Peer Review File

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Reviewer Comments

Reviewer A

This is an excellent and well written review on cTTP. I thought this covered all the key area nicely and was highly informative.

Reply: We appreciate your positive comments on our manuscript.

Comment 1: I would only recommend including a recent study published in JTH that has shown that replacing the signal peptide of ADAMTS13 can enhance its expression - especially in light of recombinant ADAMTS13 going into clinics.

Reply1: Thank you for your suggestion. We read the JTH paper with interest, particularly the point regarding the replacement of the signal peptide of ADAMTS13 leading to the improvement of ADAMTS13 expression in the culture medium, which contributed to the high productivity of recombinant ADAMTS13 protein. However, since rADAMTS13 is already in formulation and since the JTH paper has little relevance to this review, we have chosen not to include this reference. Nevertheless, we sincerely hope that the results from this basic research will be useful in the future because increasing the efficiency of rADAMTS13 production will lead to cost savings, and thus, benefiting both patients and companies.

Comment 2: I also agree with the authors that testing for ADAMTS13 deficiency itself rather than just assessing clinical presentation is important - to this end some mention of the current assays used to determine ADAMTS13 function could be included.

Reply 2: We have added information for determining ADAMTS13 activities to the current assays. We also included the pitfalls of the FRET-VWF73 assay in the section, "The history and features of congenital thrombotic thrombocytopenic purpura (cTTP)" (Page 8, lines 130–137).

Reviewer B

This is a comprehensive manuscript which covers in good detail the majority of relevant areas in the understanding of cTTP. However, some modifications are needed prior to acceptance. These are predominately minor however one section needs further work:

Reply: We appreciate your careful review of our manuscript.

Comment 1: Page 17 - long term survival and complications

The section included on renal disease is good however there is no mention of neurological and pyschological complications and only scant on cardiac complications - these need to be added.

Reply 1: Thank you for your valuable suggestion regarding complications. It should be noted that patients with cTTP (probably patients receiving an insufficient amount of FFP infusion) have a high risk of neuropsychiatric symptoms associated with

asymptomatic and symptomatic strokes. We added a recent publication that discussed neurological and psychological complications in patients with cTTP to the section “cTTP: Long-term survival rate and complications” (Page 20, lines 353-361).

Borogovac A, et al. Prevalence of Neuropsychiatric Symptoms and Stroke in Patients with Hereditary Thrombotic Thrombocytopenic Purpura. Blood. 2022 May 18.

Upreti H, Kasmani J, et al. Reduced ADAMTS13 activity during TTP remission is associated with stroke in TTP survivors. Blood 2019;134:1037-45.

Comment 2: Page 3 line 38 - remove "the"
Page 6 line 95 - change to lower case s on Spacer
Page 6 line 102 - start a new paragraph before "cTTP is diagnosed..."
Page 7 line 113 - I would reword the pentad sentence to something like "such as the pentad of clinical symptoms no longer used in clinical practice"
Page 10 line 173 - replace private with novel or unique
Page 12 line 213 - change unelucidated to "a point of discussion" or "open to discussion"
Page 13 line 217 - a few patients are infected with hepatitis C needs wording more clearly i.e. number of patients
Page 15 line 259 - change "would occur" to "occured in up to"
Page 15 line 266 - change babies to "mother and fetus"
Page 40 tables - can you explain the code column? Is it necessary?
Reply 2: We have included suggested corrections to our revised manuscript. Regarding the patient code (the last point), we would like to use this code because our previous publications used the same code to identify each patient.

Reviewer C
Comment 1: To discuss gene therapy, this would need a more extensive, expert discussion of the issues that complicate gene therapy that explain why progress in this field has been very slow. For example but not limited to the size of the gene and risk of cancer - where the gene integrates etc. One could also delete this section as, presently it does not add much.
Reply 1: Thank you for your feedback regarding gene therapy. As you mentioned, gene therapy development for cTTP has been slow compared to that for other diseases, such as hemophilia. Among previous ADAMTS13 expression studies in ADAMTS13 KO mice, no critical adverse events were observed, and long-term (more than 2 years) expression was confirmed. This suggests that the ADMATS13 transgene into host cells (mostly hepatocytes) is possible because the ADAMTS13 protein is derived from a single gene and the length of the coding sequence (3.7 kb) is acceptable for gene therapy. Authors who have published in the area of cTTP gene therapy explained that they could not proceed with gene therapy projects because pharmacological companies were not interested in supporting them financially. Companies must compensate for the huge expenses associated with drug development. Novel therapies for very rare diseases,

including cTTP, potentially have this issue.
To overcome this issue, a robust connection between international working groups and patient associations is required in the upcoming decades. We would like to retain this section because we believe there are opportunities to develop a novel therapy. We have added a more detailed discussion to the revised manuscript (page 24-25, lines 428-446).

We have also added to our discussion of drug development in extremely rare diseases.

Comment 2: You may want to mention that Joly et al have reported two cases where patients were only heterozygous for one mutation, compounded by common SNPs. In these cases pregnancy induced TTP.
Reply 2: Thank you for highlighting this reference. Generally, cTTP can be genetically diagnosed by using biallelic *ADAMTS13* mutations. Although some researchers have reported that an ADAMTS13 mutation with some common SNPs could contribute to cTTP development, there is no consensus regarding the diagnosis of cTTP with no or monoallelic ADAMTS13 mutations. Patients with only one identified causative mutation may have intronic causative mutations or large structural variants that could not be identified by regular gene analysis. Hence, we did not include the information about common SNPs and their contributions to cTTP development in this review.

Comment 3: Do Japanese cTTP patients report suffering from headaches as they do in other countries?
Reply 3: Unfortunately, we could not investigate the occurrence of headaches in Japanese patients with cTTP because most of our registered data came from physicians and they did not report on mild symptoms, such as headaches.

Comment 4: You may also want to discuss increased risk of stroke when ADAMTS13 is low (Upreti et al in TTP and the Rotterdam study)
Reply 4: Thank you for your suggestion regarding the risk of stroke. We have added more details to the section, “cTTP: Long-term survival rate and complications” section (Page 20, lines 361-362).