Reviewer’s report

Title: Genetic diagnosis of X-linked dominant hypophosphatemic rickets in a cohort study: Tubular reabsorption of phosphate and 1,25(OH)2D serum levels are associated with PHEX mutation type

Version: 2 Date: 6 April 2011

Reviewer: ERik Imel

Reviewer’s report:

The authors report on the genotype of 36 patients with XLH, and evaluate for correlation between phenotype and the severity of the genotypic defect. The mutational analysis and discussion is informative. However regarding the phenotype correlations, there are several important points that are not adequately addressed.

Major revisions:

1. The authors mention 43 patients, but focus their report on the findings in 36 index cases. Index case may not be the proper term, since 5 or 6 of the cases are Familial, and at least some of them were reported in the discussion to be tested pre-symptomatically. Despite that, some of the familial cases are listed as “severe” bone phenotype, which is not exactly pre-symptomatic. This discrepancy needs to be addressed. Given that it appears only one subject from each family was included, are both of the monozygotic twins included? If both, this needs to be justified. If only one, the decision of which one also needs to be clearly justified. If only one is included in the analysis, it may be useful to run the analysis with each one to see if the results change.

2. The age at diagnosis is listed in table 1, but it is not explicitly stated whether any of these subjects had received any previous treatment prior to actually receiving the diagnosis of XLH, as sometimes referring pediatricians may initiate some forms of treatment prior to being seen by the specialist. If a subgroup were under treatment, that may have altered some results, and they should be excluded from the analysis of phenotype correlations.

3. How were the samples collected? Were subjects fasting? This needs to be described in the methods. In addition, One table indicates that biochemical assessments were at diagnosis (should be in methods, too). However, what years were these diagnoses made? Were the samples collected and stored and parameters tested at the same time, or were clinical tests performed as samples were collected, in the clinical laboratory (and hence potentially subject to batch effects)? Were all samples run in the same clinical laboratory for the biochemistries? TmP/GFR may be a more useful assessment than %TRP. For 2 subjects, the %TRP was listed, but they do not have a phosphate listed, which was necessary to calculate the TRP.

4. The methods list quantitative assessment of severity of bone disease. I think
the authors mean qualitative, as this appears to be a subjective assessment. Please define the degrees of severity more carefully, and if this is truly quantitative, that would imply that some form of measurements were made and should be included in the results. What was the height SDS at the time of diagnosis?

5. While a genotype effect is plausible, it is difficult to draw accurate conclusions about a genotype-phenotype correlation, without the inclusion of multiple of subjects who have the same mutation. It may be necessary to identify more than 2 or 3 subjects with a given genotype to identify such variability. Indeed previous studies and clinical experience indicate a significant degree of variation in clinical severity among affected members within a family, in terms of bone phenotype and response to treatment, especially in large kindreds. Significant intra-genotype variability could completely negate the findings of a genotype-phenotype correlation. At the very least, this possibility needs to be adequately discussed.

6. Furthermore, the inclusion of only 4 to 6 subjects in the group with less deleterious mutations, may lead to inaccurate conclusions. It appears also that the ages of these subjects are in general younger than that of subjects with more deleterious mutations. Since younger subjects will have higher TRP and TmP/GFR and serum phosphate, the apparent differences might be completely due to their age. Table 3 should have the ages and the same test should be performed to see if there is a significant age difference between those groups, in which case a difference in TRP at least would not be meaningful from the point of view of genotype-phenotype correlation. If there is an age difference, the statistical analysis of the other parameters needs to be adjusted in some way for age as a continuous variable.

Minor essential revisions

1. In the beginning of the genotype –phenotype correlation section, the first sentence refers to correlating genotype with type of mutation. Please correct to correlating phenotype with the type of mutation.

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

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.