Reviewer’s report

Title: Verification of genes differentially expressed in neuroblastoma tumours; a study of potential tumour suppressor genes.

Version: 1 Date: 16 January 2009

Reviewer: Karin Klinga-Levan

Reviewer’s report:

Major compulsory revisions
1. Page 10:
The text under the subtitles
“DNA extraction, Primer design and PCR amplification”
and
“Sequencing analysis”
must be revised.
The order and position of the different parts in the descriptions is a bit mixed up, and must be arranged more logically. One example: “Eight tumours were sequenced, choosing the four favourable and four unfavourable tumours having the highest or the lowest expression values in their respective group (table 1 and 4). PCR-based DNA sequencing of the promoter region and two exons of POU4F2 was performed using ….” (under wrong subtitle and in wrong order)

2. Page 13:
The POU4F2 gene was chosen for further studies, which is strange since it was not verified in the second verification round. I can agree that it could be included in further studies due to the large fold change, but at least the three genes with the highest fold change CACNA2D3, CNTNAP2 and SLC35E, that were verified in both verification rounds should be further studied as well.

Minor Essential Revisions
1. In the abstract under the sub title Results:
"In the first verification round 81 out of 87 genes were found to be significant between groups of which 14 has been previously reported."
I suppose that significant is referred to as differences in gene expression between groups.

2. Second paragraph in the introduction (page 4-5):
"These facts suggest distinct genetic mechanisms and pathways through which low- and high stage tumour types develop."
What is distinct? I suppose you mean distinct differences between the groups
3. Page 8 paragraph 2 and 3:
The choice of tumours in the different verification rounds should be explained even if it chosen at random

4. Page 8 bottom of page:
"These genes were selected on one of three criteria; either they were among the top ten genes with the highest fold change or they had been suggested to discriminate between groups in previous NB studies."

It is not clearly understood which the three criteria are. Maybe it is a comma missing between “genes” and “with”

5. Page 13 first row:
“The microarray expression analysis has been described in a Wilzén et al. [20]. “ Change to
“The microarray expression analysis has previously been described in Wilzén et al. [20].”

6. Page 23
Figure 3. Describe in the figure text the diagram in more detail.

7. Page 26:
Table 3. The table text should be more self-explanatory as not only “QPCR results of verification group 2” is presented in the table

8. Page 24:
Figure 5 on this page looks nearly (except for the very strange figure text) the same as Table 1, which is found among the additional files. Change it to Table 1!

9. Page 25:
Table 2 should be moved to the supplementary part.

10. Page 26:
Table 3. In this table the criteria for the choice of gene for the second verification round should be described in an additional column

Discretionary Revisions
1. Page 5 below middle:
"Gene expression can be affected by genomic alterations as well as epigenetic mechanisms, such………"

Gene expression can be affected by genomic alterations as well by as epigenetic mechanisms, such

2. Page 6:
“These data were used to prioritize candidate genes for as second verification
and for further analyses by bisulphate sequencing of CpG islands as well as mutation screening."

What do you refer to when you write “these data”?

3. Page 8:
“These results were confirmed with TaqMan Low Density Array (TLDA) in a technical replicate.”

You should clarify which results you refer to. It looks like you refer to the microarray analysis, not the gene expression data.

4. Page 26:
Instead of t-test in the head of the table, it should be P<.
Instead of exact P-values, the significance levels 0.05, 0.01 and 0.001 could be used

**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, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests