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:** Kit Sing Au

**Reviewer's report:**

**General**

As of today, approximately 20 to 30% of mutations identified on TSC2 gene are missense substitutions. Many of these missense substitutions had been classified as pathogenic if only present in sporadic TSC patients and many are classified as polymorphic variants when a definite mutation identified on a separate location or separate gene. However, a significant proportion of the missense substitution remain unclassified either due to lack of parental DNA for confirmation, passing on all affected individuals in multi-generation families, or situations as reported in this article. It is to the benefit of the TSC patients and importance to medical geneticists that a battery of standardized lab assays to be established in order to identify pathogenic variants on TSC2 gene. This manuscript investigated and identified pathogenic missense variants for 4 families.

No major revision is necessary. However minor essential revisions are needed.

1. In Results section, Patient Characteristics: Family 4 II:2 was described to have epilepsy only and should not be classified as possible TSC as in figure 1, family 4 II:2. Epilepsy is not a major or minor diagnostic criteria for TSC.

2. Although the TSC1, TSC2 bands (figure 2A) showed a obvious changes of intensities, the authors may consider using densitometry tools to estimate an intensity ratio for TSC1 vs TSC bands in a similar way to estimate GDP vs GTP ratio for assaying TSC2 GAP activity on RHEB-GTPase (figure 2B).

3. Results section, under Functional analysis, 4th and 5th paragraphs: it is a bit confusing when the authors use "control" and "wild-type" to indicate differences in TSC2 GAP activities on RHEB-GTPase. It will be much clearer when GAP activity of TSC variants compared only to the wild type TSC2.

4. Discussion section, first paragraph should also comment on the application of their assay to characterize missense variant found only in affected individuals of multi-generation TSC families.

5. Instead of starting with "In 2 families" twice in paragraph 2 and 3 in the "Discussion", the family number should be used for clarity.
6. Both family 1 and 2 carry an "positive TSC complex activitor" vairants as discussed. It will be interesting to see if a neutralizing effect in trans of the "positive TSC complex activitor" on the pathogentic variant exist when both present. This is particularly relevant since members of these family do have both variants and appears to have milder phenotypes.

7. It was discussed in the third page of Discussion the 3rd paragraph that in some assay F143L variant has lesser activity compare to wild-type TSC2. Figure 3B appears to show additive effect on the triple mutation variants (S132C/F143L/C244R). Since all experiments were done in triplicates (Figure 2D, figure 3B), the authors should be able to find if adding S132C/F143L to C244R significantly lower the inhibition. Similar statistical activities can be applied to compare F143L vs S132S/F143L and for family 4 variants. In light of this comment, it might be premature to conclude some of these variants as no-pathogenic since they may exert a much milder effect that the present assay cannot detect. Since TSC patients are known to have variable expressivity, it is not surprising to find some of these "polymorphic" variants together contribute to the atypical TSC symptoms.

8. I am not sure if Table 1 add useful information to the manuscript since the predictions do not seem to align with the experimental results.

9. Table 2. Variants for family 3 and family 4 appears to be switched.

10. Figure 2 legend should put variants immediately after family number (e.g. Family 1 (I820del and R1772C)) for clarity. Figure 2B should add label for GDP and GTP for clarity and the legend should add description for t=0.

11. Figure 3 legend should put variants immediately after family number (e.g. Family 3 (S132C, F143L, and C244R)) for clarity. Not sure if the TSC2 and TSC1 bands on Figure 3A are results of lysates or TSC1 IP. Need clarification.

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

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:

I declare that I have no competing interests.