the proportion of variance in the metabolic variables explained by the five PC-derived components, as well as the eigenvector weights.

Table A2: Principal component factor weights* for metabolic variables (n=987)

| metabolic variable          | Principal component 1 | Principal component 2 | Principal component 3 | Principal component 4 | Principal component 5 |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Waist                      | .49 (.48 to .51)      | .25 (.18 to .33)      | -.46 (-.55 to -.36)   | .39 (.10 to .64)      | .58 (.38 to .75)      |
| QUICKI                     | -.50 (-.52 to -.48)   | .04 (-.06 to .14)     | .52 (.42 to .61)      | .47 (.23 to .68)      | .51 (.27 to .72)      |
| Systolic blood pressure    | .26 (.21 to .31)      | .82 (.74 to .89)      | .41 (.28 to .54)      | .07 (-.10 to .24)     | -.30 (-.37 to -.21)   |
| HDL-cholesterol            | -.45 (-.48 to -.43)   | .47 (.39 to .55)      | -.28 (-.39 to -.16)   | -.60 (-.76 to -.41)   | .36 (.08 to .62)      |
| log(triglycerides)         | .48 (.46 to .50)      | -.21 (-.31 to -.11)   | .52 (.43 to .60)      | -.52 (-.70 to -.29)   | .43 (.19 to .65)      |
| Eigenvalue                 | 2.41                  | 1.02                  | 0.71                  | 0.45                  | 0.37                  |
| Fraction of total variance explained | 49% 20%            | 14% 9%                | 7%                   |                       |                       |

*adjusted for age, sex, and family membership, QUICKI – Quantitative Insulin Sensitivity Check Index (Quantitative Check Index of Insulin Sensitivity)

Association of Metabolic Syndrome Principal Components with New Onset Coronary Artery Disease
Exploratory analysis using PCA score quartiles showed non-linear association of CAD incidence over the range of PCA scores (data not shown), thus the PCA scores were dichotomized at the median to allow for the non-linearity of association. Figure A1 shows the Kaplan-Meier incidence curves for the PC metabolic variants dichotomized at the median PC scores. Those with high PC1 scores had a greater incidence of new onset CAD over all ages (p=0.0006). Individuals with high scores along PC2, PC3 and PC4, also had significantly higher incidence of CAD events across age.
Figure A1: Age-based incidence curves for coronary artery disease for metabolic PCA variables (PC scores above median vs. scores below median, n= 987).
Model fit analysis
The Cox regression model with dichotomous PCs fit the data much better than a model with ATP-defined MetS (sample-size corrected ΔAICc=15.1) or a model with all continuous metabolic variables included (sample-size corrected ΔAICc=9.4) instead of the PCAs.

Sensitivity Analysis
Models for CAD incidence which excluded persons with diabetes showed the same results as presented above.

References for online appendix:
1. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ: Human blood pressure determination by sphygmomanometry. *Circulation* 88:2460-2470, 1993
2. NHANES: National Health and Nutrition Examination SURVEY III : Body Measurements (Anthropometry). Centers for Disease Control, Atlanta, 1988
3. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502, 1972
4. Kannel WB, Wolf PA, Garrison RJ: Monograph Section 34: Some Risk Factors Related to the Annual Incidence of Cardiovascular Disease and Death Using Pooled Repeated Biennial Measurements: Framingham Heart Study, 30-Year Followup. Springfield, MA, National Technical Information Service, 1987, p. 1-459
5. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB: Prediction of coronary heart disease using risk factor categories. *Circulation* 97:1837-1847, 1998
6. Reaven GM: Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595-1607, 1988
7. Alberti KG, Aschner P, Assal JP, Groop L, Jervell J, Kanazawa Y, Keen H, Klein R, Mbanya JC, McCarty D, Motala A, Pan XR, Ramachandran A, Samad N, Unwin N, Vardi P, Zimmet PZ: Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications : Report of a WHO Consultation Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Organization, 1999, p. 31-33
8. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85:2402-2410, 2000
9. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419, 1985
10. Jong J-C, Kotz S: On a Relation Between Principal Components and Regression Analysis. *The American Statistician* 53:349-351, 1999
11. Comrey AL, Lee HB: *A First Course in Factor Analysis*. Hillsdale, NJ, Erlbaum, 1992
12. Hanley AJ, Karter AJ, Festa A, D'Agostino R, Jr., Wagenknecht LE, Savage P, Tracy RP, Saad MF, Haffner S: Factor analysis of metabolic syndrome using directly measured insulin sensitivity: The Insulin Resistance Atherosclerosis Study. *Diabetes* 51:2642-2647, 2002
13. Niemeier HM, Phelan S, Fava JL, Wing RR: Internal disinhibition predicts weight regain following weight loss and weight loss maintenance. *Obesity (Silver Spring)* 15:2485-2494, 2007
14. Hodge AM, Boyko EJ, de Courten M, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG: Leptin and other components of the Metabolic Syndrome in Mauritius--a factor analysis. *Int J Obes Relat Metab Disord* 25:126-131, 2001
15. Burnham KP, Anderson DR: *Model selection and multimodel inference: a practical information-theoretic approach*. New York, Springer, 2002