Reviewer’s report

Title: An Intronic Alteration of the Fibroblast Growth Factor 10 Gene Causing ALSG- (aplasia of lacrimal and salivary glands) Syndrome

Version: 1 Date: 2 February 2008

Reviewer: Hideyo Ohuchi

Reviewer’s report:

Scheckenbach et al. reported a novel type of Fgf10 mutations, a point mutation in the intron 2, from a patient and his brother of ASLG syndrome. The authors also found a decreased level of Fgf10 mRNA in the patient-derived fibroblasts. Knowledge of various allelic mutations is essentially useful for correct diagnosis of human diseases and this paper might provide such information in this sense; however, I feel that the paper is not sound in the present state and that the authors should revise the manuscript extensively before publication in the BMC journal.

Major compulsory revisions:

1. The most critical point to this paper is that there are no direct evidence that links the relationship between the intronic alteration in the Fgf10 gene and decreased level of Fgf10 mRNA. The authors have not identified the aberrant splicing pattern of the Fgf10 gene in reality. This must be clearly stated. Did the authors examine the 3\textprime UTR of the patient’s Fgf10 mRNA? Stability of mRNA does not solely depend on the length of polyA.

2. In Results, the authors say, "additional flanking intronic sequences may be inserted, or the 5\textprime-part of exon 3 may be deleted." This should be anticipated by the splice site prediction algorithms based on the sequencing data of the patient’s Fgf10 gene.

3. There are many "data not shown" data described in the results. The journal has no space constraints and is willing to accept sound negative data. Therefore, specific data and the relevant methods must be fully presented using figures if needed.

4. Methods: Please describe all the primer sequences used in this study, together with their amplicon size. This information is indispensable for reproducing the data and examining the genome from similar patients the readers would have. "Primer and amplification conditions are available on request." is not accepted. Since none of the primer sequences is mentioned or more specific information on the primer positions is not presented, it is very hard to understand various genetic studies examined in this study. Relevant figures and tables would be helpful.

5. Methods and Conclusions: The authors say, "this is the first investigation of FGF10 levels in fibroblasts of ASLG patients." However, none of the specific methodology is not described for isolation and culturing primary fibroblasts from
the biopsies of the patient's mucosa. This information is the key to reproduce the experiment. Please describe the method in detail.

6. Figure 3: I could not understand â##fold expression, %â##. The vertical axis represents 0, 1, and 2. What does â##%â## stand for? I am also wondering to know what was considered as 1 in this figure? Given the haploinsufficiency of the Fgf10 gene, it would be more reasonable if the level of the patient's Fgf10 mRNA would be half of the normal or between the half and normal. But the authorsâ## data seem to show a lower level of Fgf10 mRNA than half.

7. Overall, the manuscript including the figure legends should be rewritten in detail and well referenced to cover the full description of the studies.

Discretionary revisions:

1. Reporting and data deposition: I would recommend Figure 1 should be presented in Results and the patient's information should be briefly mentioned in Methods. Also, in Results, only experimental data along with computational analysis must be presented and speculation should be transferred to Discussion.

2. I would recommend the authors identify the direct effect (ex. alteration in splicing pattern causing the absence of the terminal exon 3 or else) of the intronic mutation described in this paper using animal models or other methods.

3. Discussion and Conclusions: The authors describe modifier genes, but I would not understand its significance in this context. Is it suggested by the phenotypes of the patient and his family of ALSG syndrome studied in this paper?

4. Figure 1c-e: Photos from normal individuals would be helpful for non-medical readers to understand the abnormalities in the patient.

5. Methods: Description on the long range RT-PCR would be informative for readers.

6. Conclusions: The authors say â##this non-functional alterationâ#\textendash;â##. How could we say non-functional?

Minor essential revisions:

1. Title page: â##equallyâ## appears twice.

2. Methods, â##biopsies of the mucosa from four healthy individualsâ## : four or three?

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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' below.