**Author's response to reviews**

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

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**Author's response to reviews:** see over
Many thanks for sending the comments from the three reviewers on our manuscript 2200778961635001, 'Functional characterisation of the TSC1-TSC2 complex to assess multiple TSC2 variants identified in single families'. We have now revised the manuscript to address the reviewers’ concerns and to ensure that the manuscript conforms with the required *BMC Medical Genetics* format.

**Reviewer 1 (Hengstschlager):**

We have included a short discussion on the potential effects of mutations on other putative (non-mTOR-related) cellular functions of TSC2 (Discussion; page 10, second paragraph).

**Reviewer 2 (Au):**

1. Figure 1 has been amended to show individuals with epilepsy only, and no other signs of TSC.
2. We have now included the densitometric analysis of the Western blot results shown in Figures 2 and 3.
3. We have replaced the value for the negative control ratio with the value for the wild-type control (Results, page 6, paragraphs 2 and 3). (see also reviewer 3).
4. As suggested by the reviewer, we have included a comment in the discussion on the ability of the assay to distinguish pathogenic and non-pathogenic missense variants that co-segregate with TSC in multi-generational families (Discussion, page 7, first paragraph).
5. In the Discussion, the family numbers have been included for clarity (Discussion, page 7, second and third paragraph).
6. We agree with the reviewer that it will be interesting to determine whether TSC2 variants have an activating effect in trans. We have now referred to this possibility in the Discussion (page 9, last paragraph).
7. We have already included reference to this problem in the discussion (Discussion, page 9, third paragraph), and have modified the text slightly according to the comments of the reviewer.
8. We have not removed Table 1, as reviewer 3 found the information useful.
9. We have corrected the mistake in Table 2, as requested.
10. Figure 2 has been altered, as requested.
11. Figure 3 has been altered, as requested.

**Reviewer 3 (Dabora):**

**Minor Essential Revisions:**
We have altered the text, as requested (see also reviewer 2). For clarity, we have now stated the wild-type control ratio, not the ratio of the negative control (Results, page 6, paragraphs 3 and 4). We have corrected the error in the introduction (5 families has been changed to 4 families; Background, page 3, last paragraph).

**Discretionary Revisions:**
We have included the details of the families and the TSC2 variants in the Abstract (page 2), as requested.
We have included details of the proportion of TSC2 missense and in-frame insertion/deletion mutations found in the TSC patient population (Background, page 3, first paragraph).
The range of scores for the PAM 250, BLOSUM 62 and Grantham matrices are given in the legend to Table 2. For the sake of brevity, we omitted a review of these protein analysis tools. Full descriptions of the methods can be found in the original articles, which are listed in the References section.

We hope that we have addressed the concerns of the reviewers sufficiently and that the revised manuscript will now be acceptable for publication. The work described in this article was performed as part of the DNA diagnostic service at the Erasmus MC. Our research has obtained ethical approval from the Medisch Ethische Toetsingcommissie (METC) at the Erasmus Medical Centre (MEC 2007 024).

Yours faithfully,

Mark Nellist (on behalf of all co-authors)