Peer Review File

Article information: https://dx.doi.org/10.21037/aob-21-49

Reviewer A

The authors have written an informative overview of the current knowledge on CD36 gene variants and associated clinical pathologies. The review is well-structured and provides the main references for further reading. However, some aspects are discussed too briefly and in some cases the proper references are not given; these points need to be addressed.

Comments

1. Abstract

- CD36 is officially known as SR-B2 (see Prabhudas, J Immunol 192: 1997, 2014). Please use this name once in the abstract so that literature searches for "SR-B2" will find your review.

Reply: SR-B2 has been added in abstract and introduction sections.

Changes in the text: line 15, and line 84-85. The line refers to the position in the revised version (with modification traces of insertion, deletion, etc.), similar below.

- Please add that, at least in parenchymal cells, CD36 also recycles between subcellular compartments (endosomes) and the plasma membrane, thereby regulating its presence and thus activity at the plasma membrane. (Reviewed in ref. 6).

Reply: The description has been added in abstract according to the reviewer's suggestion.

Changes in the text: Line 18-20.

2. Type I and type II deficiency

- The data presented are for blood cells. Is there any data on the presence, or not, on other cell types in which CD36 occurs?

Reply: In type I deficiency CD36 is not expressed in all cells, whereas in type II deficiency CD36 is expressed in monocytes but not in platelets. No data show other cells expression of type II deficiency.

Changes in the text: Not changes.

- Interestingly, in Table 2, reference 27 is on CD36 mutations affecting lipid uptake. This aspect (lipid uptake/ fatty acid metabolism) is not further discussed. (Perhaps the reference 27 should also be covered in section 4 of the review).

Reply: Mutations of the CD36 gene affecting lipid uptake in the human heart have been briefly mentioned in section 4, and the reference 27 has been covered in section 4.

Changes in the text: Line 279-281.

3. Section 4 of the review

Line 203: reference should be made to Laugerette, J Clin Invest 115: 3177, 2005.

Reply: The reference has been added according to the reviewer's suggestion.

Changes in the text: Line 236.

4. Conclusions

- Line 316: this phrase is unclear, especially “we studied”. Please revise.

Reply: This sentence has been deleted.

Changes in the text: Line 362-364.

- Lines 318-320: other papers on CD36 as drug target include: Geloen, PLoS One 7: e37633, 2012; Glatz, Expert Opinion Ther Targets 25: 393, 2021.

Reply: Those papers have been added in references.

Changes in the text: Line 370.

5. CD36 and gender

Gender-specific associations of CD36 polymorphisms with heart disease have been reported and would be of interest to include in the review. See Du et al., Gene 753: 144806, 2020.

Reply: The data of gender-specific correlation between CD36 polymorphisms and atherogenic lipid profile have been added in table 4.

Changes in the text: Table 4.

6. Textual suggestions and minor comments

- line 16: please add “skeletal and cardiac myocytes” because this occurrence is important for the role of CD36 in heart disease.

Reply: This sentence has been revised according to the reviewer's suggestion.

Changes in the text: Line 18.

- line 18: use plural, i.e., lipoproteins, fatty acids

Reply: This sentence has been deleted in revised version.

Changes in the text: Line 37-38.

- line 52: “were relevant to” is unclear. Use “were disclosed” instead.

Reply: This sentence has been revised according to another reviewer's suggestion.

Changes in the text: Line 71-72.

- line 56: “…collagen and anionic…” “…and functions as a scavenger…”

Reply: This sentence has been revised according to the reviewer's suggestion.

Changes in the text: Line 77.

- line 57: use plural

Reply: This sentence has been revised according to the reviewer's suggestion.

Changes in the text: Line 78.

- line 57: “…it may play a role…”. This role has been established and confirmed. See Bonen, J Biol Chem 275: 14501, 2000; Zhu, Theranostics 10: 1332, 2020; also reviewed in ref. 6.

Reply: The word “may” have been deleted.

Changes in the text: Line 78.

- lines 61-64: please add the official name SR-B2 and the reference to Prabhudas (see comment 1).
Reply: This sentence has been revised according to the reviewer's suggestion.
Changes in the text: Line 84-85.

- lines 70-85: (a) reference(s) are lacking. Please add Hsieh, Nature Commun 7: 12837, 2016 in which the structure of CD36 and the basis for CD36 binding by the malaria parasite are presented.
Reply: The gene structure we referred from gene information of NCBI web, and added the website as reference 5. For the description of protein structure, we mainly refer to reference 6.
Changes in the text: Line 106 and line 110.

- line 91: "20754" should read "20,754".
Reply: This sentence has been revised according to the reviewer's suggestion, and the data were updated.
Changes in the text: Line 120-121.

- line 94: "… a small number…". Please provide that number or an estimated number.
Reply: There are 590 missense mutations of CD36 gene in dbSNP database.
Changes in the text: Line 125.

- line 121: "acids" should read "acid"
Reply: This sentence has been revised according to the reviewer's suggestion.
Changes in the text: Line 154.

- line 207: "…obesity or thin." This is unclear, please explain "thin".
Reply: The sentence has been deleted by another reviewer.
Changes in the text: Line 240-241.

- line 215: "…higher plasma fatty acid and …"
Reply: This sentence has been revised according to the reviewer's suggestion.
Changes in the text: Line 249.

Reviewer B

The authors have produced a comprehensive narrative review on the CD36 gene and its various health-related associations. Overall, this review paper is informative and summarizes important findings related to the CD36 gene. The following minor suggestions can be considered by the authors:

1. Methods: While this is a narrative review without a systematic search strategy, the article would be strengthened with the inclusion of a brief description of the review methods and/or justification for the topics that are presented. This can be a new subsection titled Methods or added in to the current Introduction.
Reply: We added the search literature strategy and some key words in the end of Introduction section.
Changes in the text: Line 89-94.

2. CD36 and taste perception: The authors missed an important recent study on CD36 and lipid taste perception, that demonstrated a possible link between CD36 variation, saturated fat intake, and serum triglycerides levels among individuals with overweight/obesity (but not normal weight individuals): https://pubmed.ncbi.nlm.nih.gov/33574567/
It would be useful for this paper to be included in this section of the review and the point about adiposity potentially being a mediator of CD36 variation and taste perception.
Reply: The paper and its found have been added according to the reviewer's suggestion.
Changes in the text: Line 279-283.

3. Page 8 Lines 237-238: The authors should also add that taste acuity may decline with age (another reason why CD36 variation and taste perception may be more pronounced among children).
Reply: This sentence has been revised according to the reviewer's suggestion.
Changes in the text: Line 273-274.

4. Conclusions: The authors should note the limitations of a narrative review approach. This section could also be strengthened with mention of any common limitations to the current evidence around CD36 and health-related outcomes. Are more functional genomics studies needed to confirm effects and mechanisms?
Reply: We added the limitations of current evidence on CD36 and health-related outcomes in Conclusions section.
Changes in the text: Line 365-366.

Reviewer C

The authors provide a comprehensive review on CD36 variants and their clinical relevance in immune thrombocytopenia, malaria, lipid taste perception, obesity and other metabolic syndromes. They systematically screened recent and relevant references related to the topic.
Some minor questions or remarks are listed in the following.

1. The authors cite numerous references regarding the effect of single nucleotide variations (SNVs) on different diseases and symptoms. In terms of acuteness of thoughts it could be helpful to rate the effect of different mutations like nonsense and frame shipft mutations (more likely inducing CD36 deficiency) vs. non-synonymous (more likely to reduce expression) and regulatory SNPs with regard to severity of disease via interference with the immune response or e.g. blood clotting.
Reply: The reviewer's comments are entirely to the point.
Changes in the text: No change.

2. Please try to use the correct position numbering of variants according to http://www.hgvs.org/mutnomen/recs.html throughout the text with first mentioning of the original position. This could simplify understanding.
Reply: The website provided by the reviewer is frozen since May 1, 2016. It has been replaced by a new version at http://www.HGVS.org/varnomen. We have tried to use the correct position numbering

of variants according to www.HGVS.org and corresponding rs ID according to dbSNP database if it is possible.
Changes in the text: Whole manuscript.

3. Can you please add a reference for the presence of soluble sCD36 (lines 87/88). Do you have any information regarding the contribution of sCD36 to malaria and metabolic syndromes? Can sCD36 inhibit receptor binding?
Reply: A reference and related description have been added according to reviewer's suggestion.
Changes in the text: Line 116-117.

4. Please explain the relationship between type II deficiency and autoimmune tolerance (lines108/109). If CD36 is expressen on monocytes and other cells and tissues, CD36 should be recognized by the immune system as self antigen.
Reply: We understand that in type II deficiency, CD36 is expressed on monocytes and the immune system recognizes CD36 as its own antigen and does not produce anti-CD36. This is autoimmune tolerance to the CD36 antigen in type II deficient individuals.
Changes in the text: No change.

5. It could be helpful to add recent references regarding CD36 deficiency in Arabs including the identified variants (e.g. Flesch et al. Transfusion 2021) (lines 112/113 and Table 2).
Reply: The data of Arabians have been added in corresponding paragraph and table, and the paper has been inserted into the references.
Changes in the text: Line 142-144 and table 2.

6. Please comment on variation within the CD36 regulatory/promoter region and possible effects onto CD36 expression, e.g. Xu X et al. (your own paper, ref. 18) or Kashiwagi H et al. Thromb Haemost. 1995.
Reply: We have added relevant descriptions and references according to reviewer's suggestion.
Changes in the text: line 149-151.

7. Please include the respective reference(s) for the first paragraph of sub-heading 4 (lines 197-207).
Reply: Two references have been inserted in this paragraph.
Changes in the text: Line 236 and 241.

8. Conclusions: There is significant evidence that CD36 on various cells and tissues is involved in the development of different disorders. However, from my point of view it seems that anti-CD36 induced thrombocytopenia is the only undoubtful relation between complete CD36 absence and disease. But even within thrombocytopenia the impact of single SNV on type I deficiency is under discussion. Concerning disorders other than immune thrombocytopenia data are much more conflicting not only with regard to single variations but also with respect to general inhibitory or inducing effects. Maybe the authors like to stress this point within the conclusion.
Reply: Thanks to the reviewer's comments, we have added this viewpoint to the conclusion section.
Changes in the text: Line 355-361.

Reviewer D

The review is a pertinent and well-organized article. I just recommend some minor modifications, particularly linguistic corrections.

-In the Introduction section, it should be mentioned that CD36 was initially also known as FAT (fatty acid translocase)

Reply: The FAT (fatty acid translocase) has been mentioned in the second paragraph of Introduction.

Changes in the text: Line 78 and 84.

-A diagram of CD36 with different binding sites is mandatory for the article.

Reply: In order not to infringe on the originality of a diagram, we only use Table 1 instead of a diagram. The data in Table 1 are derived from several different literatures and summarized.

Changes in the text: no change.

-aim “to” should be aim “at + gerund”

Reply: This sentence has been revised according to the reviewer's suggestion.

Changes in the text: Line 69.

-Studies have “found” should be studies have “reported”

Reply: This sentence has been revised according to the reviewer's suggestion.

Changes in the text: Line 73.

Reviewer E

1. The manuscript is in need of improvements in use of written English. I have corrected several instances in the attached copy “AOB-2021-ICD-05(AOB-21-49)-review comments”, but the entire manuscript should be reviewed and corrected for English.

Reply: As non-native English writers, we have done our best and made the changes according to the editor's suggestion.

Changes in the text: Whole manuscript.

2. The authors provide references in several Tables, but should also be sure to cite these references where appropriate in the body of the manuscript itself.

Reply: References are added at the location specified by the editor and several other locations.

Changes in the text: Whole manuscript.

3. Discussion of some sections can be significantly reduced, and others require additional discussion. Malaria section could be reduced significantly. Thrombocytopenia could benefit from mention of variants identified in patients that developed antibodies. Also, a bit more detail should be provided about the reported gene variants associated with Type II CD36 deficiency. Cardiovascular section could use a little more discussion.

Reply: We have tried our best to revise the manuscript.

Changes in the text: Whole manuscript.