VOLUME 9 | ISSUE 4 | OCTOBER - DECEMBER 2018

High density lipoprotein: the role of apolipoprotein A2

Eva Xepapadaki[§], Christina Kalogeropoulou[§], Evangelia Zvintzou[§], Serafoula Filou and Kyriakos E. Kypreos*

[1]*University of Patras, School of Medicine, Department of Pharmacology, Rio Achaias, TK. 26500, Greece;*

[§]*Equal contribution to this work*

Abstract

Atherosclerosis is a multistep process that progresses over a long period of time and displays a broad range of severity. In its final form, it manifests as a lesion of the intimal layer of the arterial wall. Multiple epidemiological and clinical studies in the past suggested that reduced HDL cholesterol (HDL-C) levels may correlate with increased risk for atherosclerosis. More recent data indicated that high density lipoprotein (HDL) particle functionality rather than HDL-C levels is a much more important parameter for human health and disease. Recent data from clinical paradigms and studies in mice support the interesting hypothesis that variations in HDL proteome may set the basis for its functionality. Apolipoprotein A2 (APOA2) is the second most abundant protein of HDL and plays a crucial role in HDL particle synthesis. Studies in mice suggested a proatherogenic role for APOA2 though studies in humans failed thus far to establish a clear role for APOA2 in atherosclerosis. Interestingly, though APOA2 increases HDL-C levels, the effects of this protein on HDL functionality are not adequately investigated. Understanding how APOA2 affects HDL and the lipoprotein transport system may provide another important piece in the puzzle of the mechanisms linking plasma lipoproteins with atherosclerosis.

Key words: High density lipoprotein; Atherosclerosis; Apolipoprotein A2

SUBMISSION: 25-1-2019 | ACCEPTANCE: 07-3-2019

Citation

Xepapadaki E, Kalogeropoulou C, Zvintzou E, Filou S and Kypreos KE. High density lipoprotein: the role of apolipoprotein A2. *Hell J Atheroscler* 2018; 9: 128-137

*Address correspondence at University of Patras Medical School, Department of Medicine, Pharmacology laboratory, Panepistimioupolis, Rio, TK. 26500, Greece, Tel: +302610969120, Fax: +302610996103, email: kkypreos@med.upatras.gr, web: http://www.kyriakoskypreos.com

High density lipoprotein (HDL) has been for years an intriguing lipoprotein that attracted the attention of biomedical community, mainly because of its important role in atheroprotection [1]. In particular, the inverse correlation between HDL cholesterol (HDL-C) levels and the risk for developing coronary heart disease (CHD) [2-7] suggested that high HDL-C levels in plasma may be protective against the development of atherosclerosis while lower than physiological levels of HDL-C may constitute a risk factor for this disease.

Based on these clinical observations, investigational drugs aiming at raising HDL-C levels have been tested recently as an additional mode of protection against atherosclerosis and CHD morbidity and mortality. One such approach was inhibition of cholesterol-ester transfer protein (CETP)[8]. The first 3 CETP inhibitors, torcetrapib [9], dalcetrapib [10] and evacetrapib [11] showed no clinical benefit or even increased mortality (torcetrapib), supporting the notion that CETP may not be the proper pharmacological target. More recently anacetrapib, a fourth experimental drug of this category, showed some benefit in patients under intense statin treatment [12] though it was not clear whether the benefit is a result of increased HDL-C levels or reduced non-HDL-C levels. In any case, the failure of high-dose niacin, another HDL raising drug, to reduce the risk for cardiovascular events (AIM-HIGH [13] and HPS2-THRIVE [14] clinical trials), is additional evidence that simply raising HDL-C in plasma is not an effective strategy for the prevention and treatment of CHD as once thought. These results, along with Mendelian randomization studies failing to demonstrate a causative relationship between HDL-C and cardiovascular diseases [15], and more recent epidemiological data demonstrating a U-shape correlation between all-cause mortality and HDL-C levels further supported that excessive increase in HDL-C may be detrimental to human health [16;17].

Even though HDL is usually referred to as the "good cholesterol", it is actually more than just a "cholesterol". HDL is a macromolecular assembly of proteins and lipids synthesized in the circulation and the main lipid cargo of mature HDL particles is esterified cholesterol. However, other lipids (phospholipids, sphingolipids, ceramides etc) are also part of HDL lipidome [18]. Current studies indicate that HDL is rather a mixture of lipoprotein particles with densities in the range of 1.063 to 1.21 g/ml and depending on their lipid composition these particles may assume a discoidal or spherical geometry. Proteomic analyses revealed that HDL proteome also includes more than 85 different proteins, identified in particles isolated by different methods[1;5;7]. Moreover these studies supported that HDL associated proteins may be found on structurally distinct particles, that are differentially distributed across the HDL density spectrum [5].

Recent data from experimental mice and clinical trials have indicated that HDL functionality, as determined by its lipidome and proteome, is more important in atheroprotection than HDL-C levels alone [5;7].

The main protein component of HDL is apolipoprotein A-I (APOA1) which plays a key role in the biogenesis and functions of HDL [19]. Studies in cell cultures and experimental mice showed that biogenesis of classical APOA1-containing HDL particles (APOA1-HDL) involves the lipid transporters ATP-binding cassette A1 (ABCA1) and G1 (ABCG1) and the plasma enzyme Lecithin:Cholesterol Acyl Transferase (LCAT) [20-23]. However, studies in mice showed that, other apolipoproteins such as apolipoprotein E (APOE) [24], apolipoprotein A-II (APOA2)[25], apolipoprotein C-III (APOC3) [26] and possibly other small exchangeable apolipoproteins are also capable of promoting the de novo biogenesis of HDL in the absence of a functional APOA1.

In addition to the studies in mice, we recently observed the existence of APOE-containing HDL (APOE-HDL) and APOC3-containing HDL (APOC3-HDL) particles in the plasma of morbidly obese human subjects [18]. Analysis of their HDL particle composition showed that rapid weight loss was associated with a significant switch from primarily APOE-HDL and APOC3-

HDL to primarily APOA1-HDL [18] alongside with a significant improvement of the antioxidant properties of HDL [18]. In another clinical paradigm we observed that young asymptomatic subjects (≤35 years of age) who suffered an acute non-fatal myocardial infarction had elevated plasma APOE-HDL and APOC3-HDL that correlated with reduced antioxidant potential [27]. These clinical observations supported the interesting hypothesis that variations in HDL proteome may set the basis for its functionality. To test this hypothesis, we turned back to experimental mice where we selectively produced HDL of different apolipoprotein composition using adenovirus-mediated gene transfer of APOA1, or APOE or APOC3. Indeed, our findings support the notion that APOA1-HDL is functionally distinct from APOE-HDL and APOC3-HDL [18;28]. The apparent differences in HDL apolipoprotein content, lipidome and functionality between APOE3-HDL, APOC3-HDL and APOA1-HDL, identified by our preclinical and clinical studies, reinforce our theory that not all HDL particles are equally active and that HDL proteome dictates its lipidome and subsequently its functionality [18]. Along the same line, genetic control of the mouse HDL proteome defines HDL traits, function, and heterogeneity [29].

Structural and functional changes of HDL have also been reported in the chronic inflammatory process of atherosclerosis [30;31]. In pathological states such as oxidative stress, inflammation and diabetes, HDL may undergo changes that affect their antiatherogenic properties. Moreover, it was observed that subjects at high risk of coronary artery disease (CAD), or already with CAD, possessed small HDL3 particles, which have impaired antiatherogenic properties [32]. In type 2 diabetes, the small dense HDL was found to have a diminished antioxidant activity, which was associated with oxidative stress, glycaemia and hypertriglyceridemia [33]. The HDL particles of patients with diabetes were found to have an increased content of oxidized fatty acids and impaired anti-inflammatory and antioxidant activities [34].

Apolipoprotein A2 in HDL and beyond

APOA2, the second most abundant protein of HDL [35], is a 77 aminoacid amphipathic glycoprotein [5;19] which plays a crucial role in HDL particle synthesis, structure, functions and plasma concentration [36] and is synthesized mainly by the liver and to a much lesser extent by the intestine [37;38]. The interactions of APOA2 with APOA1, APOE and other proteins of the HDL metabolic pathway may affect subpopulation distribution and functionality of HDL. In vitro studies suggested that APOA2 forms dimers with APOE and it was proposed that this interaction may affect the ability of APOE to associate with HDL particles [39].

Transgenic mice, overexpressing human APOA2, had abnormal lipoprotein composition, increased HDL-C levels and were prone to atherosclerosis (40;41). Specifically, increased APOA2 levels affected the size and subpopulation distribution of HDL particles [25;42] as well as the ratio of APOA1/APOA2 in HDL. These mice had smaller, predominantly APOA2-containing HDL (APOA2-HDL), while APOA1-HDL was significantly reduced [25;43;44] and had a decreased proportion of esterified cholesterol to total cholesterol, due to reduced LCAT activity [43;44]. In addition, in transgenic mice expressing human APOA2, very low density lipoproteins (VLDL) contained significant levels of APOA2 and the activities of both lipoprotein lipase (LpL) and hepatic lipase (HL) were reduced [44]. These mice also developed some atherosclerotic lesions even on a chow diet [37] that became more advanced and complex when fed an atherogenic diet [40;45;46].

Mice overexpressing murine APOA2 had elevated triglyceride levels in HDL [47]. Deficiency of APOA2 in mice correlated with a 50% reduction in HDL-C levels due to increased catabolism of HDL [48], raising the possibility that concomitant expression of APOA1 and APOA2 may be important in maintaining physiological plasma HDL levels [48].

In contrast, studies in humans, failed thus far to establish a clear role for APOA2 in atherosclero-

sis. APOA2 appears to impair the reverse cholesterol transport and antioxidant function of HDL, which is consistent with the observation that increased APOA2 levels promote the development of atherosclerosis [40;49]. However, in another study in 126 subjects with varying degrees of atherosclerosis (calcified and non-calcified), APOA2 appears to positively associate with reverse cholesterol transport and negatively associate with non-calcified atherosclerosis burden [50].

There are two major subsets of human APOA1-containing HDL, categorized by their apolipoprotein composition: the particles containing APOA1 but not APOA2 (LpA1) and those having both (LpA1:A2) [51;52]. These subpopulations of HDL are distinct from each other with respect to structural stability and apparent metabolic fate. It is therefore important to clarify their functional differences to understand cholesterol transport by HDL which is overall considered as antiatherogenic. It has been reported that LpA1:A2, in contrast to LpA1, could be an atherogenic rather than an antiatherogenic indicator [52]. In an additional study in 23 men with metabolic syndrome a hypercatabolism of LpA1 and LpA1:A2 particles was observed, but selective overproduction of LpA1 maintained a normal plasma concentration of this subpopulation of HDL, compared to lean controls [53].

APOA2 has also been shown to influence VLDL catabolism in humans, since a small percentage of VLDL and intermediate density lipoproteins (IDL) particles contain this apolipoprotein (54). A study in 11 subjects on controlled diet, reported that the conversion rate of VLDL to IDL remained unchanged in the absence or presence of APOA2 in these particles, suggesting that APOA2 does not inhibit lipolytic conversion of VLDL by LpL. This finding contrasts data in mice suggesting that APOA2 is an inhibitor of LpL [44]. In humans, APOA2-containing VLDL have a slow clearance rate from plasma, thus increasing the percentage that is converted to IDL. Most APOA2-containing VLDL are converted to IDL before clearance from the circulation. Given the poor clearance of APOA2-containing VLDL, the presence of APOA2 on VLDL may promote atherogenesis [55]. Additionally, in abdominally obese individuals the rate of clearance of APOA2 was positively and independently associated with both APOA1 and VLDL indirect clearance. These data suggested that, in a condition of delayed VLDL catabolism, such as abdominal obesity, retention of APOA2 on the VLDL pool may occur, reducing the rate of plasma APOA2 and VLDL catabolism. This direct association between VLDL and APOA2 catabolism suggests that the exchange of APOA2 between TG-rich lipoproteins (VLDL) and HDL may be a process regulating both VLDL and LpA1:A2 HDL particle levels in plasma.

Significant amount of data exists on the role of APOA2 polymorphisms in the development of obesity. Specifically, the APOA2 c.-492T>C genotype has been linked to the development of obesity and the regulation of food intake, since homozygous individuals for the C allele have higher body mass index (BMI) and obesity risk than the carriers of the T allele (56-60). However, there is a study that reported that APOA2 -265 T/T genotype carriers had a higher intake of polyunsaturated fatty acids than the T/C+C/C carriers which then resulted in the obese phenotype [61].

Conclusions

Studies showed that HDL proteome includes more than 85 different proteins, identified in particles isolated by different methods.[1;5;7]. The plasma abundance of these proteins is insufficient to permit one copy of protein for each HDL particle, suggesting that different proteins may be associated with different HDL particles that are differentially distributed across the HDL density spectrum [5]. Our data indicate that HDL proteome dictates its lipidome and subsequently HDL particle functionality [18], suggesting that the understanding of HDL proteome and the factors affecting it, are crucial steps in successfully improving HDL functionality. In support of this hypothesis, another recent study indicates that genetic control of the mouse HDL proteome defines HDL traits, functionality, and heterogeneity [29].

Alterations in the HDL metabolic pathway as

well as in HDL proteome appear to influence to a great extent its properties and functions. A key piece in the HDL puzzle, is the correlation between HDL lipid and protein content with particle functionality. To this date, such correlation remains not well understood [62-64]. The assignment of a clear role of APOA2 in HDL remains an unexplored area that may provide significant new mechanistic insights for atherosclerosis development and progression.

Conflict of interest

There is no conflict of interest.

Acknowledgments

This work was supported financially by the program "Support of Young Investigators" MIS No. 5005458, co-financed by the Operational Program "Human Resources Development, Education and Lifelong Learning" and by the European Union (European Social Fund) and Greek national funds.

Abbreviations

ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1; APOA1, Human apolipoprotein A-I; Apoa1, Mouse apolipoprotein A-I; APOA1-HDL, APOA1-containing HDL; APOA2, Human apolipoprotein A-II; APOA2-HDL, APOA2-containing HDL; APOC3, Human apolipoprotein C-III; APOC3-HDL, APOC3-containing HDL; APOE, Human apolipoprotein E; APOE-HDL, APOE-containing HDL; BMI, Body mass index; CHD, Coronary heart disease; HDL, High density lipoprotein; HDL-C, HDL cholesterol; HL, Hepatic lipase; IDL, Intermediate density lipoproteins; LCAT, Lecithin:Cholesterol Acyl Transferase; LpA1, HDL particles containing APOA1 but not APOA2; LpA1:A2, HDL particles containing both APOA1 and APOA2; LpL, Lipoprotein lipase; TG, Triglycerides; VLDL, Very low density lipoproteins

Περίληψη

Η λιποπρωτεΐνη υψηλής πυκνότητας: Ο ρόλος της απολιποπρωτεΐνης Α2

Εύα Ξεπαπαδάκη§, Χριστίνα Καλογεροπούλου§, Ευαγγελία Ζβίντζου§, Σεραφούλα Φίλου και Κυριάκος Η. Κυπραίος*

[1]*Πανεπιστήμιο Πατρών, Τμήμα Ιατρικής, Εργαστήριο Φαρμακολογίας, Ρίο Αχαΐας, ΤΚ. 26500, Ελλάς*

§Ίση συνεισφορά

Η αθηροσκλήρωση είναι μια παθολογική κατάσταση πολλαπλών σταδίων που εξελίσσεται σε βάθος χρόνου και εμφανίζεται με ένα ευρύ φάσμα δριμύτητας. Στην τελική της μορφή, εκδηλώνεται ως βλάβη στην εσωτερική στιβάδα του αρτηριακού τοιχώματος. Πολλές επιδημιολογικές και κλινικές μελέτες στο παρελθόν υπέδειξαν ότι μειωμένα επίπεδα HDL-χοληστερόλης (HDL-C) μπορεί να συσχετίζονται με αυξημένο κίνδυνο εμφάνισης αθηροσκλήρωσης. Πιο πρόσφατα δεδομένα προτείνουν ότι η λειτουργικότητα των σωματιδίων της υψηλής πυκνότητας λιποπρωτεΐνης (HDL) αποτελεί πολύ πιο σημαντική παράμετρο για

την ανθρώπινη υγεία σε σχέση με τα επίπεδα της HDL-C. Πρόσφατες μελέτες σε κλινικά περιστατικά καθώς και σε πειραματόζωα υποστηρίζουν την ενδιαφέρουσα θεωρία ότι οι μεταβολές στην πρωτεϊνική σύσταση της HDL αποτελούν τη βάση για τη λειτουργικότητα των σωματιδίων της. Η απολιποπρωτεΐνη Α2 (APOA2) είναι η δεύτερη σε συχνότητα πρωτεΐνη που συναντάται στην HDL και παίζει καθοριστικό ρόλο στη σύνθεση των σωματιδίων της. Μελέτες σε πειραματικά μοντέλα ποντικών, προτείνουν ότι η APOA2 πιθανά έχει προ-αθηρογόνο ρόλο, αν και μελέτες σε ανθρώπους απέτυχαν μέχρι στιγμής να καταστήσουν σαφή τον ρόλο της APOA2 στην αθηροσκλήρωση. Είναι ενδιαφέρον το γεγονός ότι, αν και η APOA2 αυξάνει τα επίπεδα της HDL-C, οι επιδράσεις αυτής της πρωτεΐνης στη λειτουργικότητα της HDL παραμένουν αδιερεύνητες. Η διαλεύκανση του τρόπου με τον οποίο η APOA2 επηρεάζει την HDL και το σύστημα των λιποπρωτεϊνών μπορεί να αποτελέσει ένα σημαντικό κομμάτι στο παζλ των μηχανισμών που συνδέουν τις λιποπρωτεΐνες του αίματος με την αθηροσκλήρωση.

Λέξεις ευρετηρίου: Λιποπρωτεΐνη Υψηλής Πυκνότητας, Αθηροσκλήρωση, Απολιποπρωτεΐνη Α2

References

1. Constantinou, C., Karavia, E. A., Xepapadaki, E., Petropoulou, P. I., Papakosta, E., Karavyraki, M., Zvintzou, E., Theodoropoulos, V., Filou, S., Hatziri, A., Kalogeropoulou, C., Panayiotakopoulos, G., and Kypreos, K. E. (2015) Advances in High Density Lipoprotein Physiology: surprises, overturns and promises, *Am. J. Physiol Endocrinol. Metab* ajpendo
2. Gofman, J. W., Glazier, F., Tamplin, A., Strisower, B., and De, L. O (1954) Lipoproteins, coronary heart disease, and atherosclerosis, *Physiol Rev. 34*, 589-607
3. Delalla, O. F., Elliott, H. A., and Gofman, J. W. (1954) Ultracentrifugal studies of high density serum lipoproteins in clinically healthy adults, *Am. J. Physiol 179*, 333-337
4. Miller, G. J. and Miller, N. E. (1975) Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease, *Lancet 1*, 16-19
5. Tsompanidi, E. M., Brinkmeier, M. S., Fotiadou, E. H., Giakoumi, S. M., and Kypreos, K. E. (2010) HDL biogenesis and functions: Role of HDL quality and quantity in atherosclerosis, *Atherosclerosis 208*, 3-9
6. Kypreos, K. E., Gkizas, S., Rallidis, L. S., and Karagiannides, I. (2013) HDL particle functionality as a primary pharmacological target for HDL-based therapies, *Biochem. Pharmacol. 85*, 1575-1578
7. Karavia, E. A., Zvintzou, E., Petropoulou, P. I., Xepapadaki, E., Constantinou, C., and Kypreos, K. E. (2014) HDL quality and functionality: what can proteins and genes predict?, *Expert. Rev. Cardiovasc. Ther. 12*, 521-532
8. Chapman, M. J., Assmann, G., Fruchart, J. C., Shepherd, J., and Sirtori, C. (2004) Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid--a position paper developed by the European Consensus Panel on HDL-C, *Curr. Med. Res. Opin. 20*, 1253-1268
9. Barter, P. J., Caulfield, M., Eriksson, M., Grundy, S. M., Kastelein, J. J., Komajda, M., Lopez-Sendon, J., Mosca, L., Tardif, J. C., Waters, D. D., Shear, C. L., Revkin, J. H., Buhr, K. A., Fisher, M. R., Tall, A. R., and Brewer, B. (2007) Effects of torcetrapib in patients at high risk for coronary events, *N. Engl. J Med. 357*, 2109-2122
10. Schwartz, G. G., Olsson, A. G., Abt, M., Ballantyne, C. M., Barter, P. J., Brumm, J., Chaitman, B. R., Holme, I. M., Kallend, D., Leiter, L. A., Leitersdorf, E., McMurray, J. J., Mundl, H., Nicholls, S. J., Shah, P. K., Tardif, J. C., and Wright, R. S. (2012) Effects of dalcetrapib in patients with a recent acute coronary syndrome, *N. Engl. J Med. 367*, 2089-2099
11. Lincoff, A. M., Nicholls, S. J., Riesmeyer, J. S., Barter, P.

J., Brewer, H. B., Fox, K. A. A., Gibson, C. M., Granger, C., Menon, V., Montalescot, G., Rader, D., Tall, A. R., McErlean, E., Wolski, K., Ruotolo, G., Vangerow, B., Weerakkody, G., Goodman, S. G., Conde, D., McGuire, D. K., Nicolau, J. C., Leiva-Pons, J. L., Pesant, Y., Li, W., Kandath, D., Kouz, S., Tahirkheli, N., Mason, D., and Nissen, S. E. (2017) Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease, *N. Engl. J. Med. 376*, 1933-1942

12. Bowman, L., Hopewell, J. C., Chen, F., Wallendszus, K., Stevens, W., Collins, R., Wiviott, S. D., Cannon, C. P., Braunwald, E., Sammons, E., and Landray, M. J. (2017) Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease, *N. Engl. J. Med. 377*, 1217-1227
13. Boden, W. E., Probstfield, J. L., Anderson, T., Chaitman, B. R., Desvignes-Nickens, P., Koprowicz, K., McBride, R., Teo, K., and Weintraub, W. (2011) Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy, *N. Engl. J. Med. 365*, 2255-2267
14. Landray, M. J., Haynes, R., Hopewell, J. C., Parish, S., Aung, T., Tomson, J., Wallendszus, K., Craig, M., Jiang, L., Collins, R., and Armitage, J. (2014) Effects of extended-release niacin with laropiprant in high-risk patients, *N. Engl. J. Med. 371*, 203-212
15. Voight, B. F., Peloso, G. M., Orho-Melander, M., Frikke-Schmidt, R., Barbalic, M., Jensen, M. K., Hindy, G., Holm, H., Ding, E. L., Johnson, T., Schunkert, H., Samani, N. J., Clarke, R., Hopewell, J. C., Thompson, J. F., Li, M., Thorleifsson, G., Newton-Cheh, C., Musunuru, K., Pirruccello, J. P., Saleheen, D., Chen, L., Stewart, A., Schillert, A., Thorsteinsdottir, U., Thorgeirsson, G., Anand, S., Engert, J. C., Morgan, T., Spertus, J., Stoll, M., Berger, K., Martinelli, N., Girelli, D., McKeown, P. P., Patterson, C. C., Epstein, S. E., Devaney, J., Burnett, M. S., Mooser, V., Ripatti, S., Surakka, I., Nieminen, M. S., Sinisalo, J., Lokki, M. L., Perola, M., Havulinna, A., de, F. U., Gigante, B., Ingelsson, E., Zeller, T., Wild, P., de Bakker, P. I., Klungel, O. H., Maitland-van der Zee AH, Peters, B. J., de, B. A., Grobbee, D. E., Kamphuisen, P. W., Deneer, V. H., Elbers, C. C., Onland-Moret, N. C., Hofker, M. H., Wijmenga, C., Verschuren, W. M., Boer, J. M., van der Schouw, Y. T., Rasheed, A., Frossard, P., Demissie, S., Willer, C., Do, R., Ordovas, J. M., Abecasis, G. R., Boehnke, M., Mohlke, K. L., Daly, M. J., Guiducci, C., Burtt, N. P., Surti, A., Gonzalez, E., Purcell, S., Gabriel, S., Marrugat, J., Peden, J., Erdmann, J., Diemert, P., Willenborg, C., Konig, I. R., Fischer, M., Hengstenberg, C., Ziegler, A., Buysschaert, I., Lambrechts, D., Van de Werf, F., Fox, K. A., El Mokhtari, N. E., Rubin, D., Schrezenmeir, J., Schreiber, S., Schafer, A., Danesh, J., Blankenberg, S., Roberts, R., McPherson, R., Watkins, H., Hall, A. S., Overvad, K., Rimm, E., Boerwinkle, E., Tybjaerg-Hansen, A., Cupples, L. A., Reilly, M. P., Melander, O., Mannucci, P. M., Ardissino, D., Siscovick, D., Elosua, R., Stefansson, K., O'Donnell, C. J., Salomaa, V., Rader, D. J., Peltonen, L., Schwartz, S. M., Altshuler, D., and Kathiresan, S. (2012) Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study, *Lancet 380*, 572-580
16. Madsen, C. M., Varbo, A., and Nordestgaard, B. G. (2017) Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies, *Eur. Heart J. 38*, 2478-2486
17. Bowe, B., Xie, Y., Xian, H., Balasubramanian, S., Zayed, M. A., and Al-Aly, Z. (2016) High Density Lipoprotein Cholesterol and the Risk of All-Cause Mortality among U.S. Veterans, *Clin. J. Am. Soc. Nephrol. 11*, 1784-1793
18. Filou, S., Lhomme, M., Karavia, E. A., Kalogeropoulou, C., Theodoropoulos, V., Zvintzou, E., Sakellaropoulos, G. C., Petropoulou, P. I., Constantinou, C., Kontush, A., and Kypreos, K. E. (2016) Distinct Roles of Apolipoproteins A1 and E in the Modulation of High-Density Lipoprotein Composition and Function, *Biochemistry 55*, 3752-3762
19. Zannis, V. I., Kypreos, K. E., Chroni, A., Kardassis, D., and Zanni, E. E. (2004) in *Molecular Mechanisms of Atherosclerosis* (Loscalzo, J., Ed.) pp 111-174, Taylor & Francis, New York, NY
20. Soutar, A. K., Garner, C. W., Baker, H. N., Sparrow, J. T., Jackson, R. L., Gotto, A. M., and Smith, L. C. (1975) Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin:

cholesterol acyltransferase, *Biochemistry 14*, 3057-3064

21. Chroni, A., Liu, T., Gorshkova, I., Kan, H. Y., Uehara, Y., von Eckardstein, A., and Zannis, V. I. (2003) The central helices of apoA-I can promote ATP-binding cassette transporter A1 (ABCA1)-mediated lipid efflux. Amino acid residues 220-231 of the wild-type apoA-I are required for lipid efflux in vitro and high density lipoprotein formation in vivo, *J. Biol. Chem. 278*, 6719-6730
22. Fitzgerald, M. L., Morris, A. L., Chroni, A., Mendez, A. J., Zannis, V. I., and Freeman, M. W. (2004) ABCA1 and amphipathic apolipoproteins form high-affinity molecular complexes required for cholesterol efflux, *J. Lipid Res. 45*, 287-294
23. Ji, A., Wroblewski, J. M., Cai, L., de Beer, M. C., Webb, N. R., and van der Westhuyzen, D. R. (2012) Nascent HDL formation in hepatocytes and role of ABCA1, ABCG1, and SR-BI, *J. Lipid Res. 53*, 446-455
24. Kypreos, K. E. and Zannis, V. I. (2007) Pathway of biogenesis of apolipoprotein E-containing HDL in vivo with the participation of ABCA1 and LCAT, *Biochem. J. 403*, 359-367
25. Schultz, J. R., Gong, E. L., McCall, M. R., Nichols, A. V., Clift, S. M., and Rubin, E. M. (1992) Expression of human apolipoprotein A-II and its effect on high density lipoproteins in transgenic mice, *J. Biol. Chem. 267*, 21630-21636
26. Kypreos, K. E. (2008) ABCA1 Promotes the de Novo Biogenesis of Apolipoprotein CIII-Containing HDL Particles in Vivo and Modulates the Severity of Apolipoprotein CIII-Induced Hypertriglyceridemia, *Biochemistry 47*, 10491-10502
27. Kavo, A. E., Rallidis, L. S., Sakellaropoulos, G. C., Lehr, S., Hartwig, S., Eckel, J., Bozatzi, P. I., Anastasiou-Nana, M., Tsikrika, P., and Kypreos, K. E. (2012) Qualitative characteristics of HDL in young patients of an acute myocardial infarction, *Atherosclerosis 220*, 257-264
28. Zvintzou, E., Lhomme, M., Chasapi, S., Filou, S., Theodoropoulos, V., Xapapadaki, E., Kontush, A., Spyroulias, G., Tellis, C. C., Tselepis, A. D., Constantinou, C., and Kypreos, K. E. (2017) Pleiotropic effects of apolipoprotein C3 on HDL functionality and adipose tissue metabolic activity, *J. Lipid Res. 58*, 1869-1883
29. Pamir, N., Pan, C., Plubell, D. L., Hutchins, P. M., Tang, C., Wimberger, J., Irwin, A., Vallim, T. Q. A., Heinecke, J. W., and Lusis, A. J. (2019) Genetic control of the mouse HDL proteome defines HDL traits, function, and heterogeneity, *J. Lipid Res*
30. Katsiki, N., Athyros, V. G., Karagiannis, A., and Mikhailidis, D. P. (2014) High-density lipoprotein, vascular risk, cancer and infection: a case of quantity and quality?, *Curr. Med. Chem. 21*, 2917-2926
31. Otocka-Kmiecik, A., Mikhailidis, D. P., Nicholls, S. J., Davidson, M., Rysz, J., and Banach, M. (2012) Dysfunctional HDL: a novel important diagnostic and therapeutic target in cardiovascular disease?, *Prog. Lipid Res. 51*, 314-324
32. Lagos, K. G., Filippatos, T. D., Tsimihodimos, V., Gazi, I. F., Rizos, C., Tselepis, A. D., Mikhailidis, D. P., and Elisaf, M. S. (2009) Alterations in the high density lipoprotein phenotype and HDL-associated enzymes in subjects with metabolic syndrome, *Lipids 44*, 9-16
33. Nobecourt, E., Jacqueminet, S., Hansel, B., Chantepie, S., Grimaldi, A., Chapman, M. J., and Kontush, A. (2005) Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia, *Diabetologia 48*, 529-538
34. Morgantini, C., Natali, A., Boldrini, B., Imaizumi, S., Navab, M., Fogelman, A. M., Ferrannini, E., and Reddy, S. T. (2011) Anti-inflammatory and antioxidant properties of HDLs are impaired in type 2 diabetes, *Diabetes 60*, 2617-2623
35. Brewer, H. B., Jr., Lux, S. E., Ronan, R., and John, K. M. (1972) Amino acid sequence of human apoLp-Gln-II (apoA-II), an apolipoprotein isolated from the high-density lipoprotein complex, *Proc. Natl. Acad. Sci. U. S. A 69*, 1304-1308
36. Pownall, H. J., Gillard, B. K., and Gotto, A. M., Jr. (2013) Setting the course for apoAII: a port in sight?, *Clin. Lipidol. 8*, 551-560
37. Schonfeld, G., Patsch, W., Rudel, L. L., Nelson, C., Epstein, M., and Olson, R. E. (1982) Effects of dietary cho-

lesterol and fatty acids on plasma lipoproteins, *J. Clin. Invest 69*, 1072-1080

38. Hussain, M. M. and Zannis, V. I. (1990) Intracellular modification of human apolipoprotein AII (apoAII) and sites of apoAII mRNA synthesis: comparison of apoAII with apoCII and apoCIII isoproteins, *Biochemistry 29*, 209-217
39. Borghini, I., Barja, F., Pometta, D., and James, R. W. (1995) Characterization of subpopulations of lipoprotein particles isolated from human cerebrospinal fluid, *Biochim. Biophys. Acta 1255*, 192-200
40. Warden, C. H., Hedrick, C. C., Qiao, J. H., Castellani, L. W., and Lusis, A. J. (1993) Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II, *Science 261*, 469-472
41. Kalopissis, A. D., Pastier, D., and Chambaz, J. (2003) Apolipoprotein A-II: beyond genetic associations with lipid disorders and insulin resistance, *Curr Opin Lipidol. 14*, 165-172
42. Lusis, A. J. (1988) Genetic factors affecting blood lipoproteins: the candidate gene approach, *J. Lipid Res. 29*, 397-429
43. Marzal-Casacuberta, A., Blanco-Vaca, F., Ishida, B. Y., Julve-Gil, J., Shen, J., Calvet-Marquez, S., Gonzalez-Sastre, F., and Chan, L. (1996) Functional lecithin:cholesterol acyltransferase deficiency and high density lipoprotein deficiency in transgenic mice overexpressing human apolipoprotein A-II, *J. Biol. Chem. 271*, 6720-6728
44. Boisfer, E., Lambert, G., Atger, V., Tran, N. Q., Pastier, D., Benetollo, C., Trottier, J. F., Beaucamps, I., Antonucci, M., Laplaud, M., Griglio, S., Chambaz, J., and Kalopissis, A. D. (1999) Overexpression of human apolipoprotein A-II in mice induces hypertriglyceridemia due to defective very low density lipoprotein hydrolysis, *J. Biol. Chem. 274*, 11564-11572
45. Schultz, J. R., Verstuyft, J. G., Gong, E. L., Nichols, A. V., and Rubin, E. M. (1993) Protein composition determines the anti-atherogenic properties of HDL in transgenic mice, *Nature 365*, 762-764
46. Escola-Gil, J. C., Marzal-Casacuberta, A., Julve-Gil, J., Ishida, B. Y., Ordonez-Llanos, J., Chan, L., Gonzalez-Sastre, F., and Blanco-Vaca, F. (1998) Human apolipoprotein A-II is a pro-atherogenic molecule when it is expressed in transgenic mice at a level similar to that in humans: evidence of a potentially relevant species-specific interaction with diet, *J. Lipid Res. 39*, 457-462
47. Hedrick, C. C., Castellani, L. W., Warden, C. H., Puppione, D. L., and Lusis, A. J. (1993) Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice, *J. Biol. Chem. 268*, 20676-20682
48. Weng, W. and Breslow, J. L. (1996) Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility, *Proc. Natl. Acad. Sci. U. S. A 93*, 14788-14794
49. van 't Hooft, F. M., Ruotolo, G., Boquist, S., de, F. U., Eggertsen, G., and Hamsten, A. (2001) Human evidence that the apolipoprotein a-II gene is implicated in visceral fat accumulation and metabolism of triglyceride-rich lipoproteins, *Circulation 104*, 1223-1228
50. Gordon, S. M., Chung, J. H., Playford, M. P., Dey, A. K., Sviridov, D., Seifuddin, F., Chen, Y. C., Pirooznia, M., Chen, M. Y., Mehta, N. N., and Remaley, A. T. (2018) High density lipoprotein proteome is associated with cardiovascular risk factors and atherosclerosis burden as evaluated by coronary CT angiography, *Atherosclerosis 278*, 278-285
51. Kido, T., Kurata, H., Kondo, K., Itakura, H., Okazaki, M., Urata, T., and Yokoyama, S. (2016) Bioinformatic Analysis of Plasma Apolipoproteins A-I and A-II Revealed Unique Features of A-I/A-II HDL Particles in Human Plasma, *Sci. Rep. 6*, 31532
52. Kido, T., Kondo, K., Kurata, H., Fujiwara, Y., Urata, T., Itakura, H., and Yokoyama, S. (2017) ApoA-I/A-II-HDL positively associates with apoB-lipoproteins as a potential atherogenic indicator, *Lipids Health Dis. 16*, 225
53. Ji, J., Watts, G. F., Johnson, A. G., Chan, D. C., Ooi, E. M., Rye, K. A., Serone, A. P., and Barrett, P. H. (2006) High-density lipoprotein (HDL) transport in the metabolic syndrome: application of a new model for HDL particle kinetics, *J. Clin. Endocrinol. Metab 91*, 973-979
54. Alaupovic, P., Knight-Gibson, C., Wang, C. S., Downs, D., Koren, E., Brewer, H. B., Jr., and Gregg, R. E. (1991) Isolation and characterization of an apoA-II-contain-

ing lipoprotein (LP-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia, *J. Lipid Res. 32*, 9-19

55. Desai, N. K., Ooi, E. M., Mitchell, P. D., Furtado, J., and Sacks, F. M. (2015) Metabolism of apolipoprotein A-II containing triglyceride rich ApoB lipoproteins in humans, *Atherosclerosis 241*, 326-333
56. Corella, D., Arnett, D. K., Tsai, M. Y., Kabagambe, E. K., Peacock, J. M., Hixson, J. E., Straka, R. J., Province, M., Lai, C. Q., Parnell, L. D., Borecki, I., and Ordovas, J. M. (2007) The -256T>C polymorphism in the apolipoprotein A-II gene promoter is associated with body mass index and food intake in the genetics of lipid lowering drugs and diet network study, *Clin. Chem. 53*, 1144-1152
57. Corella, D., Peloso, G., Arnett, D. K., Demissie, S., Cupples, L. A., Tucker, K., Lai, C. Q., Parnell, L. D., Coltell, O., Lee, Y. C., and Ordovas, J. M. (2009) APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations, *Arch. Intern. Med. 169*, 1897-1906
58. Zaki, M. E., Amr, K. S., and Abdel-Hamid, M. (2013) APOA2 Polymorphism in Relation to Obesity and Lipid Metabolism, *Cholesterol. 2013*, 289481
59. Smith, C. E., Tucker, K. L., Arnett, D. K., Noel, S. E., Corella, D., Borecki, I. B., Feitosa, M. F., Aslibekyan, S., Parnell, L. D., Lai, C. Q., Lee, Y. C., and Ordovas, J. M. (2013) Apolipoprotein A2 polymorphism interacts with intakes of dairy foods to influence body weight in 2 U.S. populations, *J. Nutr. 143*, 1865-1871
60. Lai, C. Q., Smith, C. E., Parnell, L. D., Lee, Y. C., Corella, D., Hopkins, P., Hidalgo, B. A., Aslibekyan, S., Province, M. A., Absher, D., Arnett, D. K., Tucker, K. L., and Ordovas, J. M. (2018) Epigenomics and metabolomics reveal the mechanism of the APOA2-saturated fat intake interaction affecting obesity, *Am. J. Clin. Nutr. 108*, 188-200
61. Dominguez-Reyes, T., Astudillo-Lopez, C. C., Salgado-Goytia, L., Munoz-Valle, J. F., Salgado-Bernabe, A. B., Guzman-Guzman, I. P., Castro-Alarcon, N., Moreno-Godinez, M. E., and Parra-Rojas, I. (2015) Interaction of dietary fat intake with APOA2, APOA5 and LEPR polymorphisms and its relationship with obesity and dyslipidemia in young subjects, *Lipids Health Dis. 14*, 106
62. Kontush, A., Lindahl, M., Lhomme, M., Calabresi, L., Chapman, M. J., and Davidson, W. S. (2015) Structure of HDL: particle subclasses and molecular components, *Handb. Exp. Pharmacol. 224*, 3-51
63. Kontush, A., Lhomme, M., and Chapman, M. J. (2013) Unraveling the complexities of the HDL lipidome, *J. Lipid Res. 54*, 2950-2963
64. Kontush, A. and Chapman, M. J. (2010) Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities, *Curr. Opin. Lipidol. 21*, 312-318

Original article

VOLUME 9 | ISSUE 4 | OCTOBER - DECEMBER 2018

A case of uncommon cause of hypercholesterolemia

[1]Sebastian Filippas-Ntekouan, MD
[1]Maria Kosmidou, MD, PhD, Associate Professor of Internal Medicine
[2]Constantinos Tellis, PhD
[2]Alexandros Tselepis, MD, PhD, Professor of Biochemistry
[1]Haralampos Milionis, MD, PhD, Professor of Internal Medicine

[1]*Department of Internal Medicine, School of Medicine, and*
[2]*Atherothrombosis Research Centre, Department of Chemistry, University of Ioannina, Ioannina, Greece*

Abstract

Cholestasis is a rare cause of secondary hypercholesterolemia which may be attributed to increased production of an abnormal lipoprotein (apo), known as Lp-X. We present the case of a patient admitted due to cholestatic jaundice and pruritus and showed excess hypercholesterolemia due to increased production of Lp-X. The patient was diagnosed with an adenocarcinoma of the pancreas. Surgical restoration of the bile flow resulted in normalization of lipids.

Key words: Secondary hypercholesterolemia; lipoprotein X; cholestasis; jaundice

SUBMISSION: 27-2-2019 | ACCEPTANCE: 19-3-2019

Introduction

Hypercholesterolemia is a metabolic disorder affecting millions of individuals around the globe and can be mainly attributed to either increased concentration of low density lipoprotein cholesterol (LDL-C),) or very low density lipoprotein cholesterol (VLDL-C). Increased levels of cholesterol can be due to either primary or secondary

Citation

Xepapadaki E, Kalogeropoulou C, Zvintzou E, Filou S and Kypreos KE. High density lipoprotein: the role of apolipoprotein A2. *Hell J Atheroscler* 2018; 9: 138-143

*Corresponding author: Haralampos Milionis, MD, PhD, Professor of Internal Medicine, Department of Internal Medicine, School of Medicine, University of Ioannina, 45110 Ioannina, Greece, Email: hmilioni@uoi.gr

causes; the latter must be always ruled out before a diagnosis of a primary hypercholesterolemia is established. Common causes of secondary hypercholesterolemia include nephrotic syndrome, chronic kidney disease, hypothyroidism, metabolic syndrome and type 2 diabetes mellitus, multiple drugs and cholestasis; these should be ruled out by using simple tests (such as thyroid stimulating hormone levels, glucose levels, kidney function, liver function tests assessments) before the diagnosis of primary hypercholesterolemia syndrome is established [1, 2].

Cholestasis, intra-hepatic and extra-hepatic, is a rather uncommon cause of secondary hypercholesterolemia characterized by an increased LDL-C concentration and total cholesterol (T-CHOL) levels. The observed increased of LDL-C is due to the production of an abnormal lipoprotein particle, known as Lipoprotein-X (Lp-X) [3]. Lp-X is consisted mostly of phospholipids (approximately 60% w/w) and free cholesterol (FC-25% w/w) as well as by small amounts of protein, triglycerides and cholesterol esters [4]. The outer membrane constitutes of a mixture of an abnormal apolipoprotein, apolipoprotein X (60%), and albumin (40%) [5]. Herein, we describe the case of a patient who presented with excess hypercholesterolemia, jaundice and pruritus.

Case presentation

A 78-year old woman was referred to our hospital for the evaluation of a new onset pruritus and 'painless' jaundice. Two weeks before, the patient complained of severe itching on her feet and hands, while her family noticed a yellow discoloration of her eyes and skin. Anorexia starting a month prior to admission was also reported by the patient. Her medical history involved type 2 diabetes mellitus treated with insulin glargine s.c. (glycated hemoglobulin 6%), cholecystectomy and a right inguinal hernia repair. The family history was unremarkable and she did not report any allergies, smoking or alcohol drinking. Jaundice (sclerae and skin) was evident on physical examination.

Laboratory work-up (**Table 1**) revealed in-

Table 1: **Baseline laboratory work up**

Hematocrit	31.7 %
Hemoglobulin	10.3 g/dl
WBC	5950/μl
Platelets	252000/μl
Glucose	133 mg/dl
Urea	15 mg/dl
Creatinine	0.71 mg/dl
AST	135 IU/l
ALT	194 IU/l
ALP	604 IU/l
γGT	655 IU/l
Tbil	21.7 mg/dl
Dbil	12.6 mg/dl
Uric acid	2.6 mg/dl
Total protein	5.6 g/dl
Albumin	3 g/dl
CK	15 IU/l
LDH	291 U/l
T-CHOL	636 mg/dl
Triglycerides	275 mg/dl
HDL-C	38 mg/dl
LDL-C	544 mg/dl
Apolipoprotein-A1	27.8 mg/dl
Apolipoprotein-B	200 mg/dl
Apolipoprotein-E	355 mg/l
Lipoprotein (a)	2 mg/dl
TSH	0.76 μIU/ml

Abbreviations: WBC: White blood cells; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; γGT: γ-glutamyl-transferase; Tbil: Total bilirubin; Dbil: Direct bilirubin; CK: Creatine kinase; LDH: Lactate dehydrogenase; T-CHOL: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; TSH: Thyroid stimulating hormone.