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Reviewer A

Major comment

Comment: "Although the authors describe three transmembrane glycoproteins complexes and one GPI-linked glycoprotein which harbor the different platelet antigens, they do not discuss antibodies against the antigens within the HPA-2 and HPA-15 systems. In such a comprehensive review, it would be natural also to include sections about antibodies against the antigens on GPIb/IX/V and CD109."

Reply: HPA-2a and other antigens on GP Ib/IX/V and CD 109 are of minor importance in FNAIT. We have, however, added to sections on these two groups of antigens (page 17-19).

Minor comments

1) Page 3, line 71: Please explain the abbreviation "GPI".

Reply: The GPI abbreviation "glycosylphosphatidylinositol" is given.
Changes in the Text: "a glycosylphosphatidylinositol (GPI)-linked glycoprotein" (page 3, line 78)

2) Page 4, lines 90 and 91: "In 85 newborn babies with FNAIT (anti-HPA-1a), 2 (2.4%) suffered from ICH" should be changed to "In 79 newborn babies with FNAIT (anti-HPA-1a), 2 (2.5%) suffered from ICH".

Reply: Thank you for this rectification. The numbers are corrected.

Changes in the Text: In 79 newborn babies with FNAIT (anti-HPA-1a), 2 (2.5%) developed ICH." (page 4, lines 99 and 100).

3) Page 4, lines 145 to 149: It has been speculated that the immunizing agent during pregnancy could be HPA-1a expressed on the fetal trophoblast or on microparticles from the fetal trophoblast that are released to the maternal circulation during pregnancy. The phrases used by the authors seem to indicate that this is a fact based on experimental or clinical data; if so, they need to refer to publications that support this statement; references 20 to 22 do not provide firm evidence about the nature of the immunizing agent. Please add appropriate references or rephrase the text.

Reply: Thank you for drawing attention to this important issue. The text is now rephrased.

Changes in the Text: Line 147 to 150: "However ... generated." is changed to: "As HPA-1a is expressed on αvβ3 integrin in the fetal trophoblast, one might speculate that contact of maternal blood with trophoblast or circulating microparticles in maternal blood result in maternal alloimmunization against HPA-1a on β3 and αvβ3 complex (21-23)" (page 6, lines 158 to 161).

4) Page 6, line 162: "0.38" should be changed to "0.39".

Reply: The number is corrected.
Changes in the Text: "...and 0.39 to 0.50, respectively" (page 6, line 173).

5) Page 6, lines 167 to 169: The sentence "Ongoing observations from animal models suggest that the rarity of FNAIT mediated by anti-αIIb antibodies may be attributed to life-threatening phenotypes, resulting in underreported miscarriages" is almost identical with the following sentence from reference no. 17: "Ongoing studies from animal models suggest that the rarity of anti-αIIb and anti-GPIbα-mediated FNAIT may be attributed to life-threatening phenotypes, resulting in underreported miscarriages". Instead of referring to another review, the authors should rather refer to reports containing original data and explain more thoroughly what is meant by this sentence.

Reply: Thank you for pointing out this inaccuracy. The sentence is now rephrased.
Changes in the Text: The text "Ongoing observations from animal models suggest that the rarity of FNAIT mediated by anti-αIIb antibodies may be attributed to life-threatening phenotypes, resulting in underreported

miscarriages" in lines 167 to 169 is now deleted from the page 6. Instead of this a new sentence is inserted in page 7. ".... differentiating mouse trophoblast (46). Ongoing study in animal model using aIIb deficient and human aIIb transgenic mice indicated that maternal anti-aIIb may target trophoblast cells and lead to placenta dysfunction (Vadaz B, Master Thesis, 2015; reference no. 47)." (page 7, lines 207 to 209).

6) Page 8, lines 259 to 261: The authors state: "In accordance with the previous results, anti-HPA-1a antibodies reacting with αvβ3 derived from these GT patients impacted the angiogenesis process". This aspect was neither examined by Jacquelin et al. 2003 (reference no. 69) nor Fiore et al. 2021 (reference no. 13). Please provide an appropriate reference or rephrase.

Reply: We apologize for this inaccuracy. The sentence is now corrected.
Changes in the Text: The sentence "In accordance with the previous results, anti-HPA-1a antibodies reacting with αvβ3 derived from these GT patients impacted the angiogenesis process" is now deleted in the revised version (page 9; line 270).

7) Page 12, lines 350 to 371: This comprehensive list of details in these two paragraphs about frequencies of different types of CD36 deficiency in different populations, could be reduced significantly or reserved for a table that could be published on-line as Supplementary information.

Reply: Thank you for pointing out this issue. These paragraphs about the frequencies of CD36 deficiency are reduced.
Changes in the Text: The sentence "However, a higher incidence of CD36 deficiency was found in the Chinese Zhuang ethnic group living in South China in Guangxi province (5.76%) (116). The frequencies of type I-deficient individuals in Han and Zhuang ethnic groups were about 0.5% and 1.3%, respectively, which are lower than the frequency in the Japanese (1.50%; 79, 112, 116)" and "Homozygous or compound heterozygous mutations in the CD36 gene associated with the total absence of CD36 surface expression on platelets and monocytes have been discovered." are now deleted in the revised version.
The sentence "The highest frequency of CD36-deficient individuals (type I and type II) was reported in Japan (8.15%) …...Thailand (2.14%) (79, 98, 103, 113-115)" is now changed into "A higher frequency of CD36-deficient individuals (type I and type II) was reported in Japan (6.8%) (114). In China, the frequency of CD36 deficiency on platelets ranges from 1.8% to 3.6% in largest ethnic group, the Han population (81, 115,116). Similar frequency (2.64%) was reported in the Arabian population (117)."(page 12, lines 361 to 365).

8) Page 13, lines 399 to 401: The authors state: "…other mechanisms, including hemolysis due to complement activation by anti-CD36, may also contribute to the pathogenesis of AHF." Complement activation is not a necessary requirement for AHF. Untreated RhD-immunization is often associated with AHF and anti-D does not activate complement. Does anti-CD36 activate complement?

Reply: Thank you for pointing out this issue. We have strong evidence that anti-CD36 antibodies could induce complement activation (paper in preparation). Since the direct impact of complement activation and the development of AHF is not shown yet, we delete this speculation in the revised version.
Changes in the text: "However, other mechanisms, including hemolysis due to complement activation by anti-CD36, may also contribute to the pathogenesis of AHF" is deleted (page 13, line 403).

9) Page 13, line 403: The authors state: "CD36 is found on … basal membrane". The CD36 molecule is anchored in the cell membrane. How come that it is also found on an extracellular structure such as the basal membrane? How is CD36 anchored to this structure? Please, explain or rephrase.

Reply: This is issue is mentioned in the review article (Duttaroy et. Al., 2009) and reported by Campbell and coworkers (Placenta 1998;19:409-415). In this study, the authors proved the presence of CD36 in the purified microvillous and basal membrane fraction using Western blot analysis. They found CD36/FAT, FATP (fatty acid transport protein) in basal membrane, but not in p_FABPpm (placental membrane fatty acid-binding) (Campbell et al. Figure 1). Besides as membrane anchored protein, CD36 also exists as soluble form (Little et al. Sem Thromb Haemost 2010; 36:845-856). It is theoretically possible that this soluble CD36 is absorbed by the basal membrane.

10) Page 14, line 427: “47.2%” should be changed to “49”.

Reply: agree
Changes in the text: This part is deleted.

11) Page 14, line 428 to 431: In this context, it would be natural also to mention that not only the risk of HPA-1a-immunization, but also neonatal outcome is associated with the HLA-DRB3*01:01 carrier status (Kjeldsen-Kragh et al. Blood Adv 2019;4(14):3368-3377).

Reply: Thank you for pointing out this important issue. This fact is now added in the revised version.
Changes in the text: The following text is added “Accordingly, the outcome of the neonates is associated with the HLA-DRB3*01:01 (new citation Kjeldsen-Kragh et al. Blood Adv 2019; 3:945-951; reference no. 138) (page 14, line 432).

12) Page 14, line 430 and 431: A narrative review (reference no. 137) is used to support the statement: “the risk of immunization is about 25 times higher if the mothers are a carrier of HLA-DRB3*01:01”. It would be more appropriate to refer to the manuscript that originally reported this estimate (Kjeldsen-Kragh & Olsen. Transfusion, 2019;59(4):1344-52).

Reply: Thank you for this suggestion. The reference no.137 is changed to a new one (reference no.137).
Changes in the text: No change in the text. Only the reference no. 137 is now substituted by the new reference (Kjeldsen-Kragh & Olsen. Transfusion, 2019;59:1344-1352).

13) Page 14, lines 438 and 440: As a supplement or an alternative to HPA-1a-typing of the father it should be mentioned that more recent methods allow prediction of the fetal HPA-1 type based on cell-free fetal DNA collected from the mother’s plasma. Determination of the fetal platelet type by such non-invasive methods will avoid the ethical challenges that may occur when clinical decisions are based on samples collected from the (presumptive) father.

Reply: We followed the reviewer’s proposal and included noninvasive typing of the fetus and changed the text from line 431 to 436 “In HPA-1a immunized women ... the following child” including the comments in no 14). Reference 138 has been replaced by Radder et al., 2003 (pmid: 12757506; reference no.142)
Changes in the Text: “In HPA-1a immunized women with at least one previously affected child, the risk for the next pregnancy depends upon the paternal genotype (HPA-1aa or HPA-1ab). In case of a heterozygous father (HPA-1ab) the fetal HPA genotype should be determined, preferably by (noninvasive) typing of cell free fetal DNA in maternal plasma (Wienzek-Lischka S ,2015;Nogués, 2020 pmid:31953107, Ouzegdouh Mammasse et al., 2020 pmid:32266719; reference no.139-141). The risk of fetal ICH is enhanced, if a previous sibling with FNAIT suffered from ICH (Radder et al., 2003 pmid: 12757506; reference no.142) and the clinical outcome of the newborn seems to be worse, if the mother carries the DRB3*01:01 antigen (Kjeldsen-Kragh J et al, pmid: 31919011; reference no.143).” (page 14, lines 439 to 445).

14) Page 14, line 441: To what extend can neonatal platelet count or duration of thrombocytopenia be used for the prediction of the severity of FNAIT in a subsequent pregnancy? Instead, HLA-DRB3*01:01 typing of the mother may provide important information about risk of severe FNAIT in a subsequent pregnancy. Please provide relevant references or rephrase.

Reply: We followed the reviewer’s recommendations.
Changes in the Text: See changed text in “no. 13)”.

15) Page 14, lines 443 and 444: It is important to mention that IVIg is used off-label for treatment of HPA-1a-immunized pregnant women, and that the evidence for the efficacy of IVIg for this indication is very weak as IVIg has never been tested in a placebo-controlled clinical trial.

Reply: This is true, we amended the text accordingly.
Changes in the Text: The sentence “For pregnancies at risk ... therapy with corticosteroids”(Lines 439 to 441) is now changed to “For pregnancies at risk, maternal IVIG therapy (1 g/kg once a week) is considered to be effective in avoiding cerebral bleeding. Due to the lack of randomized studies with a control group, maternal IVIG for prevention fetal ICH in NAIT is used off-label. It is not clear whether additional maternal therapy with corticosteroids is effective (144).” (page 14, lines 445 to 449).

16) Page 14, line 454: "…are also effective" should be changed to "…are usually also effective".

Reply: We agree with the suggestion of the reviewer.
Changes in the Text: The text is now changed into "However, in urgent situations, platelet concentrates from random donors are usually also effective."(page 15, line 458).

17) Page 16, lines 487 and 488: Modified human recombinant anti-HPA-1a, such as B2G1Δnab has never been tested in pregnant HPA-1a-immunized women. Please delete or rephrase.

Reply: Thank you for pointing out this wrong information. We deleted the wrong sentence.
Changes in the Text: The sentence "These modified mAbs against HPA-1a …...(149-151)" is deleted in the revised version. (page 16, line 492).

18) Page 16, lines 498 to 503: Why are the fetal death rates provided with two decimals? One or no decimals would suffice.

Reply: The number is originally from our experimental results published recently by Xu et al., 2021. Indeed, it is not mandatory to provide fetal death rates with two decimals. We decide to mention without decimal.
Changes in the Text: We changed overall 40.00% into 40% (page 16, lines 506, 509, 510), 12.70% into 13% (page 16, line 510) and 2.17% into 2% (page 16, line 507).

19) Page 16, line 507: "… is visible" should be changed to "… could be envisaged".

Reply: Correct.
Changes in the Text: The sentence "...as a drug to prevent severe FNAIT caused by anti-CD36 could be envisaged." (page 16, line 513).

20) Page 16, lines 512 to 515: It should also be mentioned that NAITgam is in clinical trial (EudraCT Number: 2019-003459-12) and that the first results from the phase 1/2 trial have shown that NAITgam can reduce the half-life HPA-1a positive platelets transfused to HPA-1a negative males from 2.7 days to 19 min. (Geisen C, Fleck E, Schäfer SMG, Walter C, Braeuninger S, Olsen K, Bhagwagar Z, Mortberg A, Wikman A, Kjaer M, Kjeldsen-Kragh J, Behrens F, Seifried E, Köhm M. Rapid and complete clearance of HPA-1a mismatched platelets in a human model of fetal and neonatal alloimmune thrombocytopenia by a hyperimmune plasma derived polyclonal anti HPA-1a antibody [abstract]. Res Pract Thromb Haemost 2021; 5 (Suppl 1); https://abstracts.isth.org/abstract/rapid-and-complete-clearance-of-hpa-1a-mismatched-platelets-in-a-human-model-of-fetal-and-neonatal-alloimmune-thrombocytopenia-by-a-hyperimmune-plasma-derived-polyclonal-anti-hpa-1a-antibody/).

Reply: This information is now added in the revised version.
Changes in the Text: Line 514 .. line 515: "However ... prophylactic therapy": First results from a clinical trial show (EudraCT number 2019-003459-12) show that NAITgam is able to reduce the half life of HPA-1a positive platelets transfused to HPA-1a-negative subjects (Geisen et al., 2021; reference No.163). However, more trials are mandatory to show both safety and clinical efficiency of this prophylactic therapy (page 16, lines 521-524).

21) Page 22, line 696: "tw1o-" should be changed to "two-".

Reply: Thank you for pointing out this wrong information.
Changes in the Reference: The word "tw1o-" is now changed to "two-" (page 24, line 779).

22) Page 28, lines 909 and 910: "[published online ahead of print, 2021 Apr 8]. Am J Obstet Gynecol 2021; S0002-9378(21)00436-1" should be changed to "Am J Obstet Gynecol 2021;225 (2):120-127".

Reply: This reference is now updated.
Changes in the Reference: This reference is changed into Am J Obstet Gynecol 2021;225 (2):120-127 (page 30, line 999).

23) Page 31, Figure 2: What is the grey area between the cytotrophoblast and the endothelium?

Reply: The grey area represents embryonal connective tissue. We therefore labeled this area with “stroma” in figure 2.
Changes in the Legend: Figure 2: Possible alloimmunization of HPA-1a (-) mother by HPA-1a (+) fetus. Simplified representation of fetal-maternal placenta interface is illustrated. HPA-1a (+) fetal and HPA-1a (-) maternal platelets expressed abundantly on □IIb□3, but little on □v□3 are indicated. The expression of HPA-1a (+) on □v□3 in syncytiotrophoblast is shown. Theoretically, sensitization of the mother may occur by direct contact of maternal blood with syncytiotrophoblast or/and through the shedding of trophoblast microparticles into the maternal circulation or by fetal platelets resulting from obstetrical, trauma related complications or at parturition (page 34, lines 1127 to 1134).

24) Page 31, lines 973 to 975: It should be emphasized that it has been speculated that immunization could occur by direct contact of maternal blood with syncytiotrophoblast or/and through the shedding of trophoblast microparticles into the maternal circulation, but this has so far not been demonstrated experimentally or clinically.

Reply: The impeccable of the reviewer is justified. The legend is now modified.
Changes in the legend: see “No.23”.

25) Page 33, Figure 4: This figure does not add much that has not already been explained in the text and for this reason this figure could be deleted.

Reply: We agree with the suggestion of the reviewer.
Changes in the Figure: Figure 4 is now deleted in the revised version.

26) Page 34, Figure 5: This figure does not add much that has not already been explained in the text and for this reason this figure could be deleted.

Reply: We agree with the suggestion of the reviewer.
Changes in the Figure: Figure 5 is now deleted in the revised version.

27) Pages 35 to 37, Table 1: This table provides very detailed information about 14 case reports about anti-CD36-associated FNAIT and could be published on-line as Supplementary information. In addition, the abbreviations used in this table should be explained.

Reply: We agree with the suggestion of the reviewer.
Changes in the Figure: Table 1 is now published on-line as Supplementary information, and the abbreviations used in this table are explained.

Reviewer B

The manuscript entitled "Fetal and neonatal immune thrombocytopenia caused by maternal alloantibodies and isoantibodies in Caucasian and Asian populations" broadly introduced recent findings/knowledge related to pathophysiology of various alloantibody and isoantibody – mediated FNAIT, particularly focused on the alloimmunization against human platelet antigens located on αIIbβ3 and α2β1, and isoimmunization against CD36. In the latter half of the paper, the authors covered some current and emerging therapies including FcRn blockage, AMIS, and various competitive monoclonal antibodies with modified Fc region including deglycosylated monoclonal antibody. Overall, this reviewer feels the review article contains interesting knowledge, there are, however, some minor concerns and suggestions, which may be useful for authors to further enhance the quality of the manuscript.

Minor concerns/suggestions:

1) The authors should pay more attention to their citations:
Reply: We apologize for this accuracy in our citations. We rechecked our citations and corrected as suggested by the reviewer.

a) For instance, line 125, ref 13 should be listed before ref 16, the same is true for other places (they should list smaller numbers first); Line 128, the authors should also cite Yougbaré I, J Clin Invest. 2015 Apr;125(4):1545-56.

Reply: The order of citation number (small number first) is corrected throughout the manuscript. *Changes in the text:* line 184 (28, 29, 36-38), line 217 (3, 50, 51), line 361 (74, 75, 114), line 364 (81, 115,116), line 458 (145, 146), line 473 (17,132,150-152). Line 137, the reference "Yougbaré I, J Clin Invest. 2015;125:1545-1556 (reference No.18)"is now cited.

b) In the section of CD36, the authors should also cite: Wu G et al. Transfus Med Rev. 2017 Apr;31

Reply: We followed the suggestion of the reviewer.
Changes in the text: The work of Wu et al in "Transfus Med Rev. 2017 Apr;31" is now added in line 381 ".....were identified in China (Wu G et al, 2017; reference No.119)".

c) line 468 should also cite "Chen P et al., Blood, 2010 Nov 4;116(18):3660-8" and Li C et al, J Clin Invest. 2011 Nov;121(11):4537-47, which presented significant original data for anti-FcRn therapy.

Reply: We followed the suggestion of the reviewer both references (No.150 &151) are now added.
Changes in the text: "by inhibiting fetal FcRn (17, 132, 150 plus Chen P et al., Blood, 2010 Nov 4;116(18):3660-8" and Li C et al, J Clin Invest. 2011 Nov;121(11):4537-47." (page 15, line 473).

d) In line 488, I did not see within the cited refs, evidence of the statement "these modified mAbs were able to pass through the placenta and ease FNAIT…" The authors should look into more recent papers as one of the cited articles (ref 149) mentioned in the Discussion section that research assessing the efficiency of placental transport are still ongoing.

Reply: Thank you for pointing out this issue.
Changes in the text: The sentence "these modified mAbs were able to pass through the placenta and ease FNAIT…" is changed into "This modified mAb against HPA-1a would be an excellent candidate for treatment of FNAIT if it could be shown in humans that it is able to cross the placenta in sufficient amounts and can displace effectively bound to fetal platelets (156)."(page 16, lines 492 to 494.

In the "New therapies approaches" section, line 508-515, discussing AMIS potential for FNAIT, the authors should cite "Tiller H et al., Transfusion, 2012 Jul;52(7):1446-57" which demonstrated that AMIS against both human and mouse antigens can be induced, and subsequently rescued pups' platelet count in mouse model of FNAIT.

Reply: We included the paper by Tiller et al. (reference No.161) in the text.
Changes in the Text: Line 512 "... from the maternal circulation (156). Currently, a plasma collection program ..." changed to: "... from the maternal circulation (161). Tiller et al. (Tiller et al., pmid: 22251227) were able to transfer this situation of antibody-mediated immune suppression (AMIS) to a mouse model for FNAIT: they

reduced the immunizing effects of human platelet transfusions to β3-/- mice with infusions of human anti-HPA-1a and with infusions of mouse monoclonal anti-β3 antibodies (162). (page 16, lines 518 to 521).

2) This reviewer feels that the authors should take some time to proofread the review article to minimize spelling errors/incorrect statements. Some selected examples are below:

a) Lines 64-66 repeats "(such as HPAs)". The authors may eliminate one of them.
Reply: Typo is corrected.
Changes in the Text: "such as HPA" in line 72 is deleted.

b) Line 67 implies that isoantigens are only restricted to blood cells which is untrue.
Reply: We corrected the sentence.
Changes in the text: This sentence "isoantigens are recognized…carrying this glycoprotein" is changed to "isoantigens are recognized by individuals lacking an entire cellular glycoprotein present in cells of normal individuals."(page 3, lines 73 to 74).

c) Line 98, "Lue33Pro" contains a spelling mistake for "Leu" amino acid.
Reply: Typo is corrected.
Changes in the Text: Lue33Pro is now changed into Leu33Pro (page 4, line 107).

d) Line 98, "PSI" should be defined for the readers.
Reply: The abbreviation of PSI is now given. PSI (Plexins, Semaphorins and Integrins) domain (page 4, line 107).

e) Line 99, what is "antigenic constellation"?
Reply: Indeed, the word constellation is not clear.
Changes in the Text: constellation is now changed into "incompatibility" (page 4, line 109).

f) Lines 125-127 include two findings. One is the NK cell – trophoblast story, which is cited, but the angiogenesis story should additionally cite "Yougbaré I et al., JCI, 2015 Apr 1;125(4):1545-56".
Reply: Thank you for this clarification. The paper of Yougbare and coworkers is now included in this citation.
Changes in the Text: "placenta dysfunction and miscarriage (17 plus Yougbaré I et al., JCI, 2015 Apr 1;125(4):1545-56; reference No.18) ." (page 5, line 138).

g) Line 148 "throphoblast" should be corrected to "trophoblast".
Reply: Typo is corrected.
Changes in the text: "throphoblast" into "trophoblast"(page 6, line 160).

h) Line 158, what does it mean "different types"? Different epitope targets, different constant regions of the antibodies…?
Reply: Indeed, the word type is not clear, and therefore is now changed.
Changes in the Text: The sentence "it is unknown whether different types of anti-HPA-4 antibodies exist." Is changed to".....unknown whether different types of anti-HPA-4 antibodies recognizing different epitopes exist." (page 6, lines 169 to 170).

i) Line 258, cannot say "novel" GT patients because according to the paper the authors cited, these mutations are thought to occur 300-400 years ago…
Reply: This is true, and the word "novel" is now deleted.
Changes in the Text: "Interestingly, these GT patients developed..."(page 9, line 270).

The last sentence (lines 259-261), the authors of the study (ref 13) never explored effects on angiogenesis.
Reply: Thank you for pointing out this error. Indeed, angiogenesis experiment with these serum samples was not included in this paper (reference 13).
Changes in the Text: The sentence "In accordance with the previous results, anti-HPA-1a antibodies reacting with αvβ3 derived from these GT patients impacted the angiogenesis process" is now deleted in the revised version (page 9; line 269).

j) Line 290, “Mr” is usually “MW” to signify “molecular weight”.
Reply: Typo is corrected. Change Mr is now changed into “MW” (page 10, line 299).

k) Line 299, “that 500 million years ago” should be changed to “than 500 million years ago”.
Reply: Typo is corrected.
Changes in the Text: The text is now corrected into “than 500 million years ago.”(page 10, line 308).

l) Lines 311-312 mention “immunizing with fetal erythrocytes”, why is CD36 on erythrocytes not mentioned in lines 280-282 in the background for CD36?
Reply: Indeed, this information is missing in lines 280-282. The information that CD36 is also found on fetal erythrocytes is now added in lines 280 to 283.
Changes in the Text: the text is now corrected into “.....endothelial cells, macrophages, platelets and fetal erythrocytes.”(page 10, line 291-292).

m) Line 344, should be Bernard-Soulier Syndrome.
Reply: Typo is corrected.
Changes in the Text: changed “Bernard Soulier” into “Bernard-Soulier syndrome” (page 11, line 353).

n) Line 359, “(1.50%; 79, 116, 112)” should be separated into “(1.50%) (79, 116, 112)”.
Reply: Typo is corrected.
Changes in the Text: This sentence is now deleted.

o) Lines 428-430, it would be helpful to briefly introduce immunology background of what is Class II MHC and how is that important for FNAIT.
Reply: We are confident that readers of this manuscript are acquainted with the role of HLA class molecules in (allo) immune reactions. Therefore, we did not include further explanations of the role of MHC class II in immune reactions.

p) Line 468, “bind IgG” should be changed to “binds IgG”.
Reply: Typo is corrected.
Changes in the Text: the text in now changed into “....that only binds IgG at....”(page 15, line 473).

q) Line 469, the authors should eliminate “the” in the sentence “The following pinocytosis…” to make it grammatically correct.
Reply: Typo is corrected.
*Changes in the Text: t*he text is now changed into “Following pinocytosis into endosomes of endothelial cells....” (page 15, line 474).

r) Line 474, “This effect reduces anti-platelet antibodies…”
Reply: Typo is corrected.
Changes in the Text: The text is now corrected into “This effect reduces anti-platelet antibodies, which...”(page 15, line 479).

Reviewer C

It is a good review of FNAIT with a special focus on CD36, an important antigen especially in Asian and African populations, but also reviewing the other antigens of importance, and the current and new potential therapeutic approaches. The authors describe in detail the HPA-1 to -5 systems and the CD36 antigen, but there is no description of HPA-15. There are many points that need to be addressed before the paper is acceptable for publication.

Major points

1. There is no description about HPA-15. I would suggest adding a section related to HPA-15 antibodies in FNAIT.
Reply: HPA-2a and other antigens on GP Ib/IX/V and CD 109 are of minor importance in FNAIT. We, however, added to sections on these two groups of antigens (see pages17-19).

2. The frequency of CD36 deficiency in Japan is based on 3 references (70, 105, and 112). The authors mention on different references in different sections, which makes the readers confused. The frequency described in reference 105 is based on the following paper (Shibata Y, Kim N, Morita S, Ishijima A, Okazaki S: Monoclonal antibody OKM5 and platelet alloantibody anti-Nak(a) have the same specificity. Proc Jpn Acad 66:41, 1990; https://www.jstage.jst.go.jp/article/pjab1977/66/2/66_2_41/_article/-char/en). Thus, I recommend adding this paper to the reference instead of reference 105. Another reference that could be cited is (Thomb Haemost 2000 Sep; 84(3): 436-441), which reports frequencies of 1.0% and 5.8%, respectively for type I and type II deficiencies in Japan. The same frequencies (with the same references) should be used along the text to avoid confusion. The frequency of 1.50% (L359) for type I deficiency in Japan is very high. Usually, it is reported to be less than 1.0%. Please check.
Reply: Thank you for pointing out this issue. The references are now updated.
Changes in the Text: The reference 105(Yamamoto et al, Blood, 1990) is changed into "Shibata Y et al., Proc Japan Acad,1990; reference No. 107".
The reference 112 (Masuda et al, Thromb Res, 2015) is changed into "Yanai H, et al. Phenotype-genotype correlation in CD36 deficiency types I and II (Thromb Haemost 2000;84(3):436-441)". The frequency of 1.5% for type I deficiency in Japan is from reference No.112 (Masuda Y et al, Thromb Res 2015). Indeed, this reported frequency is high. So we changed to another reference (Yanai H et al, Thromb Haemost 2000; reference No.114).

3. In the section [CD36 deficiency]: It is worth mention that the C268T, the most common mutation in Japan, seems to be not associated with antibody production (J Clin Invest 1995 Mar; 95(3): 1040-1046), and all the TRALI cases reported from Japan are associated with the 3290-330 delAC, the most common mutation in China.
Reply: Indeed, the information about the common C268T mutation on antibody production is important to mention in this paragraph.
Changes in the Text: The sentence "Three common mutations..... most frequent mutation (>50%)" is now changed into "Three mutations of C268T, 949insA, and 329–330 delAC responsible for CD36 deficiency are common in Japanese individuals (118). Although the C268T mutation represents the most frequent substitution (>50%), this polymorphism did not lead to the production of anti-CD36 isoantibodies in reported cases (Supplementary Table 1)."(page 12, lines 367-370).

4. The section "Natural history of maternal immunization" is difficult to follow. This section should be elaborated better. Especially in L421-424, why is the antibody detection rate higher in primipara (20-26%), compared to 9.7% in reference 133. Is 9.7% for all pregnancies? Data from different references (133, 134, 135) are listed, but there is no explanation for the discrepant results. Where does the 84% chance of HPA-1a (-) women giving birth to an HPA-1a (+) child come from? Is there any reference?
Reply: The section was partly rewritten; we tried to be more clear ant related the data to the pertinent references.
Changes in the Text: Lines 417: "Meanwhile, comprehensive data..." to line 428: "...counts were higher than 150,000/µL" were changed to "Prospective studies on FNAIT in Caucasian populations provided information on the natural history of FNAIT. Cumulative data from ten prospective studies (Kamphuis et al., 2010) show that 9.7 % of all HPA-1a (-) negative mothers are immunized during pregnancy. The chance that a HPA-1a (-) mother gives birth to a HPA-1a (+) child is approximately 84%, given a gene frequency of 0.839 for HPA-1a (Vorholt et al., 2020 pmid:32110192). In the large Norwegian study (Kjeldsen-Kragh et al., 2007), 34.2 % of all HPA-1a (+) neonates of immunized mothers were severely thrombocytopenic (platelet counts < 50,000/µL), whereas 47.2 % had normal counts with platelets > 150,000/µL. In this study the rate of FNAIT cases with ICH (platelet counts < 150,000/µL) was 2,4 %, This rate may be the consequence of the screening and treatment

strategy applied by the investigators: as children of all immunized mothers were delivered two to four weeks prior to term by cesarean section." (pages13 to14, lines 419-428).

5. The section "New therapy approaches" is quite confusing because there are descriptions related to different experiments, without mention to the target of the antibody (anti-HPA-1 or anti-CD36). They should be mentioned and the extrapolation to other antibody specificities should be done at the end. The section subtitle would be better "New therapeutic approaches"
Reply: We follow the suggestion of the reviewer.
Changes in the Text: We changed the subtitle into "New therapeutic approaches"(page 15, line 462).

Minor points

Lines 36-39 (Abstract): Restructure the sentence. Suggestion: "Maternal antibodies crossing the placenta via FcRn-mediated transport during pregnancy enhance the clearance of fetal platelet and cause endothelial dysfunction, resulting in fetal neonatal alloimmune thrombocytopenia (FNAIT). This is often associated with bleeding complications of the fetus and neonate."

Reply: Thank you. This sentence is clearer.
Changes in the Text: The sentence is changed to "Maternal antibodies crossing the placenta via FcRn-mediated transport during pregnancy enhance the clearance of fetal platelets and cause endothelial dysfunction, resulting in fetal and neonatal alloimmune thrombocytopenia (FNAIT). This is often associated with bleeding complications of the fetus and neonate."(page 2, line 41 to 44).

Line 56: "diallelic" should be "biallelic"
Reply: I am not sure which terminology is more correct. As the meaning of both prefixes "bi-" and "di-" is "two", both forms should be correct. We decide to keep our terminology.

Line 72: better restructure the sentence as follows: "HPA-1, HPA-3 and HPA-4 reside on αIIbβ3, whereas HPA-5 resides on α2β1, and HPA-15 is found on CD109."
Reply: Thank you. This sentence is clearer.
Changes in the Text: The text is changed into "HPA-1, HPA-3 and HPA-4 reside on αIIbβ3, whereas HPA-5 resides on α2β1, and HPA-15 is found on CD109."(page 3, line 79 to 80).

Line 82: better restructure the sentence as follows: "namely, HPA-1, -3 and -4 residing on integrin αIIbβ3, and HPA-5 on α2β1"
Reply: Thank you. This sentence is clearer.
Changes in the Text: The text is now changed into "namely, HPA-1, -3 and -4 residing on integrin αIIbβ3, and HPA-5 on α2β1(Figures 1A and 1B)."(pages 3 and 4, line 90 to 92).

Line 87: better restructure the sentence as follows: "FNAIT is observed in about 1:1,000 of live births"
Reply: The sentence is ok.
Changes in the Text: none.

Line 91: "suffered from ICH" should be better "developed ICH"
Reply: agree
Changes in the Text: change the text into "developed ICH" (page 4, line 99).

Line 91: platelet count is expressed as <50.000/mL. It should be expressed as <50x10^9/L
Reply: agree
Changes in the Text: change the platelet count into <50x10^9/L (page 4, line 100).

Line 121: "antibody reacted with" would be better "antibody recognizing"
Reply: agree
Changes in the Text: change "antibody reacted with" into "antibody recognizing" (page 5, line 131).

Line 123: "predominantly with ICH" should be "predominantly those with ICH"
Reply: The text is modified.
Changes in the Text: change the text into " … predominantly found in FNAIT cases with ICH" (page 5, line 132).

Line 128: “It remains to be elucidated that which” should be “It remains to be elucidated which”
Reply: The text is corrected.
Changes in the Text: delete “that” and change the text into “It remains to be elucidated which types” (page 5, line 138).

Line 137: “fetal/neonate” should be “fetal/neonatal”
Reply: Typo is corrected.
Changes in the Text: change “fetal/neonate” into “fetal/neonatal” (page 5, line 148).

Line 149: “via microparticles into the maternal circulation” should be “via microparticles entering into the maternal circulation”
Reply: agree
Changes in the Text: This sentence is now changed into “one might speculate that contact of maternal blood with trophoblast or circulating foetal microparticles result in maternal alloimmunization against HPA-1a on β3 and αvβ3 complex ”. (pages 5 and 6, lines 159 to 161).

Line 181: “regulated differentially” should be “regulated differently”
Reply: Typo is corrected.
Changes in the Text: change “differentially” into “differently” (page 6, line 190).

Lines 183-184: better restructure the sentence as follows: “anti-HPA-30b antibodies did react with HPA-30 expressed in human (HEK-293) cell line but not non-human (CHO-K1) cells”
Reply: The suggested sentence is clearer.
Changes in the Text: We changed the sentence into “anti-HPA-30b antibodies did react with HPA-30b expressed in human (HEK-293) cell line but not in non-human (CHO-K1) cells” (pages 6 and 7, lines 191 to 193).

Line 195: “consequently bind some anti-HPA-3” should be “consequently the binding of some anti-HPA-3”
Reply: The suggested sentence is clearer.
Changes in the Text: change the sentence into “...consequently the binding of some anti-HPA-3 antibodies” (page 7, line 204).

Line 210: “Accordingly” does not make sense. Should it be “On the other hand” (?)
Reply: Thank you for pointing out this error.
Changes in the Text: change the text into “However, the chance for a thrombocytopenic child in immunized pregnant women with anti-HPA-5b is low” (page 7, line 221).

Line 230: “on platelet surface” should be “on the platelet surface”
Reply: Typo is corrected.
Changes in the Text: change the text into “on the platelet surface” (page 8, line 242).

Lines 282-283: “binds many different ligands” should be “binds to many different ligands”
Reply: Typo is corrected.
Changes in the Text: change the text into “binds to many different ligands” (page 10, line 292).

Lines 284-286: CD36 also mediates the adhesion of Plasmodium falciparum-infected erythrocytes, Staphylococcus, and Mycobacterium bacterial components to what? Or do you mean, binding of platelets to Plasmodium falciparum-infected erythrocytes, Staphylococcus, and Mycobacterium bacterial components?
Reply: Indeed, this sentence is confusing.
Changes in the Text: change the text into “CD36 also mediates the adhesion of Plasmodium falciparum, Staphylococcus, and Mycobacterium to infected erythrocytes.” (page 10, lines 294 to 295).

Line 287: “defence” should be “defense”
Reply: Typo is corrected.
Changes in the Text: change into “defense” (page 10, line 297).

Line 316: “plasma (5F1; IgM) induced platelet aggregation” should be “plasma (5F1; IgM), induced platelet aggregation”
Reply: This sentence is incorrect.
Changes in the Text: We changed the text into “some anti-CD36 mAb (5F1; IgM) lysed platelets in plasma and some (ES IVC7; IgG) induced platelet aggregation.” (page 11, lines 324 to 325).

Line 325: “binding of human sera containing anti-CD36 antibodies” would be better “binding of anti-CD36 antibodies in human sera”
Reply: agree
Changes in the Text: change into “...binding of anti-CD36 antibodies in human sera” (page 11, line 334).

Line 327: “anti-CD36 serum” would be better “anti-CD36 in (human?) serum”
Reply: Indeed, the sentence is not clear.
Changes in the Text: change into “...for the binding of anti-CD36 antibodies” (page 11, line 336).

Line 354-355: the frequency in Taiwan (1.56%) is not included in the interval 1.8%-3.65%. Please check.
Reply: Thank you for pointing out this issue.
Changes in the Text: The frequency of CD36 deficient in Taiwanese (1.56) is deleted.

Line 358-359: the references 79 and 116 are not related to the frequency of type I deficiency in Japan. They should be cited appropriately after the respective populations, as follows: “Han and Zhuang ethnic groups was about 0.5% (79) and 1.3% (116)”.
Reply: The references 79 and 116 cited in this sentence are not appropriate.
Changes in the Text: This information “Han and Zhuang ethnic groups was about 0.5% (79) and 1.3% (116)” is now deleted.

Line 381: it is mentioned that “the CD36 antigen and antibody will be discussed separately in another review chapter” followed by references. Do authors mean that there will be a different chapter in the journal related to these topics? References are not required in such case.
Reply: The reviewer is correct. References are not required.
Changes in the Text: references (87, 88,117, 119-124) is now deleted.

Line 393: “could cause” should be “can cause”
Reply: agree
Changes in the Text: the sentence “could cause anemic hydrops fetalis” is now changed into “can cause anemic hydrops fetalis” (page 13, line 394).

Line 395: better restructure the sentence as “anti-CD36 antibodies are associated with AHF”
Reply: the sentence is restructured
Changes in the Text: "several studies reported an association between anti-CD36 antibodies and AHF" (page 13, line 396 to 397).

Line 425: platelet count is expressed as <50.000/mL. Better describe as 50x10^9/L
Reply: agree
Changes in the Text: change the platelet count into 50x10^9/L (page 13, line 424).

Line 442: “Cerebral bleeding of a previous child is considered to be predictive for” would be better “ICH in a previous child is considered to be predictive of”
Reply: agree
Changes in the Text: This sentence is changed (see Reviewer A, no. 13).

Line 443-444: “maternal IVIG therapy (1g/kg one time per week is effective in avoiding cerebral bleeding” would be better “maternal IVIG therapy (1g/kg once a week) is effective in preventing ICH” (ICH should be used instead of cerebral bleeding)
Reply: agree
Changes in the Text: change the text into “maternal IVIG therapy (1g/kg once a week) is considered to be effective in avoiding cerebral bleeding.” (page 14. Lines 446 to 447).

Line 447: “high risk for” should be “high risk of”
Reply: agree
Changes in the Text: change the text into “the high risk of fetal complications” (page 14, line 451).

Line 449: “pregnant women with their first pregnancy are not aware of the risk of this immunization” should be “pregnant women in their first pregnancy are not aware of the risk of immunization”
Reply: agree
Changes in the Text: change the text into “pregnant women in their first pregnancy are not aware of the risk of immunization.” (page 14, line 453 to 454).

Line 450-451: “platelet alloimmunization is deliberated by still intensively debated” would be better “platelet alloimmunization is deliberately performed, but it is still intensively debated”
Reply: agree
Changes in the Text: change the text into “the screening program for platelet alloimmunization is deliberately performed, but it is still intensively debated.” (page 14, line 454 to 455).

Line 451: “at risk for” should be “at risk of”
Reply: agree
Changes in the Text: change the text into “If thrombocytopenic newborns of alloimmunised mothers are at risk of serious bleeding” (page 14, line 456).

Line 452: “transfusion of platelet antigen-compatible platelets” would be better “transfusion of antigen-compatible platelets, including maternal platelets”
Reply: agree
Changes in the Text: change the text into “the therapy of choice is the transfusion of antigen-compatible platelets, including maternal platelets” (pages 14 and 15, lines 456 to 457).

Lines 454-455: “In case of platelet concentrates are not available” would be better “In case platelet concentrates are not available”
Reply: agree
Changes in the Text: change the text into “In case platelet concentrates are not available, IVIG may be used” (page 15, lines 458 to 459).

Line 456: better restructure as follows “Consensus for standard pre- and postnatal therapies for other antibodies, except HPA-1, is not yet available”

Reply: agree
Changes in the Text: change the text into “Consensus for standard pre- and postnatal therapies for other antibodies, except HPA-1, is not yet available.” (page 15, lines 460 to 461).

Line 458: “IVIG for the treatment of FNAIT” should be “IVIG for the prevention/treatment of FNAIT”
Reply: agree
Changes in the Text: change the text into “IVIG for the prevention/treatment of FNAIT” (page 15, line 463).

Lines 460-461: better restructure the sentence as “including decrease of maternal antibody production by inducing immune tolerance or reduction of placental transfer, and enhancement of maternal IgG catabolism”
Reply: agree
Changes in the Text: change the text into “Several mechanisms have been proposed, including decreasing of maternal antibody production by inducing immune tolerance or reduction of placental transfer, and enhancement of maternal IgG catabolism” (page 15, lines 464 to 466).

Lines 462-464: better restructure the sentence as “immune-protective maternal IgG, with potential increased risk of infections by the locally dominant pathogens during pregnancy and during the first weeks after the birth”
Reply: agree
Changes in the Text: change the text into “such strategies may inhibit the transfer of immune-protective maternal IgG, with potentially increased risk of infections by the locally dominant pathogens during pregnancy and during the first weeks after birth” (page 15, lines 467 to 469).

Line 469: “The following pinocytosis” should be “Following pinocytosis”
Reply: agree
Changes in the Text: delete “the" and change the text into "Following pinocytosis...." (page 15, line 474).

Line 483: “can inhibit the pathogenic maternal” should be “can inhibit the binding of the pathogenic maternal”
Reply: agree
Changes in the Text: change the text into “can inhibit the binding of the pathogenic maternal alloantibody to fetal platelets” (page 15, line 488).

Lines 484-485: “effector function as it cannot interact with fetal FcγRs but” should be “effector function, by e.g. inability to interact with fetal FcγRs, but”
Reply: the text is modified.
Changes in the Text: change the text into “...effector function by inability to interact with fetal Fc□Rs, but still retain the ability …..” (pages 15 and 16, lines 489 to 490).

Line 487: “pass the placenta” would be better “cross the placenta”
Reply: agree
Changes in the Text: change the text into “cross the placenta” (page 16, line 490).

Line 490: “autoimmunity treatment” would be better “treatment of autoimmune disorders”
Reply: agree
Changes in the Text: change the text into “treatment of autoimmune disorders” (page 16, line 496).

Line 495: “pass through the placenta” would be better “cross the placenta”
Reply: agree
Changes in the Text: change the text into “cross the placenta” (page 16, line 501).

Lines 500-503: the description is difficult to follow. They mention “three days earlier to” but earlier than what?

Reply: The text is modified.
Changes in the Text: change the text into “Only IVIG administration three days earlier at days 7, 12, and 17 instead at days 10, 15, and 20 reduced fetal death....” (page 16, lines 509 to 510).

Line 507: I suppose “is visible” should be “is feasible”. Is it correct?
Reply: agree
Changes in the Text: change the text into “the development of humanized deg-32-106 as a drug to prevent severe FNAIT caused by anti-CD36 could be envisaged.” (page 16, line 513).

Line 509: should be restructured for a better understanding. Suggestion “reduce the risk of alloimmunization by administering antibodies to eliminate fetal platelets from the maternal circulation, which has been attempted by administration of anti-HPA-1a antibodies”
Reply: agree
Changes in the Text: Line 508...509: change the text “Another strategy to prevent ... anti-HPA-1a antibodies” into “Another strategy to prevent fetal bleeding complications is to reduce the risk of alloimmunization by administering antibodies to eliminate fetal platelets and fetal cellular particles from the maternal circulation. This has been attempted by administration of anti-HPA-1a antibodies.” (page 16, lines 514-517).

Line 525: “as considered before” would be better “as confirmed previously”
Reply: agree
Changes in the Text: change the text into “Platelet alloantibodies and isoantibodies not only bind to platelets, as confirmed previously” (page 19, lines 599 to 600).

Line 526: “and also other blood cells” would be better “as well as other cell types”
Reply: agree
Changes in the Text: change the text into “... as well as other blood cells (such as anti-CD36)” (page 19, line 600).

Line 532: “one to other cases” would be better “one case to other”

Reply: agree
Changes in the Text: change the text into “their ratio may differ from one case to other” (page 19, line 606).

Line 534: “is mandatory for better” should be “is mandatory for the better”

Reply: agree
Changes in the Text: add “the” before “better”, the text is changed into “... is mandatory for the better prediction and management of FNAIT.” (page 19, line 608).

Line 536: “nature of pathogenic” should be “nature of the pathogenic”
Reply: agree
Changes in the Text: change the text into “... a further understanding of the nature of the pathogenic antibodies will be necessary” (page 19, line 610).

The numbers must be standardized. For example, in line 91, it is described as 50.000, in line 225 as 2.000 – 6.000, and in line 428 as 150,000.
Reply: agree
Changes in the Text: change the numbers into “~50x10^9/L (line 100); ~150x10^9/L (line 225); 2,000-6,000 (line 237); ~50x10^9/L (line 424) and ~150x10^9/L (line 425).”