Reviewer's report

Title: Gene expression profile of peripheral blood mononuclear cells in the x-linked alpha thalassemia mental retardation syndrome

Version: 2 Date: 20 April 2010

Reviewer: Jonathan Flanagan

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Background: The manuscript by Barresi et al., entitled “Gene expression profile of peripheral blood mononuclear cells in the x-linked alpha thalassemia mental retardation syndrome” is a study looking a rare but interesting disorder. This ATRX syndrome is typified by mental retardation and alpha-thalassemia. While the disorder is not very prevalent, this manuscript provides an interesting insight to the disorder. The authors use modern techniques to examine gene expression in peripheral blood cells from ATRX patients compared to control individuals. This provides a route to examine any genes that may be differentially expressed in the ATRX disorder. The authors find a small number of genes that have altered expression profiles in ATRX and investigate one particular gene in detail. This GRAF/OPHN-1-L gene has three splice isoforms, one of which appears to be highly expressed in the brain compared to other tissues. This exploratory study provides a number of interesting avenues for future research. While the GRAF/OPHN-1-L gene seems interesting from the development of mental retardation aspect, its role (and the role of other genes identified by microarray) are interesting as to why this disorder is typified by alpha-thalassemia without deletion of #-globin gene. The defects in this syndrome may also help elucidate pathways that could reduce #-globin expression in patients who have an imbalance of #-globin expression

Major Compulsory Revisions

1) The authors used a microarray system to perform a global gene expression analysis. This system has 8524 unique expressed gene probes. Clearly this does not represent the approximated 18,000 plus expressed genes in the human genome (~47%). In addition some probes may detect genes which are not expressed in peripheral blood monocytes. The authors should address these issues in their Results/Discussion as a potential drawback to the study.

2) In tables 1 and 2, the transcripts with decreased and increased expression in ATRX are given. However, there is no description of whether these are mean or median differences between the ATRX samples (n=3) and the control samples (pooled n=42). In addition, no statistics are given for this analysis. While this reviewer believes that no statistical test can be done between a group with n==3 and a pooled group with a technical n=1, the authors do not overtly state how many genes they would expect to find by chance doing this type of analysis. This statement would lead into their subsequent validation steps.

3) The microarray analysis detected a decrease in OPHN-1 expression. The
authors could not validate this by qRT-PCR but could find the decreased expression of homolog GRAF/OPHN-1-L. The specific OPHN-1 probe sequence from the microarray should be known. Is the probe binding region homologous between OPHN-1 and GRAF/OPHN-1-L? In addition, from the subsequent splice isoform analysis of GRAF/OPHN-1-L, does the microarray probe pick up all isoforms of GRAF/OPHN-1-L? These points may be included in the results.

4) The authors should also be clearer on why they chose to only validate genes which were downregulated in ATRX. There did not seem to be any specific reason for choosing their genes of interest. In addition, the vast majority of the results involve investigation into GRAF/OPHN-1-L, with very little follow up into the G0S2 and HAIK1 genes. I wonder whether these genes were also expressed in the brain and could therefore also have a potential role in the development of mental retardation.

5) The authors show the expression of β-globin and δ-globin in ATRX patients and controls. However the data is expressed as a β-globin:δ-globin ratio. Particularly as the authors state the absolute values of the β-globin and δ-globin transcripts were too variable to determine if the modification is due to decreased β-globin or increased δ-globin expression. From the microarray, β-globin, δ-globin and ε-globin are all increased in ATRX. While this is suggestive that the change in β-globin:δ-globin ratio is due to increased δ-globin expression, why did the authors not normalize gene expression between patients by using a housekeeping gene (e.g. β-actin, RPLP2)? This would allow “absolute” values to be shown and possible establish the reason for the change in the β-globin:δ-globin ratios.

Minor Essential Revisions

1) On page 1 of the results, there is a sentence which seems out of place. “Quantification of oligophrenin-1, GRAF/Oligophrenin-1-like…” does not describe any data but merely seems like a title.

2) In Table 1, gene # 6 is GRAF/OPHN-1-L but from the Results section this should be OPHN-1. Although this was not proven by qRT-PCR, the subsequent analysis showed that this probe most likely detected GRAF/OPHN-1-L. As above, the homology in where the microarray probe binds should be described somewhere in the manuscript.

3) In addition, in tables 1 and 2 there are genes highlighted in bold. These should be described in the legends.

4) In Figure 1, the change in GRAF/OPHN-1-L is shown. This appears to be approximately 40% compared to controls. However this is described as over 58% in the results section.

5) The p-values given in Figure 1 are chosen oddly. The three categories are P<0.03; P<0.01 and P<0.05. This should be re-classified, e.g. P<0.05; P<0.01, P<0.005.

6) Figures 1B, 3 and 4 do not have any statistics and should be included.

This work is a good exploratory analysis of the alterations in gene expression in
ATRX patients. This reviewer feels that this work is suitable for publication for
general interest, but with some changes to the manuscript. The novel data of this
study are the discovery of the GRAF/OPHN-1-L splice isoforms and their
potential role in development of mental retardation in ATRX and other disorders.

**Level of interest:** An article whose findings are important to those with closely
related research interests

**Quality of written English:** Needs some language corrections before being
published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the
statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.