Materials and Methods

The Penn Coronary Artery Calcification sample included predominantly European Caucasian ancestry subjects recruited from three separate parallel studies: the Study of Inherited Risk of Coronary Atherosclerosis (N=617), the Penn Diabetes Heart Study (N=711), and the Philadelphia Area Metabolic Syndrome Network (N=442). Details of each of these studies have been discussed previously. [1,2]

The Study of Inherited Risk of Coronary Atherosclerosis is a cross-sectional study of factors associated with coronary calcium in a community-based sample of asymptomatic subjects and their families. Subjects were healthy adults aged 30-75 who had a family history of premature coronary artery disease (CAD). Subjects were excluded if they reported evidence of CAD on screening questionnaire, total cholesterol higher than 300 g/dl, cigarette smoking of pack or more per day, blood pressure higher than 160/100 mmHg, had reported a history of diabetes mellitus, or had a serum creatinine >3.0 mg/dl.

The Penn Diabetes Heart Study is a cross-sectional community-based study of type 2 diabetic subjects without clinical evidence of CAD or overt chronic kidney disease. Subjects were aged 35 to 75 years; had a clinical diagnosis of type 2 diabetes (defined as fasting blood glucose >126 mg/dl, 2-h postprandial glucose >200 mg/dl, or use of oral hypoglycemic agents/insulin in a subject greater than age 40 years); and had negative pregnancy test if female. Subjects were excluded if they had evidence for clinical CAD, a clinical diagnosis of type 1 diabetes (insulin use prior to age 35), a serum creatinine >2.5 mg/dl, or weight >300 pounds.

The Philadelphia Area Metabolic Syndrome Network is a cross-sectional study of patients with one or more metabolic syndrome risk factors, as defined by the National Cholesterol Education Program Adult Treatment Panel III. Participants were recruited between 2004-2009 via the University of Pennsylvania Health System primary care providers, word of mouth in the community, and Penn health fairs for cardiovascular disease risk factors. Subjects were aged 18-75 years and had one or more metabolic syndrome risk factors. Subjects with known type 1 diabetes or clinical atherosclerotic coronary vascular disease were excluded. All Penn study protocols were approved by the Penn IRB, and all subjects provided written informed consent.

After a12-hour overnight fast, clinical parameters such as blood pressures, laboratory values, and body mass index were measured as described previously. Plasma lipids were measured enzymatically (Hitachi 912 AutoAnalyzer; Roche Diagnostics GmbH, Basel, Switzerland) in lipoprotein fractions after ultracentrifugation (B-quantification technique) at the University of Pennsylvania Center for Disease Control and Prevention-certified lipid laboratory. Subjects were classified as having metabolic syndrome using the definition of the National Cholesterol Education Program. All subjects were classified as having National Cholesterol Education program metabolic syndrome glucose criteria.

Plasma levels of ANGPTL3 and ANGPTL4 were measured according to the manufacturer's instructions (Biovendor Laboratory Medicine, Prague, Czech Republic). All samples were assayed and pooled human plasma samples were included to assess variability. Intra- and interassay coefficients of variation were 3.2% and 18.7% for ANGPTL3 and 3.8% and 17.5% for ANGPTL4. The manufacturer performed cross-reactivity analysis and found no cross-reactivity between ANGPTL3, ANGPTL4, and angiopoietin-like protein 2 assays. Testing with other commercialized ELISA kits are described in the Supplemental material. Radioimmunoassay was performed to measure insulin levels.

The statistical analysis was performed with R statistics software. Descriptive characteristics were performed for key clinical risk factors through the use of mean, median, and interquartile ranges for continuous variables and percentages for categorical variables. Plasma ANGPTL4, TG, VLDL-C, apolipoprotein C-III, fasting blood glucose, insulin, free fatty acids, leptin, and adiponectin concentrations did not follow a normal distribution and a natural logarithmic (log) transformation was performed. An unadjusted Pearson's correlation was performed to summarize associations between continuous risk factors and plasma ANGPTL3 and ANGPTL4 levels. A linear and logistic regression analysis was performed for the key clinical risk factors adjusting for age, gender, and race.

References

1. Reilly MP, Wolfe ML, Localio AR, Rader DJ. C-reactive protein and coronary artery calcification: The study of inherited risk of coronary atherosclerosis (sirca). *Arteriosclerosis, thrombosis, and vascular biology*. 2003;23:1851-1856
2. Reilly MP, Iqbal N, Schutta M, Wolfe ML, Scally M, Localio AR, Rader DJ, Kimmel SE. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. *The Journal of clinical endocrinology and metabolism*. 2004;89:3872-3878