Reviewer's report

**Title:** A novel c.-22T>C mutation in GALK1 promoter is associated with elevated galactokinase phenotype

**Version:** 2 **Date:** 21 November 2008

**Reviewer:** Richard Reece

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The manuscript by Park et al, describes the identification of a novel mutation within the promoter of the human galactokinase gene (T22C) that would appear to be associated with an elevated level of galactokinase activity. In this study, 63 individuals with positive newborn screening galactosaemia results but who did not have attenuated galactose degradation enzymes were chosen to study. Three mutations were identified within the promoter region of GALK1. Of these, the c.-22T>C change was found to be associated with an approximately two-fold increase in galactokinase activity. Finally, the authors have performed a mobility-shift assay to show that some unknown protein from a Hep3B nuclear extract is able to bind to this region of the GALK1 promoter. Competition with either oligonucleotides representing 22C or 22T would appear to suggest that this protein binds somewhat more strongly to the version of the promoter containing the 22C change. This data has been used to imply that an as yet unidentified nuclear protein is responsible for the increased level of galactokinase in these patients.

This paper is reasonably well written. The experiments performed clear, and most of the figures (with the possible exception of Fig. 1 and Fig. 4) present a ready summary of the experiment undertaken and results obtained. I do worry, however, that the paper does not go quite far enough to draw any firm conclusions. The mutation within the promoter of the human galactokinase gene is not associated with a phenotype. In addition, the nuclear protein to which the expression of GALK1 has been ascribed is not identified.

**Major Compulsory Revisions:**

1. This paper would be considerably strengthened if the identity of the transcription factor regulating the expression of the gene was know. In addition, the data relating to the mobility-shift competition is not at all convincing. As it stands, the paper is limited by these facts. The assertion that the c-22C DNA-protein complex was completed more efficiently with cold c-22C rather than c-22T (Fig. 4) is dubious. This effect, if it exists, is modest and would need to be quantified before any conclusions could be drawn. The authors may also wish to try and identify, for example using specific (and commercially available) antibodies to, say, Sp1 or Egr1, the nature of the protein DNA complex.

**Minor essential revisions:**
1. I am not sure why, in Figure 3, the data labelled 'AAT' in both panels have no error bars. I understand that the data has been normalised relative to the activity of the wild-type construct, but there still should be an error associated with this calculation.

2. The lanes in Figure 4 are referred to in the text as being numbered, but no numbers are on the figure itself.

**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.