Peer-Review Record

Circulating culprit or therapeutic bullseye: lipoprotein(a) in cardiovascular risk assessment and novel therapeutic prospects

by Arturo Cesaro, Gianmaria Scherillo, Gianantonio De Michele, Vincenzo Acerbo, Giovanni Signore, Domenico Panico, Gennaro Porcelli, Francesco Scialla, Giuseppe Raucci, Francesco Paolo Rotolo, Marco Tontodonato, Antonio De Pasquale, Andrea Vergara, Danilo Lisi, Mario Massimo Mensorio, Fabio Fimiani, Paolo Calabrò

Academic Editor: Ali J. Marian

Reviewer 1: Anonymous
Reviewer 2: Anonymous

Round 1

Reviewer 1 Report

In this invited review, the authors thoroughly discuss the genetic, clinical, and therapeutic aspects of Lp(a), whose positive correlation with ASCVD, thrombosis, AV stenosis, inflammation, and aging has been well established.

The carefully written review may offer the readers an updated panoramic view of this critically important topic.

The following suggestions may help to increase the impact of this review further:
1. If several types of treatment can either increase or decrease the plasma level of Lp(a), why is it so tightly controlled by genetic factors?
2. Is apo(a) exclusively associated with Lp(a), or can it exist by itself or be associated with other lipoproteins or other structures?
3. Please provide more discussion about the mechanisms underlying the various clinical manifestations.
4. Figure 1 can be further improved: fewer words, more information. The blue background color does not enhance the clarity of the figure.
5. Create a new table to associate various types of Lp(a)-lowering treatment with clinical benefits (or adverse effects), such as MACE event reduction.

Author Response

In this invited review, the authors thoroughly discuss the genetic, clinical, and therapeutic aspects of Lp(a), whose positive correlation with ASCVD, thrombosis, AV stenosis, inflammation, and aging has been well established.

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The following suggestions may help to increase the impact of this review further:

1. If several types of treatment can either increase or decrease the plasma level of Lp(a), why is it so tightly controlled by genetic factors?

Thanks for the comment. Despite several treatments may influence Lp(a) circulating levels, ones that act on genetic determinants of Lp(a) level have pointed out the strongest clinical results. This finding underlines the link between Lp(a) level and genetic factors.

To make the concept clearer, we added lines 152-154 :

"The link between genetic factors and circulating levels of Lp(a) has led researchers to look for genetic therapies for lowering Lp(a) level, such as RNA-targeted therapies, in particular antisense oligonucleotides (ASOs) or small interfering RNAs (siRNAs), to regulate apo(a) mRNA production."

2. Is apo(a) exclusively associated with Lp(a), or can it exist by itself or be associated with other lipoproteins or other structures?

Apo(a) is one of the Lp(a) components. Its structure is similar to other proteins with different functions. To provide additional information about apo (a), we have decided to add to our manuscript lines 93-97 :

Kringle domains are also present in proteins such as angiostatin, hepatocyte growth factor, prothrombin, urokinase, and tissue-type plasminogen activator. The common structure of apo(a) and angiogenic factors such as plasminogen and angiotensin has led to a possible anti-angiogenic role of apo(a). About this association, contradictive results are available in the current literature.[8]

3. Please provide more discussion about the mechanisms underlying the various clinical manifestations.

Thanks to reviewer for her/his comment. This gives us the opportunity to better elucidate the mechanisms behind the different manifestations.

To this end, we have added to the text : " *Lp(a) is the principal lipoprotein carrier for OxPL and its role adversely affects arterial wall. In fact OxPLs, preferentially carried by Lp(a) in the*

plasma, are supposed to play a crucial role in the Lp(a) pro-inflammatory properties: several studies suggest that the OxPL component of Lp(a) particles promotes endothelial cells dysfunction, autotaxin and chemo-attractants delivery, transendothelial migration of monocytes and macrophage apoptosis.

Such cellular mechanisms could underlie atherosclerosis through increased plaque vulnerability, enhanced foam cell formation, increased endothelial cell permeability and vascular smooth muscle cell proliferation and migration [46].

Schnitzler et al.[47] in an intriguing study highlight another metabolic pathway associated with Lp(a) pathogenicity: 6-phophofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB3) is considered the main glycolytic player of Lp(a)-induced endothelial inflammation. Increased endothelial PFKFB3 and ICAM (intercellular adhesion molecule)-1 expression has been detected in carotid endarterectomy patients with high Lp(a) levels and blocking PFKFB3 activity seems to reduce the inflammatory burden of OxPLs associated with Lp(a)[48].

Lp(a) promotes its atherogenic actions when transferred from circulation to the arterial wall: high Lp(a) levels result in an excessive amount of OxPLs in the arterial wall leading to plaque instability and subsequent CV events mainly represented by myocardial infarction and ischemic stroke [49]–[52]." (lines 254-271).

"*Lp(a)-transported OxPL is considered a key factor contributing to the development of AVS because it induces the expression of calcification genes in vascular and valvular cells promoting AVS, increasing the incidence of aortic valve replacement and death [57].*

Pro-osteogenic effects of Lp(a) and OxPL on valvular interstitial cells promote the progression of AVS through an increased calcification activity and a faster progression in calcium score and in hemodynamic progression assessed both by echocardiography and computed tomography leading to an enhanced aortic valve replacement and death [57].

In addition, it is interesting to refer to a brilliant study by Bouchareb et al. [58], in which they identified that autotaxin and lysophosphatidic acid are involved in the pathobiology of calcific aortic valve disease by promoting inflammation and mineralization of the aortic valve. Therefore, those findings have a potentially relevant translational impact because autotaxin or lysophosphatidic receptors could represent novel therapeutic targets for the management of calcific aortic valve disease [59]" (lines 302-315).

4. Create a new table to associate various types of Lp(a)-lowering treatment with clinical benefits (or adverse effects), such as MACE event reduction.

Dear reviewer, thank you for your comment. We have added the table as suggested. It is identified in the text as table 1.

Drug/Class	Effects on Lp(a) concentration	Proven MACE reduction
Statins	8.5 – 19.6 % increase	Yes
Ezetimibe	No effect	Yes
Niacin	20-30% reduction	No
CETP Inhibitors	32-56% reduction	No
PCSK9i	26% reduction	Yes
Lp(a) apheresis	78-94% reduction	Yes (retrospective trials only)
Inclisiran	18.6-25.6% reduction	No
Bempedoic Acid	No effect	Yes
Pelacarsen	77.8% reduction	Unknown
Olpasiran	100% reduction	Unknown
Muvalaplin	63-65% reduction	Unknown

Reviewer 2 Report

This is a comprehensive and informative review by Dr. Calabro's group. It provides an up to date and balance view of the field. The reviewer has minor suggestions a s follows:

1. The reviewer suggests writing the gene name in italics.
2. Line 39-abstract. This sentence is confusing. Please clarify. It may be clearer if you describe the components of the Lp(a) first, as done in the introduction section. To clarify you might wish to state that the LPA gene encodes apo(a) and the variation in the kringle domains is the main determinant of plasma lp(a) level. A figure might be useful to the un-initiated.
3. Line 85. Period is missing at the end of the sentence.
4. Regarding association of the single nucleotide variants with the plasma Lp(a), it might be useful to point out only those that have been replicated.
5. Lp(a) levels are reported as mg/dl or nmol/L. This might confuse the reviewers.
6. Discussion on Lp(a) and risk of atherosclerosis emphasizes the need for aggressive therapy of other risk factors. It is worthwhile noting in this section that potent therapies are emerging, which enable directly targeting the Lp(a) levels.
7. Graphic presentation of the Lp(a) levels and risk of atherosclerosis will be helpful.
8. Ditto for the association of Lp(a) levels and aortic valve stenosis.
9. Line 434. The reviewer suggests introducing Inclisiran by describing what it is and how it works.

10. Ditto for Bempedoic acid, as some readers might not be familiar with it.

11. Regarding new therapies for Lp(a), you might wish to discuss the following study: Clinical Trial JAMA. 2023 Sep 19;330(11):1042-1053. doi: 10.1001/jama.2023.16503. Muvalaplin, an Oral Small Molecule Inhibitor of Lipoprotein(a) Formation: A Randomized Clinical Trial

Stephen J Nicholls 1, Steven E Nissen 2, Cynthia Fleming 3, Shweta Urva 3, Jeffrey Suico 3, Paul H Berg 3, Helle Linnebjerg 3, Giacomo Ruotolo 3, P Kellie Turner 3, Laura F Michael 3

PMID: 37638695 PMCID: PMC10463176 DOI: 10.1001/jama.2023.16503.

Author Response

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2. Line 39-abstract. This sentence is confusing. Please clarify. It may be clearer if you describe the components of the Lp(a) first, as done in the introduction section. To clarify you might wish to state that the LPA gene encodes apo(a) and the variation in the kringle domains is the main determinant of plasma lp(a) level. A figure might be useful to the un-initiated.

Many thanks to the reviewer for his suggestions. The abstract has been edited as suggested.

3. Line 85. Period is missing at the end of the sentence.

Thank you for your suggestion. We have modified the line 85:

Lp(a) assembly occurs at the hepatocyte cell membrane surface or in the space of Disse.

4. Regarding association of the single nucleotide variants with the plasma Lp(a), it might be useful to point out only those that have been replicated.

Thank you for your suggestion. In our manuscript, we have decided to include all the single nucleotide polymorphisms (SNPs) that have been associated with higher or lower Lp(a) circulating levels.

5. Lp(a) levels are reported as mg/dl or nmol/L. This might confuse the reviewers.

Thanks for the comment. The different unit of measurement comes from reporting data from verses studies that used sometimes mg/dl and sometimes nmol/L. However, to convert nmol/L of Lp(a) to mg/dL, we can multiply the nmol/L value by 0.4. (or divide by 2.5). To convert mg/dL of Lp(a) to nmol/L, we can multiply the mg/dL value by 2.5 (or divide by 0.4). To simplify the reading, we have reported next to the values in nmol/L also the values in mg/dL.

6. Discussion on Lp(a) and risk of atherosclerosis emphasizes the need for aggressive therapy of other risk factors. It is worthwhile noting in this section that potent therapies are emerging, which enable directly targeting the Lp(a) levels.

Thanks for the comment. As suggested, we added a sentence to text to emphasize the opportunity to use new therapeutic weapons directed against Lp(a). (lines 273-274).

7. Graphic presentation of the Lp(a) levels and risk of atherosclerosis will be helpful.
8. Ditto for the association of Lp(a) levels and aortic valve stenosis.

Thanks for the comment. A new figure 1 as provided.

9. Line 434. The reviewer suggests introducing Inclisiran by describing what it is and how it works.

Thank to reviewer for the comment. This provide us the opportunity to better explain the mechanism of action of inclisiran. For this purpose we added to text: "*Inclisiran is a novel drug for lipid lowering that uses small interfering RNA (siRNA) technology. It is designed to specifically inhibit the production of PCSK9 in the liver via RNA interference, promoting PCSK9 mRNA degradation.[84]*"

10. Ditto for Bempedoic acid, as some readers might not be familiar with it.

Likewise, we added information regarding bempedoic acid.

Bempedoic acid is a novel drug for LDL-C lowering that acts by inhibiting ATP citrate lyase (ACL) in the liver, an enzyme that is featured upstream of HMG-CoA reductase in the cholesterol synthesis pathway, studied within the CLEAR program [81–85]. (lines 484-486)

11. Regarding new therapies for Lp(a), you might wish to discuss the following study:
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Muvalaplin, an Oral Small Molecule Inhibitor of Lipoprotein(a) Formation: A Randomized Clinical Trial
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Thank you for the comment. It allows us to make our review more complete and up-to-date. We have added the suggested study to the discussion. (lines 575-585)