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Resolution of Apolipoprotein A1 and A2 Proteoforms: Their Cardiometabolic Correlates and Implications for Future Research

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Abstract

Purpose of Review—A "**proteoform**" is defined as one specific protein structural form that results from the combination of allelic variation, alternative RNA splicing, and/or post-translational modifications (PTMs) in specific locations on the amino acid backbone. Apolipoproteins A1 and A2 are highly abundant apolipoproteins that mediate HDL structure and function. ApoA1 and A2 are known to undergo PTMs, which results in multiple proteoforms. However, the catalogue of apoA1 and A2 proteoforms as well as their associations with cardiometabolic health characteristics has not until recently been described. In this brief review, we discuss recent efforts to catalogue the spectrum of apoA1 and A2 proteoforms, to understand the relationships between the relative abundance of these proteoforms with cardiometabolic phenotypic characteristics, and we will discuss the implications of these findings to future research.

Recent Findings: A broad spectrum of apoA1 and A2 proteoforms has been characterized. Although, the types of apoA1 and A2 proteoforms are consistent across individuals, the relative abundances of proteoforms can vary substantially between individuals. Proteoform-specific associations with cardiometabolic characteristics in humans, independent of absolute apolipoprotein abundance, have been described. These recent findings suggest multiple levels of protein structural variation that arise from known and unknown metabolic pathways may be important markers or mediators of cardiometabolic health.

Summary: Understanding the associations between apolipoprotein proteoforms and phenotype may lead to enhanced understanding of how apolipoproteins mediate lipid metabolism and affect ASCVD risk, which may lead to discovery of novel markers of risk and/or key mechanistic insights that may drive further druggable targets for modifying lipid metabolism and reducing ASCVD risk.

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Keywords

Proteoform; Apolipoprotein A1; Apolipoprotein A2; Cardiometabolic Health

Introduction

Apolipoproteins are a structurally diverse family of serum proteins involved human lipid transport and metabolism and several are mediators of atherosclerosis and down-stream ASCVD events (among other clinical endpoints)[1]. Apolipoprotein A1 (apoA1) is a highly abundant apolipoprotein in blood that is the primary structural protein of High-Density Lipoprotein (HDL) particles[2, 3]. ApoA1 mediates several of HDL particles' atheroprotective functions, like cholesterol efflux capacity, and triglyceride/cholesterol exchange[4–6]. ApoA2 is the second most abundant HDL-associated protein, with a less well described role in HDL constitutive function, lipid homeostasis, and ASCVD pathogeneses[7–10].

Structural variation in apolipoproteins A1 and A2 is well described. In addition to rare missense mutations that lead to differences in the amino acid backbone, more commonly observed post-translational modifications (PTMs) have been described and several studies suggest that PTMs alter protein function and observed phenotype[10–15]. However, the complete spectrum of PTMs, the combinations of PTMs, as well as their effects upon observed phenotype have not, until recently, been well systematically characterized. Understanding the potential mediating effects of different PTMs (and all their combinations) may lead to enhanced understanding of how apolipoproteins (apolipoproteins A1, A2, and others) mediate lipid metabolism and affect ASCVD risk, which may lead to discovery of novel markers of risk and/or key mechanistic insights that may drive further druggable targets for modifying lipid metabolism and reducing ASCVD risk.

In this brief review, we discuss recent efforts to catalogue the spectrum of structural variation in apoA1, A2, to understand the relationships between the relative abundance of these variants with cardiometabolic phenotypic characteristics, and we will discuss the implications of these findings to future research.

Proteoform Definition and Significance

Variation in protein primary structure is the result of allelic variation in the protein coding gene, alternative mRNA splicing, and PTMs. Any one protein-coding gene can yield many different structural forms that ultimately determine phenotype. A "**proteoform**" is defined as one specific protein structural form that results from the combination of allelic variation, alternative RNA splicing, and/or PTMs in specific locations on the amino acid backbone[16]. For example, an individual who is heterozygous for a missense mutation in the APOA1 gene would create two mRNA strands that are translated into two amino acid backbones that vary by one amino acid. Each of these amino acid strands represents a distinct proteoform of apoA1. Some of these amino acid strands then undergo PTMs, which furthers their variation and structural complexity. In the case of apoA1, about 1 to 2% of apoA1 undergoes acylation at the lysine 88 residue (K88AcylA1), 3–20% has the terminal glutamine residue truncated, 4–25% is oxidized, 0.2–2% is glycated, among other PTMs that occur at discrete

amino acid residue and in different combinations[14]. Each one of these resultant distinct structural variants of apoA1 represents an apoA1 proteoform. Thus far, we have identified 15 different combinations of PTMs on the amino acid backbones, and one participant was an allelic heterozygote with a missense mutation with the identical compliment of PTMs that were observed on wild type apoA1, resulting in a total of 30 proteoforms of apoA1[14].

Post-translational modification occurs because of non-enzymatic or enzymatically mediated addition (or subtraction) of ligands to the protein that are related to the exposome, metabolome, proteome, and genome. These additions often alter protein function[17], which may ultimately affect HDL function, lipid metabolism, and potentially ASCVD risk. For example, glycation (a spontaneous addition of glucose to an amino acid residue) of apolipoprotein A1 (apoA1) has been shown to reduce apoA1's anti-inflammatory properties as well as its phospholipid affinity, which may increase ASCVD risk[18, 19].

Broadly speaking, resolution of proteoform-level structure (and thus understanding of the PTM's along an amino acid backbone) can be important for several reasons: 1) Since PTMs can affect protein localization, function, and overall phenotype, characterizing diverse proteoform profiles can reveal associations that cannot be observed through measures of total protein concentration alone; 2) proteoform-level resolution may reveal novel biologic and potentially mechanistic insights into the determinants of phenotype; and 3) measurement of proteoforms provides direct evidence of interactions between the proteome and the metabolome, exposome, and other aspects of the proteome (and by extension the genome) enhancing our understanding of how genetic, the environmental factors, and metabolism directly affects protein structure and function.

Proteoform-level characterization can also be used be used in clinical practice. For example, one of the most well-known (and commonly measured) proteoforms is hemoglobin A1C (HgbA1c), a proteoform of hemoglobin characterized by the relative amount (expressed as a percentage) of the hemoglobin protein beta chain that has undergone glycation (non-enzymatic addition of glucose)[20]. The relative abundance of this proteoform is an accurate and precise marker of longitudinal serum glucose levels and it provides clear evidence of a direct interaction between the metabolome and the proteome, given the concentration-dependent nature of glycation in this instance[21]. In contrast, measurement of total hemoglobin concentration alone (without proteoform-level resolution) provides information about the oxygen carrying capacity of blood, but no information about mean glucose serum level nor its interaction with serum proteins. This example suggests that proteoform level characterization of the proteome may provide novel biological insights and new clinical diagnostics and/or prognostic markers.

Techniques Use to Characterize and Quantify Apolipoprotein Proteoforms

Commercially available affinity-based proteomic technologies (e.g., aptamers and proximity extension assays) do not allow for proteoform-level characterization. Similarly, traditional bottom-up proteomic approaches (protein digest followed by mass spectrometric analysis) are excellent at profiling the spectrum of proteins present in a sample and to some extent they can identify post-translational modification on peptide fragments, but they cannot provide proteoform-level resolution[22]. However, top-down proteomic techniques allow

for the full characterization *and quantification* of proteoforms. In contrast to bottom-up techniques, top-down initially keeps proteins intact, fragments them in the gas phase, and utilizes tandem mass spectroscopy that allows for precise measurement of protein mass-shifts, which equal the mass of all chemical groups added to the amino acid backbone[23]. These diverse mass-shifted protein variants are then isolated and fragmented, which produces protein fragments that have individual masses and mass-shifts. The mass shifts of different protein fragments allow for the determination of the *location* of different PTMs. Then, through bioinformatic interrogation of these spectra the full secondary structure and quantities of all proteoforms present in the sample can be resolved[24, 25].

Although top-down approaches provide greatly enhanced insight into protein structural variation compared to bottom up for affinity-based platforms, this improved resolution using current, though rapidly evolving, technology comes at the expense of limited breadth of protein assessment and throughput. However, when specific protein targets that serve as either a marker or mediator of phenotypes of interest are known, as is the case for apolipoproteins, top-down approaches can provide novel insights into protein structure and biology.

Due to the central roles of apolipoproteins A1 and A2 in HDL metabolism, their well-known associations with ASCVD risk, as well as their high abundance in human serum we have focused on characterizing their proteoforms and quantifying their associations with cholesterol fractions, HDL function, markers of insulin resistance, and other cardiovascular health traits.

Apolipoprotein A1 and A2 Proteoform Characterization and Clinical Correlates

ApoA1 is the primary structural and functional protein of high-density lipoprotein (HDL) particles[2]. ApoA1 is synthesized in hepatocytes and small intestinal cells as a 30.7KD proto protein that undergoes terminal truncation into a mature canonical variant. ApoA1 serves as a binding ligand and cofactor for many HDL functions including cholesterol efflux and triglyceride/cholesterol exchange with VLDL particles. HDL particles also have a role in vasodilatory, anti-inflammatory, and anti-thrombotic pathways as well. These functions may explain the strong associations observed between apoA1 total protein concentration and ASCVD risk[26]. However, to date randomized controlled trials of HDL-C-raising therapies (which also raise apoA1) as well as Mendelian randomization studies do not support a direct causal association between apoA1 and ASCVD risk[27, 28]. Nonetheless, HDL's central role in lipid metabolism strongly supports its role in cardiovascular risk factor development. Further, independent of its causality for ASCVD, HDL-C and apoA1 are well validated, robust, and useful biomarkers used in ASCVD risk estimation[29–31]. Therefore, more detailed understandings of its role in mediation of lipid metabolism may enhance our understandings of high cardiometabolic risk.

Post-translational modification of the apoA1 canonical proteoform is a well described, though incompletely characterized, event. *In vivo* and *in vitro* studies suggest that apoA1's biological effects are at least partially mediated by PTM. For example, an apoA1 proteoform (W72-Oxidized A1) was isolated in abundance from human atheroma but was found in very low abundance in human serum[11]. Higher quantities of this proteoform were associated

with lower HDL functionality characterized by HDL efflux capacity. Similarly, glycation of apoA1 has also been shown to reduce anti-inflammatory properties and other athero-protective characteristics[18, 19].

Until the advent of top-down proteomic approaches, resolution of apoA1 proteoforms – the catalogue of detectable forms in serum, their relative abundance, as well as their associations with phenotypic characteristics of interest was not known. However, in separate recent studies, using human serum samples from Chicago Healthy Aging Study and the Coronary Artery Risk Development of Young Adults Cohort study, Wilkins, Seckler and colleagues characterized the spectrum of apoA1 proteoforms and quantified their associations with cardiometabolic characteristics of interest[14, 15]. Up to 30 distinct proteoforms of apoA1 were identified, and PTMs included a canonical or unmodified variant and combinations of truncation, oxidation, glycation, and/or acylation on the amino acid backbone[14]. The most abundant form identified was the canonical, or unmodified, apoA1 proteoform. Other variations of the amino acid backbone included a truncated variant (missing the c-terminal glutamine), and the pro-peptide that contained the 6-resideue-longer propeptide of apoA1. Post-translational modifications observed included glycation, methionine sulphoxidations, as well as 7 different acylated variants at the K88 residue, which occurred on both canonical and truncated amino acid backbones (K88AcylA1). Interestingly, these protoform profiles were preserved across all 150 individuals, though their relative abundance varied. The intra-individual relative abundances were also relatively stable over 5 years of follow-up[14].

When their associations with participant characteristics were assessed, the apoA1 proteoforms clustered into 5 motifs: canonical, glycation, truncations, oxidations, and K88acylA1. Glycation had the expected positive association with serum glucose level and prevalent DM. Interestingly, the relative abundance of K88acylA1 was directly associated with HDL-C, HDL efflux capacity and inversely associated with elevated triglyceride level, waist circumference, and BMI. Whereas the relative abundance of the canonical variant had the opposite pattern of association (canonical was inversely associated with HDL-C and positively associated with waist circumference). This finding suggests that the patterns of association between total apoA1 and cardiometabolic characteristics (generally inverse associations with markers of insulin resistance) may be driven by the relative abundance of the K88acylA1 and not the canonical proteoform. Interestingly, the fatty acids that were bound to the lysine 88 residue are the most abundant fatty acids in HDL particles and apoA1 is known to exist in bound and unbound forms. Thus, K88acylA1 may be a marker of apoA1's association with the HDL particle, and the overall metabolic activity of the HDL subfraction.

ApoA2 is the second most abundant protein on HDL particles. It serves a role in HDL particle stabilization. It is also a competitive antagonist to apoA1 and mediates functions of lecithin-cholesterol acyltransferase and hepatic lipase that use apoA1 as a cofactor[7, 9]. ApoA2 is an approximately 11KD protein synthesized in the liver and intestine. It is known to exist in various truncated forms as both monomers and disulfide linked dimeric forms. In the CARDIA study, ApoA2 proteoforms that were characterized by terminal truncation and dimerization were differentially associated with cardiometabolic characteristics[14]. Based on these observations Seckler and Wilkins posited that terminal glutamine truncation and

dimerization may be involved with activating apoA2's role in lipid metabolism. Therefore, cysteinylation of monomeric chains (which is needed for dimerization of this molecule) may regulate dimerization and thus affect its HDL-associated function. Importantly, none of the side chain PTM's that were seen on apoA1 were present on apoA2, suggesting that the PTMs observed are not an occurrence for all HDL-associated proteins, furthering the inference that apolipoprotein PTM is a specific, regulated, non-random process.

Implications

Proteoform level characterization of apolipopoproteins A1 and A2 provides proof of concept that proteoform level resolution may provide novel insights into apolipoprotein metabolism that could not be derived by measures of total protein abundance alone. ApoA1 and A2 proteoform characterization suggests a potential role of glycation, oxidation, truncation, and acylation in the mediation of apolipoprotein function.

Studies of apolipoprotein proteoforms conducted thus far consistently suggest that proteoforms vary by apolipoprotein as there is minimal overlap (other than terminal glutamine truncation) in the types of PTM on apoA1 and apoA2, despite both being associated with the HDL fraction. Furthermore, the interindividual proteoform abundance can vary substantially and this variation is associated with markers of insulin resistance and ASCVD risk. Thus, apolipoprotein proteoform profile appears to be a non-random, specific, dynamic, and likely regulated process. Furthermore, proteoform resolution of apoA1 and apoA2 provide direct evidence of specific interactions between the proteome and metabolome. For example, the specific and reproducible acylation of apoA1 at the lysine 88 residue suggests a regulated biochemical interaction between fatty acids and apoA1, potentially mediated by a previously uncharacterized acyl transferase enzyme. Likewise, although the drivers of oxidation were not well understood, it could be due to environmental and behavioral characteristics that were not well characterized in the CARDIA or CHAS studies.

In this era of high-throughput genomic, epigenomic, proteomic, and metabolomic profiling on thousands of research participants integrating these datasets and understanding their interactions to weave together a cohesive understanding of the biology of health and disease is a major challenge. The findings discussed above support the notion that obtaining proteoform resolution, when possible, will enhance our understanding of human biology and may provide novel marker of risk and targets for therapy.

Future Directions

Post-translational modification is not limited to apoA1 and apoA2, as several studies – utilizing different methods to identify PTM – have described a broad range of PTMs of apoC3[32–34], apoB[35], and apoE[36] suggesting that PTM occurs, at least to some extent, on all human apolipoproteins. A catalogue of these proteoforms and an understanding of their associations with characteristics in humans is needed to begin this investigation into the role of post-translational modification in the regulation of apolipoprotein function.

The association studies discussed above do not allow for causal inference; apoA1 and apoA2 proteoforms may be the cause or consequence of cardiometabolic characteristics.

Clarifying the precise mechanistic roles of apolipoprotein protoeforms will require detailed studies that manipulate apolipoprotein proteoform abundance and assess the effect upon outcomes. Such studies will require the development of techniques that allow for synthesis, isolation, and manipulation of apolipoprotein proteoform levels. Furthermore, investigation into apolipoprotein proteoform profile across different tissue types (vascular endothelium, valve tissue, hepatic tissue etc.) may also provide mechanistic insights and targets for intervention.

Although the associations between cardiometabolic characteristics and apoA1 and A2 proteoform relative abundances were striking, further work will need to be completed to assess their associations with different clinical endpoints including ASCVD. However, direct associations with ASCVD events are not a prerequisite to demonstrate a significant role of apolipoprotein proteoforms in the regulation of lipid metabolism and cardiometabolic health. For example, associations with other incident or prevalent disease states like diabetes, kidney disease, and heart failure, may yield insights into the causes of these central and highly prevalent determinants of cardiovascular risk.

In summary, variation in protein structure and abundance that results from allelic variation in a protein coding gene is just one of many sources of variation within the proteome. PTMs are ubiquitous, specific, and regulated processes that modify protein structure and function. This level of protein regulation must be understood if we are to understand the role of the proteome in mediating phenotype. Top-down proteomic techniques can provide proteoform resolution (and knowledge of PTMs along the protein backbone) of specific protein targets, which may enhance our understanding of the biology of human proteins. Work thus far on apolipoproteins A1 and A2, suggest multiple levels of protein structural variation that arise from known and unknown metabolic pathways may be important mediators of cardiometabolic health. However, continued research is needed to further unlock this level of proteomic structure and its complex interactions with other genomic, metabolomic, and environmental causes of health and disease.

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References

1. Collaboration* TERF. Major Lipids, Apolipoproteins, and Risk of Vascular Disease. Jama. 2009;302:1993–2000. [PubMed: 19903920]

2. Pourmousa M, Song HD, He Y, Heinecke JW, Segrest JP and Pastor RW. Tertiary structure of apolipoprotein A-I in nascent high-density lipoproteins. Proceedings of the National Academy of Sciences of the United States of America. 2018;115:5163–5168. [PubMed: 29712830]

3. Davidson WS and Thompson TB. The structure of apolipoprotein A-I in high density lipoproteins. The Journal of biological chemistry. 2007;282:22249–53. [PubMed: 17526499]

4. Wilkins JT and Seckler HS. HDL modification: recent developments and their relevance to atherosclerotic cardiovascular disease. Current opinion in lipidology. 2019;30:24–29. [PubMed: 30531230]

5. Lund-Katz S and Phillips MC. High density lipoprotein structure-function and role in reverse cholesterol transport. Sub-cellular biochemistry. 2010;51:183–227. [PubMed: 20213545]

6. Fazio S and Pamir N. HDL Particle Size and Functional Heterogeneity. Circulation research. 2016;119:704–7. [PubMed: 27587407]

7. Melchior JT, Street SE, Andraski AB, Furtado JD, Sacks FM, Shute RL, Greve EI, Swertfeger DK, Li H, Shah AS, Lu LJ and Davidson WS. Apolipoprotein A-II alters the proteome of human lipoproteins and enhances cholesterol efflux from ABCA1. Journal of lipid research. 2017;58:1374–1385. [PubMed: 28476857]

8. Fullerton SM, Clark AG, Weiss KM, Taylor SL, Stengard JH, Salomaa V, Boerwinkle E and Nickerson DA. Sequence polymorphism at the human apolipoprotein AII gene (APOA2): unexpected deficit of variation in an African-American sample. Human genetics. 2002;111:75–87. [PubMed: 12136239]

9. Castellani LW, Nguyen CN, Charugundla S, Weinstein MM, Doan CX, Blaner WS, Wongsiriroj N and Lusis AJ. Apolipoprotein AII is a regulator of very low density lipoprotein metabolism and insulin resistance. The Journal of biological chemistry. 2008;283:11633–44. [PubMed: 18160395]

10. Xiao J, Zhang F, Wiltshire S, Hung J, Jennens M, Beilby JP, Thompson PL, McQuillan BM, McCaskie PA, Carter KW, Palmer LJ and Powell BL. The apolipoprotein AII rs5082 variant is associated with reduced risk of coronary artery disease in an Australian male population. Atherosclerosis. 2008;199:333–9. [PubMed: 18179799]

11. Huang Y, DiDonato JA, Levison BS, Schmitt D, Li L, Wu Y, Buffa J, Kim T, Gerstenecker GS, Gu X, Kadiyala CS, Wang Z, Culley MK, Hazen JE, DiDonato AJ, Fu X, Berisha SZ, Peng D, Nguyen TT, Liang S, Chuang C-C, Cho L, Plow EF, Fox PL, Gogonea V, Tang WHW, Parks JS, Fisher EA, Smith JD and Hazen SL. An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med. 2014;20:193–203. [PubMed: 24464187]

12. Haase CL, Frikke-Schmidt R, Nordestgaard BG and Tybjærg-Hansen A. Population-based resequencing of APOA1 in 10,330 individuals: spectrum of genetic variation, phenotype, and comparison with extreme phenotype approach. PLoS genetics. 2012;8:e1003063-e1003063.

13. Ladias JA, Kwiterovich PO Jr., Smith HH, Karathanasis SK and Antonarakis SE. Apolipoprotein A1 Baltimore (Arg10----Leu), a new ApoA1 variant. Human genetics. 1990;84:439–45. [PubMed: 2108924]

14. Wilkins JT, Seckler HS, Rink J, Compton PD, Fornelli L, Thaxton CS, LeDuc R, Jacobs D, Doubleday PF, Sniderman A, Lloyd-Jones DM and Kelleher NL. Spectrum of Apolipoprotein AI and Apolipoprotein AII Proteoforms and Their Associations With Indices of Cardiometabolic Health: The CARDIA Study. Journal of the American Heart Association. 2021;10:e019890.

15. Seckler HDS, Fornelli L, Mutharasan RK, Thaxton CS, Fellers R, Daviglus M, Sniderman A, Rader D, Kelleher NL, Lloyd-Jones DM, Compton PD and Wilkins JT. A Targeted, Differential Top-Down Proteomic Methodology for Comparison of ApoA-I Proteoforms in Individuals with High and Low HDL Efflux Capacity. Journal of proteome research. 2018;17:2156–2164. [PubMed: 29649363]

16. Smith LM and Kelleher NL. Proteoform: a single term describing protein complexity. Nature methods. 2013;10:186–7. [PubMed: 23443629]

17. Knorre DG, Kudryashova NV and Godovikova TS. Chemical and Functional Aspects of Posttranslational Modification of Proteins. Acta Naturae. 2009;1:29–51. [PubMed: 22649613]

18. Nobecourt E, Tabet F, Lambert G, Puranik R, Bao S, Yan L, Davies MJ, Brown BE, Jenkins AJ, Dusting GJ, Bonnet DJ, Curtiss LK, Barter PJ and Rye KA. Nonenzymatic glycation impairs

the antiinflammatory properties of apolipoprotein A-I. Arteriosclerosis, thrombosis, and vascular biology. 2010;30:766–72. [PubMed: 20110571]

19. Brown BE, Nobecourt E, Zeng J, Jenkins AJ, Rye K-A and Davies MJ. Apolipoprotein A-I glycation by Glucose and Reactive Aldehydes Alters Phospholipid Affinity but Not Cholesterol Export from Lipid-Laden Macrophages. PLOS ONE. 2013;8:e65430.
20. Bunn HF, Haney DN, Kamin S, Gabbay KH and Gallop PM. The biosynthesis of human hemoglobin A1c. Slow glycosylation of hemoglobin in vivo. J Clin Invest. 1976;57:1652–9. [PubMed: 932199]
21. Fievet C, Igau B, Bresson R, Drouin P and Fruchart JC. Non-enzymatic glycosylation of apolipoprotein A-I and its functional consequences. Diabete & metabolisme. 1995;21:95–8. [PubMed: 7621978]
22. Toby TK, Fornelli L and Kelleher NL. Progress in Top-Down Proteomics and the Analysis of Proteoforms. Annual review of analytical chemistry (Palo Alto, Calif). 2016;9:499–519.
23. Tran JC, Zamdborg L, Ahlf DR, Lee JE, Catherman AD, Durbin KR, Tipton JD, Vellaichamy A, Kellie JF, Li M, Wu C, Sweet SM, Early BP, Siuti N, LeDuc RD, Compton PD, Thomas PM and Kelleher NL. Mapping intact protein isoforms in discovery mode using top-down proteomics. Nature. 2011;480:254–8. [PubMed: 22037311]
24. Fellers RT, Greer JB, Early BP, Yu X, LeDuc RD, Kelleher NL and Thomas PM. ProSight Lite: graphical software to analyze top-down mass spectrometry data. Proteomics. 2015;15:1235–8. [PubMed: 25828799]
25. LeDuc RD, Fellers RT, Early BP, Greer JB, Thomas PM and Kelleher NL. The C-score: a Bayesian framework to sharply improve proteoform scoring in high-throughput top down proteomics. Journal of proteome research. 2014;13:3231–40. [PubMed: 24922115]
26. McQueen MJ, Hawken S, Wang X, Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazmi K and Yusuf S. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. The Lancet. 2008;372:224–233.
27. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Holm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart A, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki ML, Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Zee AH, Peters BJ, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VH, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WM, Boer JM, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burtt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, Konig IR, Fischer M, Hengstenberg C, Ziegler A, Buysschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D, Schrezenmeir J, Schreiber S, Schafer A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Elosua R, Stefansson K, O'Donnell CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Altshuler D and Kathiresan S. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet (London, England). 2012;380:572–80. [PubMed: 22607825]
28. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K and Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. The New England journal of medicine. 2011;365:2255–67. [PubMed: 22085343]
29. Wilkins JT, Ning H, Stone NJ, Criqui MH, Zhao L, Greenland P and Lloyd-Jones DM. Coronary heart disease risks associated with high levels of HDL cholesterol. Journal of the American Heart Association. 2014;3:e000519.
30. <ERFC JAMA 2009.pdf>.

31. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr., Watson K, Wilson PW, Eddleman KM, Jarrett NM, LaBresh K, Nevo L, Wnek J, Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Hochman JS, Kovacs RJ, Ohman EM, Pressler SJ, Sellke FW, Shen WK, Smith SC Jr., Tomaselli GF and American College of Cardiology/American Heart Association Task Force on Practice G. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129:S1–45. [PubMed: 24222016]

32. Santos Seckler HD, Park HM, Lloyd-Jones CM, Melani RD, Camarillo JM, Wilkins JT, Compton PD and Kelleher NL. New Interface for Faster Proteoform Analysis: Immunoprecipitation Coupled with SampleStream-Mass Spectrometry. J Am Soc Mass Spectrom. 2021;32:1659–1670. [PubMed: 34043341]

33. Jian W, Edom RW, Wang D, Weng N and Zhang SW. Relative quantitation of glycoisoforms of intact apolipoprotein C3 in human plasma by liquid chromatography-high-resolution mass spectrometry. Analytical chemistry. 2013;85:2867–74. [PubMed: 23368640]

34. Zhang Y, Sinaiko AR and Nelsestuen GL. Glycoproteins and glycosylation: apolipoprotein c3 glycoforms by top-down maldi-tof mass spectrometry. Methods in molecular biology (Clifton, NJ). 2012;909:141–50.

35. Harazono A, Kawasaki N, Kawanishi T and Hayakawa T. Site-specific glycosylation analysis of human apolipoprotein B100 using LC/ESI MS/MS. Glycobiology. 2005;15:447–62. [PubMed: 15616123]

36. Patra K, Giannisis A, Edlund AK, Sando SB, Lauridsen C, Berge G, Grontvedt GR, Brathen G, White LR and Nielsen HM. Plasma Apolipoprotein E Monomer and Dimer Profile and Relevance to Alzheimer's Disease. J Alzheimers Dis. 2019.

Key Points:

- A "**proteoform**" is defined as one specific protein structural form that results from the combination of allelic variation at a protein-coding gene, alternative RNA splicing, and/or post-translational modifications (PTMs) in specific locations on the amino acid backbone of a protein.
- Variation in apolipoprotein structure due to missense mutations as well as post-translational modification are known to occur, but the catalogue of apolipoprotein proteoforms and their associations with phenotype have not been well dscribed.
- The catalogue of apoA1 and apoA2 proteoforms in humans have recently been described, and the relative abundance of apoA1 and apoA2 proteoforms have differential associations with indices of cardiovascular risk.
- Proteoform level characterization of apolipoproteins A1 and A2, and their proteoform-specific associations with phenotype provides proof of concept that proteoform level resolution may provide novel insights into apolipoprotein metabolism that could not be derived by measures of total protein abundance alone.