Clinical validation of protein components of high-density lipoproteins as novel biomarkers for cardiovascular disease and diabetes

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Current state of research in the field

According to many clinical and epidemiological studies low levels of high-density lipoprotein cholesterol (HDL-C) are associated with increased risks of cardiovascular diseases (CVD) and type 2 diabetes mellitus (T2DM).[1,2] HDL particles exert diverse anti-oxidative, anti-inflammatory, anti-thrombotic and cytoprotective effects, which suggest direct anti-atherogenic and anti-diabetic roles.[3,4,5] Furthermore, the development of atherosclerotic lesions or hyperglycaemia could be decreased or even reverted in several animal models by transgenic overexpression or exogenous application of apolipoprotein A-I (apoA-I), i.e. the most abundant protein of HDLs.[3,4,5] For LDL-cholesterol (LDL-C) this type of epidemiological and biological evidence has been successfully translated into drugs that lower CVD risk.[6] To date, however, drugs increasing HDL-C such as fibrates, niacin, cholesteryl ester transfer protein inhibitors and infusions of reconstituted HDLs have failed to prevent CVD endpoints.[1,7] Moreover, in several inborn errors of human HDL metabolism and genetic mouse models with altered HDL metabolism, the changes in HDL-C levels were not associated with the opposite changes in CVD risk and atherosclerotic plaque load expected from epidemiological studies.[1,4] It is important to note that previous intervention and genetic studies targeted LDL-C and HDL-C that is the cholesterol measured by clinical laboratories in LDLs and HDLs. By contrast to the disease causing cholesterol in LDLs, which after internalization turns macrophages of the arterial intima into pro-inflammatory foam cells, the cholesterol in HDLs neither exerts nor reflects any of the anti-atherogenic activities of HDLs.[4] HDL-C is only a non-functional surrogate marker for estimating the HDL pool size without deciphering the heterogeneous composition and functionality of HDLs.[1,4] As a result, there is a great need for biomarkers that reflect the functionality of HDLs better than HDL-C.

HDLs are heterogeneous and complex macromolecules carrying hundreds of lipid species and proteins.[4] Many of these molecules are not passive cargo but biologically active and contribute to the anti-atherogenic or anti-diabetic properties of HDLs. This physiological heterogeneity is further increased in HDLs of patients, for example with CVD or T2DM, by the loss or structural modification of typical HDL constituents or by the acquisition of atypical constituents.[8] As a consequence, HDL loses physiological functionality and gains pathological dysfunction. Detailed knowledge of structure-function-relationships of HDL-associated molecules is a pre-requisite to test them for their relative importance in the pathogenesis of CVD and T2DM and to exploit them for treatment and diagnostics.

Research findings leading to the project

Recently, the lab of the applicant together with partners from the UZH and ETH addressed the complexity of HDLs through a systems medicine approach.[9] HDL particles were isolated from plasma of 50 healthy subjects as well as 110 patients with coronary heart disease (CHD) and/or T2DM. The protein and lipid abundances were characterized by mass spectrometry. In addition, the capacity of HDLs to induce cholesterol efflux from macrophages, to inhibit the apoptosis of endothelial cells (ECs) and pancreatic beta cells and to promote mitochondrial potential and respiration in skeletal myotubes and brown

adipocytes was recorded. Data on 182 proteins and 227 lipids species as well as 14 functional read-outs of HDLs were integrated by probabilistic models to identify molecules, which define the functionality of HDLs and differentiate HDLs of patients and controls. Bioinformatic modelling led to the identification of several proteins and lipid species that are associated with disease status or HDL function. Both HDL_{CHD} and HDL_{T2DM} were deprived of apoA-IV but enriched with pulmonary surfactant protein B. Interestingly, apoA-IV has been recently identified as a binding protein of sphingosine-1-phosphate (S1P), which elicits several of HDL's protective functions.[10] Moreover, apoM, the canonical S1P binding protein, as well as several sphingomyelins, which are precursors for S1P, were inversely associated with the presence of T2DM. Also of note, the atypical sphingomyelin SM42:3, the precursor of an atypical 18:2-S1P, was the strongest determinant of HDL's ability to inhibit EC apoptosis, which in turn was decreased in HDL_{T2DM}. In a previous study, my lab identified both the typical 18:1-S1P and the atypical 18:2-S1P as determinants of HDL's ability to inhibit EC apoptosis.[11] Finally unpublished work of our lab showed that apoM, 18:1-S1P and 18:2-S1P content in HDLs are strong and significant determinants of changes in HDL's ability to inhibit EC apoptosis upon bariatric surgery.

Research aim

The aim of this project is the clinical validation of selected biomarker candidates, which were identified by the studies described above. We have already started the analysis of lipids by a broad lipidomics approach. As such a broad approach is not feasible for the analysis of proteins, at least not within the financial scope of the AGLA award, I want to focus on proteins that have significant association with disease status, that either directly or indirectly affect HDL functions and for which good quality immunoassays are available. These criteria are fulfilled by apoA-IV and apoM.

Methods

To validate whether HDL-associated apoA-IV and apoM are potential useful biomarkers of CHD or T2DM and are predictive for the development of major adverse cardiovascular events (MACE) and T2DM samples from the VIVIT study (Profs. Drexel and Säly, Feldkirch, Austria) will be analysed. The VIVIT study is an observational cohort study that includes nearly 600 patients that were enrolled between November 1999 and October 2000 while they underwent coronary angiography for the evaluation of CHD. Clinical examinations were performed at enrolment and every two years thereafter during 10 years follow-up. The study was approved by the Ethics Committee of the University of Innsbruck (Austria) and all participants gave written informed consent. Because of the extensive clinical characterization at baseline and the systematic follow-up, both cross-sectional and longitudinal data analyses are possible.

Concentrations of apoA-IV and apoM will be measured in apoB-free plasma samples of the VIVIT study using specific immunoassays (a commercial kit from Abcam for apoA-IV and an ELISA provided by Roche for apoM[12]). ApoB-free plasma is considered a surrogate for

HDLs and obtained by the precipitation of VLDLs and LDLs with 20% polyethylenglycol. Univariate and multivariate statistical analyses as well as regression analyses will be performed to test the association of apoA-IV and apoM with gender, smoking and presence of CHD, T2DM or the metabolic syndrome and to unravel any correlation with CVD risk factors such as age, lipids or renal function. Kaplan-Meier survival and Cox-proportional hazard analyses will be used to investigate the association of HDL-associated apoA-IV and apoM with incidence of MACE and T2DM. Additionally, interactions between apoA-IV/apoM and S1P and other sphingolipids will be modelled with respect to their diagnostic and prognostic performance.

Potential significance

HDL-associated proteins, which are confirmed by the proposed validation studies to be associated with CHD/MACE or T2DM, would have a great potential to become diagnostic biomarkers, either alone or in combination with other known cardiovascular risk markers, for the identification, personalized treatment stratification and monitoring of patients at increased risk for CVD and T2DM. Additionally, these proteins may also serve as therapeutic targets. This can be the molecule itself, for example by integration into HDL-like nanoparticles, or it can reflect metabolic pathways that lead to the formation of protective molecules.

References

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