Author's response to reviews

Title: Identification of a region required for TSC1 stability by functional analysis of TSC1 missense mutations found in individuals with tuberous sclerosis complex

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Author's response to reviews: see over
Dear Dr. Edmunds,

Many thanks for sending the comments of the reviewers for our manuscript 'Identification of a region required for TSC1 stability by functional analysis of TSC1 missense mutations found in individuals with tuberous sclerosis complex' (manuscript 5466462562598871) submitted for consideration for publication in BMC Medical Genetics on 9th March 2009.

We are grateful for the input of the 3 referees and have tried to address their comments. We hope that the revised version of the manuscript will be acceptable for publication.

Referee #1

We were very pleased with the comments of referee #1.
Referee #2

Reviewer #2 asked if we determined whether MG132 treatment increased the expression of all the mutant TSC1 variants, not just the L50P and L117P variants, and also whether this treatment resulted in the formation of aggregate structures. We have now included these experiments. Inhibition of proteosome activity with MG-132 results in increased expression of all the pathogenic variants, as now shown in Figure 3. We have also altered the text in the Results section, page 10, *TSC1 variants are degraded by the proteosome*, second and third paragraphs.

We agree with referee #2's suggestion that the different localisation pattern of the TSC1 mutant variants is most likely due to their low levels of expression. However, we did not observe any aggregate formation upon treatment with MG-132. This is probably because the treatment does not increase the expression levels of the variants sufficiently to cause aggregate formation. We have now included MG-132-treated cells in Figure 4 and updated the text in the Results section, page 11, *Intracellular localisation of the TSC1 variants*, second paragraph.

We have altered Figure 3, panels B and C, and Table 1, to present the data in a more logical order, as suggested. We have added a key to Figure 1C, as requested and indicated significant differences with asterisks in Figure 2. Values in Figure 3 did not quite reach significance (p = 0.06). Scale bars have been added to Figure 4.

Referee#3

We disagree with referee #3 regarding the E51D variant. The presence of amino acid substitutions in the TSC1 N-terminal region that do not affect function, such as the E51D and R190C variants, does not mean that the region is not functionally important. The findings of our previous publication (Nellist et al. Eur. J. Hum. Genet. (2009) 17: 319-328), the current manuscript and also more recent unpublished experiments are remarkably consistent: All the amino acid substitutions that affect TSC1 function map to the N-terminal region of TSC1, reduce TSC1 expression levels and disrupt the regulation of TORC1 activity by the TSC1-TSC2 complex. The R190C substitution is a known polymorphism and the E51D substitution is a very conservative change, and is therefore
less likely to have an effect on protein function. This point was already made in the Discussion section, page 13, second paragraph.

We hope that we have addressed the comments of the referees sufficiently and that the manuscript will be reconsidered for publication in BMC Medical Genetics.

Yours sincerely,

Mark Nellist (on behalf of all co-authors)