Reviewer's report

**Title:** Decreased erythrocyte nucleoside transport and hENT1 transporter expression in glucose 6-phosphate dehydrogenase deficiency

**Version:** 2  
**Date:** 21 April 2015

**Reviewer:** Richard Francis

**Reviewer's report:**

This manuscript seeks to address the issue of the variable presentation of individuals with G6PD deficiency. Previous work by the authors has demonstrated that red blood cells (RBCs) from individuals with beta thalassemia major, another condition in which the RBCs may experience increased oxidative stress, have decreased levels of expression of the hENT1 nucleoside transporter on the cell surface as well as decreased nucleoside uptake. In the current manuscript RBCs from G6PD-deficient and G6PD-normal volunteers are evaluated for the membrane expression of hENT1 and nucleoside uptake. Their results show that there is significantly decreased amounts of hENT1 in G6PD-deficient RBCs compared to G6PD-normal RBCs, and that G6PD-deficient RBCs also have decreased nucleotide uptake. No data about the possible mechanism for the decreased presence of hENT1 on the surface of G6PD-deficient RBCs is presented. In addition no connection is made between how the decreased hENT1 expression and decreased nucleoside uptake in G6PD-deficient RBCs helps to explain “the variable pathology of this enzymopathy”.

**Major Compulsory Revisions:**

1. (Pages 3-5) The “Background” section does not mention that G6PD variants fall into different classes (Classes I-V) based upon the enzyme activity, and that a major source of the variation in phenotype in G6PD-deficient individuals is based upon the location in the gene where the mutation has occurred. Therefore the reason for a great deal of the variation in presentation is actually known and this should be made clear by the authors as that point is not made clear by the authors.

2. (Page 6): “Methods” Section in the sub-section for “Subjects and samples” - It would be helpful for the authors to provide more information about the G6PD-deficient patients. For example it would be important to give information about whether or not these patients were at steady-state or if they had recently experienced hemolytic episodes. Also since in some situations patients with G6PD deficiency may receive blood transfusions (i.e. during a hemolytic crisis) it would be helpful to provide information about whether or not any of the patients were transfused.

3. (Page 6): “Methods” Section in the sub-section for “Subjects and samples” – The authors should provide information about the degree of deficiency of their
G6PD-deficient patients. Were these patients considered to be mildly deficient, severely deficient, etc? This information would again be very helpful in strengthening the manuscript. The authors should also state (information available in the literature) about what G6PD variants are expected to be found in the population they are studying.

4. (Page 11): “Results” Section – Because the authors do not provide the actual values for the G6PD activity results, there is no way to evaluate if there was a correlation between the level of G6PD deficiency and the decrease in hENT1 expression and the nucleoside uptake. This information could be helpful in understanding the relationship between G6PD deficiency and decreased hENT1 expression and nucleoside uptake. If this information is available the authors are encouraged to provide it.

5. (Pages 11-13): “Discussion” Section – The authors never address the link between how G6PD-deficiency and differences in hENT1 expression in RBCs from various G6PD-deficient individuals. Therefore they never actually address one of their central questions of why there is variation in the presentation of G6PD-deficient individuals.

Minor Essential Revisions:
1. (Page 1) Conclusions paragraph of the Abstract: line 39 – “red metabolism” should read “red cell metabolism”.

Discretionary Revisions:
1. (Page 11): “Results” Section - Since this phenomenon has been investigated in RBCs from 2 conditions (beta thalassemia major and G6PD deficiency) that are associated with increased RBC oxidative stress it would be helpful to provide data that measures oxidative stress/oxidative damage in these RBCs and compares that information to hENT1 expression and/or nucleoside uptake. This information could possibly be helpful in understanding more about this phenomenon.

2. (Page 11): “Discussion” Section – The authors state, “Decrease in expression of hENT1 showed substantial variation between different individuals with G6PD deficiency”, however they never address why this is. It is very possible that by providing some of the additional data mentioned above that the reason for this variation may be addressed.

3. (Pages 11-13): “Discussion” Section - The possible mechanism by for how G6PD deficiency leads to or is related to decreased hENT1 expression and decreased nucleoside uptake is not discussed.

4. Figure 1: It would be helpful to provide a frame in this figure that shows the SDS-PAGE gel.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.