Title: Protein Dense Lipoproteins: The unimagined biomarker for human health assessment

Authors: Scott Altmann and Steven Fried

Institute: HDL Apomics

Introduction: An unforeseen and extensive role of high-density lipoproteins (HDL) in human health and disease is gradually emerging. Constrained in part by the continued use of traditional biomarkers such as HDL-C, HDL-P and apoA-1, there is an evolving appreciation that these HDL measurements no longer provide sufficient clinical utility and that new HDL metrics are needed. HDL is comprised of >100 proteins and ~200 lipid species in an undetermined number of combinations resulting in extensive particle diversity and HDL population heterogeneity. These two attributes are critical to HDL physiology and pose several challenges to their measurement. Molecular diagnostics capable of capturing the intricacy of particle population dynamics is the gateway to true Precision Medicine for a broad variety of diseases and multiplex antibody arrays may be one of the few means to get there.

Experimental Procedures: A decade-long effort to identify the HDL proteome by mass spectrometry has cast new light on a variety of widely studied plasma proteins. Many of these proteins already have commercially available Luminex xMAP assay kits. However, review of these xMAP kit instructions reveal protocols designed to disrupt and denature particle proteome members prior to quantification. As a result, all relational context derived from particle macromolecular assembly is lost, as well as the ability to monitor important physiological consequences ascribed to specific subpopulations.

Instead, our approach employs the opposite tack by leaving the particle population undisturbed to take advantage of both HDL proteome conformation and contextual content. This process makes sole use of “Homebrew” assay development to generate a collection of “mAb Pair Proximity Assays” that grows geometrically with each new antibody added. The measurement theory enables one to triangulate the location of proteome members allowing the paratope pair to serve as surrogate markers for specific particle subpopulations.

Creating “mAb Pair Proximity Assays” is an iterative progression that utilizes both commercial and proprietary antibodies in a two-step process. Screening and identification begin with the analysis of the unmodified mAb in both the capture and detection role prior to microsphere coupling or biotinylation. Once formulated, combinatorial antibody pairing is performed using various sources of analyte samples to focus identification of the target particle subpopulation. Both steps utilize the multiplex capacity of xMAP which condenses large complicated screening paradigms into a manageable experimental process while also providing key validation techniques.

Results/Conclusion: Development of ~200 novel mAb Pair Proximity Assays has generated xMAP assays capable of distinguishing specific and distinct HDL particle subspecies. Analysis of plasma and cerebral spinal fluid has also produced unique epitope proximity maps permitting PDL proteome interactome comparison between two biofluids regularly used for biomarker discovery and molecular diagnostics. Redefining the particle and how it is measured can guide clinical diagnostics for coronary artery disease, Alzheimer’s disease other HDL-related diseases. For such diseases, a shift in drug development and therapeutic intervention toward a Precision Medicine model is needed.