Reviewer's report

**Title:** Functional characterisation of the TSC1-TSC2 complex to assess multiple TSC2 variants identified in single families affected by tuberous sclerosis complex

**Version:** 2  **Date:** 6 November 2007

**Reviewer:** Sandra Dabora

**Reviewer's report:**

**General**

The manuscript by Nellist et al. reports the evaluation of TSC2 gene variations on tuberin function relevant to 4 families with definite, probable or possible TSC and non-truncating TSC2 gene variations. In all 4 families, there are at least two non-diagnostic TSC2 gene variations. The authors report details of clinical evaluation and TSC2 genotype for all evaluable family members. The major finding is that they have used a novel approach to functional analysis of tuberin (the TSC2 gene product) to identify the pathologic mutation in each of these families. Tuberin function was evaluated using several methods: 1) co-immunoprecipitation assay to evaluate interaction of TSC1 with overexpressed TSC2 variants; 2) Evaluation of GAP activity by comparing GDP/GTP ratios after overexpression of each TSC2 variant; 3) evaluation of mTOR activity using western blot to measure levels of pS6K (T389) after overexpression of each TSC2 variant; 4) Evaluation of mTOR activity using immunofluorescence to look for inhibition of pS6 expression in cells with overexpression of each TSC2 variant. Using these methods they evaluated 9 non-diagnostic TSC2 gene variants (8 missense mutations and 1 three bp deletion) and they were able to identify the pathogenic mutation for each family. Interestingly, their findings did not correlate well with predictions using BLOSUM 62, PAM 250, or Grantham matrices. The authors also included information about conservation of these changes across species. Although all changes were fairly well conserved across species, it was nice to see that this information was included.

Non-truncating mutations (missense and in frame deletions/insertions) are a significant fraction of the known pathogenic mutations that occur in the TSC2 gene (~32% of all TSC2 mutations and ~20% of mutations overall in TSC). These variations are problematic because it is difficult to know if they are benign polymorphisms or disease causing mutations (as illustrated in this paper). This paper is carefully reported and addresses a significant problem relevant to TSC genetic analysis. Although there were several family members with minor manifestations of TSC that appeared to be inconsistent with the authors' conclusions regarding the pathogenic mutations, in each of these cases, the authors discussed these discrepancies and I found their arguments convincing (mosaicism, possibility that other variation contributed to minor features of TSC).
Summary: This is a useful paper with convincing data that is clearly presented. The functional analysis of non-truncating variations in TSC2 is useful for both genetic diagnosis and understanding protein function. This paper will be of interest to both the TSC clinician and the basic scientist interested in the mechanism of mTOR pathway regulation by TSC2.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Page 7, line 21. It looks like there is an error here. The authors should review and clarify. Shouldn’t the wild-type control ratio be 1.4 (instead of 0.4 as stated)?

2. Introduction, last paragraph. There is an error (5 families should be changed to 4 families).

Discretionary Revisions (which the author can choose to ignore)

1. Abstract:
The abstract could be improved by including more details (4 families studied, 9 non-truncating TSC2 variations evaluated, include the details of 4 pathogenic mutations identified).

2. In the introduction, include information on the frequency of TSC2 missense mutations and in-frame insertions/deletions. This is an important problem in TSC mutation analysis and more details on this will make this point.

3. The authors state that PAM 250, BLOSUM 62, Grantham matrices were used to compare amino acids for the missense mutations without a sufficient description or review of the meaning of scores generated by these tools. The introduction should include a more detailed review on these methods. If there are examples where these tools have been useful for determining the pathogenicity of mutations in human disease, this information should be included. It would be helpful to know the range of scores for each method.

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

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.