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Original Contribution

Novel protein biomarkers associated with coronary artery disease in statin-treated patients with familial hypercholesterolemia

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BACKGROUND: Familial hypercholesterolemia (FH) is the most common and serious monogenic disorder of lipid metabolism. The incidence of coronary artery disease (CAD) varies among both treated and untreated FH patients.

OBJECTIVE: The aim of the study was to utilize proteomics to identify novel protein biomarkers that differentiate genetically confirmed heterozygous patients with FH at high CAD risk from those at low CAD risk.

METHODS: Sixty genetically confirmed FH patients were recruited and stratified into (1) asymptomatic FH with low atherosclerotic burden (FH, $n = 20$); (2) asymptomatic FH with high atherosclerotic burden (FH + Ca, $n = 20$); and (3) FH with previously confirmed symptomatic CAD (FH + CAD, $n = 20$).

RESULTS: Six new potential proteins were identified; leucine-rich alpha-2-glycoprotein (LRG1), inter-alpha-trypsin inhibitor heavy chain H3, complement C4-B (C4B), complement C1q subcomponent subunit B (C1QB), monocyte differentiation antigen (CD14), and histidine-rich glycoprotein (HRG). There were significant associations between gender and C4B ($Z = 2.31$, $P = .021$), C1QB ($Z = 2.49$, $P = .013$), CD14 ($Z = 2.17$, $P = .03$), and HRG ($Z = 2.14$, $P = .033$). There were significant associations between smoking and LRG1 ($\chi^2_2 = 6.59$, $P = .037$), CB4 ($\chi^2_2 = 7.85$, $P = .02$), and HRG ($\chi^2_2 = 6.11$, $P = .047$). All the peptides were significantly associated with advanced CAD stages, independently of age and smoking. However, the absence of the proteins was the strongest marker. The most accurate association with CAD was HRG (area under the receiver operating characteristic curve = 0.922), whereas LRG1, C4B, and C1QB were also associated with CAD (area under the

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receiver operating characteristic curve >0.9). For either coronary atherosclerosis or CAD, LRG1, C4B, C1QB, and HRG were relatively well associated.

CONCLUSIONS: The present study has identified 6 novel protein biomarkers that are associated with more advanced stages of atherosclerotic disease and subsequent coronary events in patients with heterozygous FH.

Introduction

Familial hypercholesterolemia (FH) is the most common and serious monogenic disorder of lipid metabolism[1,2] with a worldwide prevalence of at least 1 in 300.[3] It is caused by mutations in the low-density lipoprotein (LDL) receptor gene, the apolipoprotein B gene, or the proprotein convertase subtilisin/kexin type 9 gene.[4–6] These mutations result in significantly elevated LDL cholesterol levels that cause premature atherosclerotic coronary artery disease (CAD).[7]

However, FH remains a frequently under diagnosed cause of CAD, and of those diagnosed, many are inadequately treated.[8] In addition, the incidence of CAD and life expectancy varies among patients with both treated and untreated FH.[9–11] Untreated, 50% of male FH patients and 20% of female FH patients develop fatal coronary heart disease by 60 years of age. Although treatment with statins more than halves the risk of coronary events in adults with FH,[12] treated asymptomatic FH patients display significant variability in the extent of subclinical coronary atherosclerosis despite the use of aggressive statin therapy.[9] Current known plasma biomarkers, in addition to classical risk factors, do not explain the residual CAD risk in people with FH. Indeed, the large variation in CAD incidence within the FH population suggests there are other factors, in addition to elevated cholesterol, that may play a role in development of atherosclerosis in FH. There is an urgent need for improved cardiovascular screening in asymptomatic individuals; however, the development of novel markers to identify cardiovascular risk must add to the prognostic value provided by standard risk markers.[13,14]

In the past decade, quantitative proteomic techniques including isobaric tag for relative and absolute quantification (iTRAQ) have been used to identify novel biomarkers in several disease states, including CAD.[15,16] Using isotope-labeled molecules, iTRAQ allows for the quantification of multiple proteins from various sources, in a single experiment.[17] Previous iTRAQ studies have shown differences in expected CAD-associated proteins, including those involved in inflammation, coagulation, and lipid metabolism,[15,16] whereas other studies have identified novel predictors.[15] To date, no such study has investigated the use of iTRAQ proteomics in predicting CAD risk in an FH population.

Therefore, the aim of the present study was to use proteomics to identify candidate protein biomarkers that may differentiate genetically confirmed FH patients at high CAD risk from those with low CAD risk.

Materials and methods

Study population

Sixty FH patients (40–70 years) from the Vascular Genetics Outpatient Clinic at the Erasmus MC were recruited. All participants had a genetically confirmed mutation in the LDL receptor gene. The 60 patients were selected and stratified into 3 subgroups; (1) asymptomatic FH with a low atherosclerotic burden as defined a coronary diseased segment score (DSS) of 0 (FH, $n = 20$); (2) asymptomatic FH with a high atherosclerotic burden as defined by a coronary DSS >7 (FH + Ca, $n = 20$); and (3) FH with previously confirmed symptomatic CAD (myocardial infarction, percutaneous coronary intervention, or coronary bypass surgery) (FH + CAD, $n = 20$). Exclusion criteria included a secondary cause of hypercholesterolemia and renal, liver, and thyroid disease. Within the asymptomatic groups, additional exclusion criteria included symptoms of CAD, history of CAD, renal insufficiency (serum creatinine >120 mmol/L), known contrast allergy and atrial fibrillation. The study was conducted in line with the Declaration of Helsinki. All patients gave a written informed consent and the study protocol was approved by the Erasmus MC Ethical Review Board.

Coronary computed tomography angiography

Coronary computed tomography angiography (CCTA) scan protocols and outcomes have previously been described.[18] Briefly, all asymptomatic FH patients underwent CCTA to determine their atherosclerotic burden. Scans were performed on a dual source CT scanner (Somatom Definition, Siemens Medical Solutions) and analyzed separately by 2 experienced readers blinded to the patient's status. Coronary calcium was measured in Agatston units using dedicated software.[19] In addition, using a modified 17 coronary segment model,[20] the percentage of maximum luminal diameter narrowing was visually estimated and graded as either 0%, 1% to 20%, 21% to 50%, 51% to 70%, or >70%. Based on the narrowing per segment, 3 scores were then used; (1) the DSS, granting 1 point for each narrowing >20%; (2) the CAD severity score, granting 1, 2, or 3 points per segment narrowing of 21% to 50%, 51% to 70%, and 70%,