Author’s response to reviews

Title: Rubinstein-Taybi 2 associated to novel EP300 mutations: deepening the clinical and genetic spectrum

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Walter E. Kaufmann (Reviewer 1): MGTC-D-17-00130-R2

The study describes an 8-subject Spanish cohort of individuals with Rubinstein-Taybi syndrome (RSTS) and mutations in the EP300 gene that, as the authors state is the less common genotype associated with RSTS. The affected subjects demonstrated an overall typical but milder RSTS phenotype and a variety of mutations, with frameshifts being the most common as reported in the literature. All major features of the cohort were in line with previous publications, with the exception of behavioral problems that were absent in the Spanish series.
Considering that, prior to this study, only 76 patients with RSTS-EP300 had been described, the reported series represents a 10% increase in well-characterized patients with RSTS-EP300. Nonetheless, it is unclear what is novel in the study. As mentioned above, all features in the 8 patients have been described before, with (absence of) behavioral issues being the main difference.

In addition to the lack of "compelling argument" for the report, the presentation of the data (e.g. lack of table/figure comparing the reported series with the literature) and the challenging grammar are major weaknesses. For instance, the sentence "Respecting skeletal malformations, 88% of EP300-individuals displayed broad thumbs with two cases also angulated, confirming previous results that showed the uncommon presence of this characteristic in EP300-RSTS patients". What was uncommon in the reported group of patients was the angulation of thumbs and halluces, not skeletal abnormalities in general. Other issues are methodological, such as whether all parents were genotyped.

A table with comparison between our results and those previously published has been added.

This sentence has been corrected and grammar has been reviewed.

The information about which parents were also genotyped has been included.

Cristina Costanza Giovanna Gervasini (Reviewer 2): To The Authors

The manuscript by Lopez and colleagues reports on eight patients with Rubinstein-Taybi presentation, found carriers of EP300 mutation.

The patients are recruited from a cohort of 72 RSTS Spanish cases, tested and negative to major gene CREBBP. In order to detect a mutation in the known second RSTS gene, EP300, the Authors performed a molecular investigation by MLPA and EP300-targeted NGS The clinical presentation of the probands is sufficiently detailed, as the molecular test.

The discussion is superficial: the genotype-phenotype correlation is only sketched.
Several inaccuracies are present (number of patients not congruent in the text, figure legends inappropriate, etc.). It has been corrected.

There are criticisms which should be addressed in order to improve the manuscript and further support the discussion.

-A more detailed discussion about the EP300 mutation in patients without a clear RSTS phenotype should be reported. In Introduction section, the authors should describe that a different phenotype associated to EP300 mutations has also been reported. I suggest to enlarge the discussion (Discussion section) about the wide presentation of EP300 mutations.

Some sentences has been included.

-As a general comment, the genotype-phenotype correlation can be developed. The Authors report that "It is difficult to establish a genotype-phenotype correlation although the clinical evaluation of our patients corroborates that clinical features in EP300 are less marked than in CREBBP patients. It is remarkable that these findings are observed in a RSTS-diagnosed cohort; some patients harbouring EP300 mutations present a different phenotype. Broadening the knowledge about EP300-RSTS phenotype may contribute to improve the management of patients and the counselling to the families." (Abstract) and "In this report we present the clinical and molecular characterization of a Spanish cohort of 8 RSTS patients carrying novel EP300 mutation identified from a group of 72 RSTS patients. The description of more RSTS patients, and more concretely of EP300-cases may contribute to better understand the range of phenotypes in order to provide clinical pointers that would improve the earlier detection and diagnosis of these patients." (Introduction). These sentences are opposing and an effort is requested in order to comment the relationship of EP300 mutations and the corresponding phenotype.
Some sentences have been included in discussion section.

- A more detailed comment is requested to compare the CREBBP-positive and EP300-positive RSTS patients, keeping into account the recent papers by Fergelot and Hamilton.

It has been completed, although the first part of the discussion attempted to deal with this comparison.

- Patients #124 and #133 (discussion, lane 29) are not described above. I suppose that pt #124 is the patient indicated as #45 and pt #133 as #42.

It has been corrected.

- The mutation of patient #42 (alias #133) is reported in ExAc Browser. The Authors should comment the doubt of the real pathogenicity of this mutation. A sentence in the discussion with detailed comments is necessary.

A comment has been added in results, although this issue was described in discussion.

- The cited comment about the missense mutations in HAT domain is simplistic. First, the Authors report "Although this kind of mutation is not commonly found as causative of RSTS (only a single missense mutation listed in LOVD)," but there are other missense mutations reported in the literature (see pts #5, #6, #9 reported by Hamilton and colleagues and pts #4, #5, #11, #33 (reported also by Wincent et al. 2015) by Fergelot. All the mutations should be considered in the following comments.

Suggestion has been followed.

- The first lanes (mutation description: nucleotide change and protein prediction) of Table 1 is unreadable, in addition the indication about involved exon could be helpful to readers. Please complete also the text with this information. The lane Heart anomalies can be deleted (none of the patients reported this sign).

It has been corrected, and suggestion about exon number has been followed.
-Figure 2 is only sketched. Please indicated the numbering of the depicted domains and the mutation description. In legend, the corresponding colors of gene-exons and protein-domains should be detailed.

It has been completed following reviewer comment.

-The inherited mutation (pt#45) was previously described (Lopez et al. 2016). Then, this case can be cited but should be removed from the group of the described patients of this work. Alternately, the Authors should explicate that this work present novel and yet described patients.

It has been included in the text and in the table.

Additional minor revisions:

-Title (not tittle)

-Background (first page, lane 37): "The first gene associated with RSTS was CREBBP," please change in "The first gene associated with RSTS is CREBBP," It has been changed.

-Background (first page, lane 39): "Ten years later, mutations in the related gene EP300 were detected": what is the date of reference? Reference has been included.

-Background (first page, lane 54 and following): this sentence should be rewritten, as it's not clear. RSTS as epigenetic machinery disorder is now well known thanks to writer role of CREBBP and EP300.

RSTS is defined as a disorder of the histone machinery by Jill A. Fahrner and Hans T. Bjornsson

-Results (second page, lanes 19-25): the percentage can be removed. Done.