Author’s response to reviews

Title: Identification of novel isoforms in the assessment of SNF2L as an XLMR (X-linked mental retardation) candidate gene in 12 families linked to Xq25-26.

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Author’s response to reviews: see over
January 18, 2008

Dear Dr. Alexandersson,

Please find enclosed our revised manuscript entitled, "Characterization of novel isoforms and evaluation of SNF2L/SMARCA1 as a candidate gene for X-linked mental retardation in 12 families linked to Xq25-26." which we have now modified according to the concerns raised by the reviewers. We apologize for the delay in returning the manuscript but we were waiting for commercial RNA samples from various human tissues that were back ordered for a long time.

We were very pleased with the positive comments of the reviewers. On the following pages is a point-by-point description of the changes that we have made to the manuscript to address the concerns raised by each reviewer. The reviewers’ comments are summarized in italics followed by the response. We have also added a section to include the contributions of each author.

Overall, we believe that the changes have resulted in a much improved contribution.

Thank you for considering our work for publication in BMC Medical Genetics.

Regards,

David Picketts
Reviewer #1

Points 1, 2, and 3. The reviewer asked for further analysis of human tissues by RT-PCR for the SNF2LA/B variants and the SNF2L-NLS isoforms. In addition, they asked for a positive control RT reaction and a RT-minus negative control for figures 1C and 2B. For Figure 2B we have added an extensive tissue survey of the SNF2L-NLS isoforms, including 7 tissues and 8 different brain regions with the appropriate controls. We have observed that the NLS-containing isoform is the predominant isoform (similar to the cell lines and our fetal brain sample we had obtained locally). We have spent some time trying to complete a similar tissue survey for the 5’ constructs but we have determined that the commercial RNA samples were of insufficient quality for amplification of these fragments (1kb and 1.2 kb) compared to the RNA we have isolated ourselves. As such, we have revised Figure 1C to provide the controls.

4. dbSNP accession numbers should be added to Table 1. We have added the accession numbers to Table 1 as requested for the two SNPs that are listed in the database.

Reviewer #2

Reviewer 2 was very positive and had only discretionary revisions which we have addressed here.

1. Are there any candidate genes for mental retardation, which map to Xq25-26? If so, have you analyzed their genes for your patients. There are several genes that map to Xq25-26 including ARGHEF6, PHF6, SLC9A6, GPC3, and ZDHHC9. We have screened for PHF6 mutations ourselves and the other genes were screened by the groups who provided the samples, prior to us receiving them. Our interest was to evaluate the SNF2L gene as it has not previously been considered.

2. Did you do X inactivation study for an obligatory carrier female with normal IQ? No, we only requested DNA samples from families in which the XLMR had been previously mapped to Xq25-26 for our sequencing analysis. X-inactivation studies have been performed on some of the families whose clinical data has been published previously. It would be premature for us to analyze X-inactivation prior to having identified a SNF2L gene mutation and a syndrome associated with it.

3. As you mentioned in “Conclusions”, you cannot exclude intronic mutations, affecting alternative splicing. Can you confirm this by doing RT-PCR using cell lines of patients. No, given that we only received DNA samples for sequence analysis we could not analyze intronic mutations that alter alternative splicing.

4. Is it possible to analyze protein level for your patients’ diagnosis. This is something that we could pursue in the future should we obtain cell lines from patients as samples.

5. As you see in ATR-X syndrome, a mutation of genes coding chromatin remodeling protein can show various clinical phenotype. How about SNF2L? We cannot answer this question until we identify some patients with mutations in the SNF2L gene. It is certainly something to consider after mutations are identified.
Reviewer #3

1. *The study would gain much more power if a large panel of presumed XLMR patients would be investigated.* This is a valid point but we were not in a position to embark on sequencing >300 patients without first pursuing this pilot project due to limited resources. This initial analysis will also help raise the interest in this putative candidate gene for the large consortium groups that have embarked on sequencing ~750 genes on the X chromosome in upwards of 1000 XLMR patient samples.

2. *How is the NLS characterized? It could be stated in more detail that the human and mouse SNF2L genes were analyzed by Blast tool.* We have changed the text to reflect that the differences in the cDNA sequences between human and mouse were identified using the Blast tool and that we determined that these 23 amino acids coded for a putative NLS sequence using the PredictNLS program. While this did not pose a problem to the other reviewers we hope that we have clarified how the NLS was identified and which isoform contains it and which does not.

Reviewer #4

*This reviewer was not satisfied with the title and suggested an alternative title.* We used the suggested title for the revised submission.

Compulsory revisions

1. *I could not readily identify the CBP gene quoted in the abstract. Provide appropriate references.* We have removed our reference to the CBP gene in the abstract. While mutations in this gene cause the Rubinstein-Taybi mental retardation syndrome and it encodes a chromatin remodeling protein that functions to acetylate histones, we overlooked the fact that the gene is located on chromosome 16 and is thus not a cause of X-linked mental retardation and was not familiar to the reviewer. As he points out, the reference to CBP was only used in the abstract and not anywhere else in the article so it does not detract from the manuscript.

2. *At the end of the Results/Discussion section the Authors mention the Shashi syndrome and the Cilliers syndrome, but according to my latest XLMR update there is at least 1 other syndrome and 2 neuromuscular conditions mapping to Xq25. Furthermore, 8 MRX pedigrees also have mapping intervals overlapping Xq25. Maybe some of the these are indeed among the tested families but I was not able to recognize them from the cases provided in the patient material section of the methods. If this is the case, this should be made explicit.* We have referenced the samples corresponding to families which were published and are associated with specific XLMR syndromes. The other samples were reported in the text using the codes provided by the clinicians to retain anonymity. When we approached them again they did not provide any information about MRX numbers attached to our samples so we have nothing further to add to the methods. We have expanded the discussion to make reference to the other syndromes as possible families for additional testing with the following sentence: “In addition, there are 3 other syndromes (Wilson/MRXS12, Gustavson, and CMTX4/Cowchock-Fishbeck) and 8 MRX families (MRX 27, 35, 42, 62, 70, 71, 75, and 82) that map to this region that should also be considered for screening.”
3. The authors may want to quote our Review article on XLMR which is in press. We have included reference to this paper which only came online this week in the discussion.

4. The authors suggest the small size of their sample may explain the failure to detect mutations. I agree with them but would also add another explanation, namely the possibility that mutations in SNF2L could cause a more severe phenotype (maybe lethal in males) associated with mid-/hind-brain malformations. This is a good suggestion and we have included this additional explanation in the text of the conclusion by use of the following sentence: “Alternatively, our failure to identify mutations may arise from (1) the small sample size which may have prevented us from ascertaining a family with a mutation in this gene, or (2) the possibility that mutations in SNF2L could cause a more severe phenotype that may be lethal in males.”

Minor Revisions 1 and 2: We added the word rate to the sentence on page 3 and capitalized the word Rett as suggested by the reviewer.