Reviewer's report

Title: Mild forms of hypophosphatasia mostly result from dominant negative effect of severe alleles or from compound heterozygosity for severe and moderate alleles

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Reviewer: Michael Whyte

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Introduction

In the first sentence, it might be best to tell the reader that hypophosphatasia is caused by loss of function mutations.

In the middle of the first paragraph of the introduction, the authors state that there is incomplete penetrance of the trait with only a small number of carriers of such mutations expressing the disease. This statement is probably incorrect. Whether or not “carrier” individuals manifest the disease depends on how thoroughly they are studied for the dental and skeletal manifestations, etc. In fact, later we will learn from the authors that it is actually not a small number. Characterization of the clinical features of large HPP kindreds with autosomal dominant disease is only now emerging, but there is already evidence that this type of statement will prove inaccurate.

It is possible that just simple haploinsufficiency for one of the ALPL genes, without a second mutation or a dominant-negative, effect could conceivably lead to clinical manifestations during periods of rapid skeletal growth, i.e. infancy, or perhaps later in adult life when skeletal disease is most prevalent.

Perhaps, add one sentence to the bottom of the first paragraph of the Introduction to tell the reader how this "tool" might be interpreted. It is not immediately apparent how this might be helpful. Might not some of the mutations causing compound heterozygosity also affect the transfected wild type allele?. What differences would be expected for true heterozygosity compared to the compound heterozygosity in these cells?

Materials and Methods

It would be best to say that your definition of mild hypophosphatasia includes the childhood and adult forms, without using the word "moderate" to describe these forms. Otherwise, this is confusing. You might, however, also say here that is severe forms are the perinatal and infantile forms, so that these entities could be distinguished from what you choose to call "mild".

You might say apparent European ancestry, because individuals ascertained in the United States and Canada may very well have admixture of other ethnic
With regard to the SNPs, it might be best in the first sentence to say all 8 of them were studied here etc. etc.

At the top of page 5, the authors have changed their designation for the gene of interest. Be consistent throughout the manuscript, and call this either ALPL or TNAP. Also, use either AP or ALP.

Did the authors sequence the mutated cDNAs to confirm that the mutations were indeed introduced?

Be a bit more specific, how was the 40% used to define the threshold between dominant and recessive mutations. If wild-type activity was reduced to below 40%, was this the evidence of a dominant-negative effect? Please clarify.

Actually, the crystallography of placental alkaline phosphatase was identified quite some time ago, as you noted using references 11 and 12. Accordingly, delete the word "recently" from the description.

Results

It would be best, in the first sentence, not to say that the authors received 361 samples from unrelated hypophosphatasia cases. Instead, it would be best to say that they received 361 samples for ALPL analysis to explore the possible diagnosis of hypophosphatasia. Otherwise, it would seem that 120 hypophosphatasia samples went without the authors being able to detect any ALPL mutation.

It would certainly be interesting to know whether there was obvious hypophosphatasia in any of the patients who showed no ALPL mutation, among the 120 where of the mutation studies were negative. Did this diagnosis of HPP carry forward for any of the referral samples?

We now learn how 2 mutated alleles were detected in 94.9% of the 138 of the severe hypophosphatasia patients. Previously, there has been no mention of severe disease, and again here is an area that could be unclear because of the first introduction of "severe".

We learn that about 50% of patients with so-called mild hypophosphatasia carried 2 mutated alleles. This is expected because of the inclusion of patients with the childhood form of hypophosphatasia, verified by the authors who tell us that this finding occurred with increased severity of the disease.

Should not the authors consider redefining what they mean by a "moderate" and "severe" alleles? They are telling us that patients with the childhood form of hypophosphatasia have compound heterozygosity for both a moderate allele and a severe allele. Certainly, one would expect severe disease, i.e. the infantile or perinatal form, with combinations of a "moderate" and "severe" allele.

Also tell us a little bit more about the p.E190K mutation, and how this identifies backgrounds.
the patient as being of European ancestry.

We see that 30% of the parents with the heterozygous mutation report "symptoms" of hypophosphatasia. It might be true that had other methods been used to study these individuals, such as radiographic skeletal surveys, bone scans, bone densitometry etc. a higher incidence of "penetrance" might be revealed ("the harder you look, the more you find").

In the paragraph that begins in the middle of page 6, tell us if Table 3 is going to contain 35 different mutations among the patients with mild hypophosphatasia.

It will be important to describe, in a little more detail, the method used to define the dominant-negative effect. Is this by measuring total cellular alkaline phosphatase activity after cotransfection? If so, can abnormalities of cellular processing versus catalytic activity be distinguished? With lysing of cells, could a dominant-negative effect change, as homodimers are released from cellular trapping and recombine to make heterodimers?

Tell us whether the mutations that show a dominant-negative effect would have had zero alkaline phosphatase activity when transfected homozygous.

Throughout the manuscript, be consistent in using wild-type spelled out, versus abbreviated, as there is variability throughout the manuscript.

Change “coherent” to “understandable”.

For the distribution of the SNP alleles, this would be “showed” rather than “shows”.

Soon after, areas rather than the area, and compared to rather than mild.

Where the words “linkage disequilibrium” first appears, introduce the abbreviation LB parenthetically, and then used the abbreviation thereafter.

Did the authors study alkaline phosphatase activity generated from the polymorphisms? We learn about of the quantity etc. of the messenger RNA, but what was the impact on alkaline phosphatase activity?

Early on in the manuscript, there was some mention of looking at the promoter region for the ALPL gene. Was this done?

For the first sentence in the Discussion, mention again the various forms of hypophosphatasia that are considered "mild".

Considerable work has gone before this manuscript, concerning both an autosomal dominant and an autosomal recessive inheritance pattern for hypophosphatasia, as well as concerning the possibility of dominant-negative effects, yet the manuscript mentions little of this. There are only 17 references. Granted this might be a comprehensive look at a large number of patients, but precedence should not be diminished.
How reliable is the methodology of cotransfection in order to fully understand phenotype/genotype relationships for inborn errors of metabolism? Here, the authors are looking at alkaline phosphatase activity in just one cell type, and by measuring alkaline phosphatase activity alone. Presumably, they cannot detect abnormalities in the intracellular processing and migration and distinguish them from changes in catalytic activity on the external surface of cells. Also, there is the possibility that differences in the expression of the defective allele (more or less on RNA) compared to wild-type etc. that may affect in vitro assessment of the mutation. Is this problematic? Please address. The authors do touch on their previous work that showed one mutation could be considered dominant-negative because it did impact intracellular processing rather than catalytic activity.

You could probably best delete postmenopausal from the designation old postmenopausal Japanese women.

In the concluding paragraphs, it might be best to say that mild hypophosphatasia can result from either compound heterozygosity etc. etc.

In the legends to Table 1, the authors talk about untranslated exons. Should this be plural?

In Table 1, there was talk about early in the manuscript about a benign prenatal form of hypophosphatasia, yet this is not listed in the clinical forms discussed here.

**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:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Yes, I have a career-long interest in this disorder, hypophosphatasia, including improving our understanding of its molecular genetic basis.