Author's response to reviews

Title: Multilocus loss of DNA methylation in individuals with mutations in the histone H3 Lysine 4 Demethylase KDM5C

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Version: 4 Date: 9 January 2013

Author's response to reviews: see over
Dear Mr. Acosta,

Thank you for your letter. We have now revised the manuscript according to the reviewers’ suggestions and respond below to each individual comment. Please find the detailed answers below in italicized font. We have also reformatted the paper according to BMC Medical Genomics requirements.

Sincerely,
Rosanna Weksberg

Referee 3
Title: Multilocus loss of DNA methylation in individuals with mutations in the histone H3 Lysine 4 Demethylase KDM5C
Version: 3 Date: 20 November 2012
Reviewer: Cintia Santos-Rebouças
Reviewer’s report: I have suggested only minor essential corrections (please see attached file for details).
Level of interest: An article of outstanding merit and interest 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.

Minor points:
- Lines 63-64: authors should cite the MIM number of KDM5C gene.

*The MIM number was added.*

- Lines 65-67: “To date, 22 different mutations have been identified and the prevalence of KDM5C mutations in patients with XLID is estimated to be ~3% [3-11]”. Reference 6 (Adegbola et al, 2008) should be removed from the total number of mutations, since the alteration reported in this cited article was found in an individual with autism. So, authors should consider only 21 different mutations.

*The sentence was modified according to the reviewer’s suggestion.*

- Supplemental Figure S1: the schematic diagram of the KDM5C protein and positions of the 5 selected mutations and p.R1546Q variant should be included along the manuscript. These data are the basis of the research question explored in the manuscript.

*The schematic diagram of the KDM5C protein and positions of the 5 selected mutations and the p.R1546Q variant were included as Figure 1 in the manuscript.*

- Lines 102-103: “Recent studies suggest that there is interplay between histone modifications and DNA methylation”. Authors could add at least two recent references to support these data.
Two references (Cedar & Bergman, Linking DNA methylation and histone modifications: patterns and paradigms, Nature Reviews, 2009; Hashimoto et al. Molecular coupling of DNA methylation and histone methylation, Epigenomics, 2010) were added to support the aforementioned statement.

- Line 346: substitute ” wich” by “which”.
The spelling mistake has been corrected.

- Lines 493-496: “… suggesting that potentially altered DNA methylation at these three genes could be used for molecular diagnosis of KDM5C mutation cases, which are frequently indistinguishable from other forms of non-syndromic ID based on clinical phenotype[15]”.
I totally agree with the authors that DNA methylation patterns concerning FBXL5, SCMH1 and CACYB could be useful in distinguishing benign from pathogenic KDM5C mutations and represent a great finding for X-Linked Intellectual Disability (XLID) understanding. However, the use of such strategy as a pre-screening test for routine diagnosis is really a strong affirmation and should not be proposed until other KDM5C mutations have been tested for this basis. Moreover, if we consider that KDM5C is one of more than 20 epigenetic regulators involved in ID, we can not exclude that these locus-specific loss of DNA methylation could be a consequence of mutation in other genes than KDM5C, mainly if we take into account that the three top candidate genes are part of ubiquitin-ligase protein degradation pathways. By this way, I suggest that the authors re-write this paragraph, concentrating on the benefits to XLID and in the use of FBXL5, SCMH1 and CACYB methylation patterns to preview the pathological role of an unclassified KDM5C mutation.

We have rewritten the paragraph as suggested by the reviewer focusing on the utility of DNA methylation for distinguishing pathogenic and benign variants of KDM5C.

- Line 517: correct “in of”.
The spelling mistake has been corrected.

Referee 4
Title: Multilocus loss of DNA methylation in individuals with mutations in the histone H3 Lysine 4 Demethylase KDM5C
Version: 3 Date: 12 December 2012
Reviewer: Shigeaki Kato
Reviewer's report:
Major Compulsory Revisions

Grafodatskaya et al., surveyed loss of DNA methylation in the gene promoters on whole genome of the intellectual disability and genetic mutations in the KDM5C gene. Since this KDM5C gene encodes the H3K4 demethylase, it is conceivable
that malfunction of this enzyme induces the aberrant shifts in the epigenetic modifications on chromatin including DNA methylation. From the analyses of the twelve patients, three genes were identified to lose DNA methylation, while such loss was not found in the control examinees. They claim that DNA methylation patterns from blood sample of the patients are epigenetic biomarkers for the diagnosis of the intellectual disability.

The present study is interesting and informative for audience. However, the alterations in DNA methylation pattern in certain gene promoters are just consequence of impaired histone demetylation by KDM5C, and looks unlikely to directly reflect the direct actions of KDM5C. This point appears to be overinterpreted by the authors, and needs to be carefully re-described.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: 'I declare that I have no competing interests'

We completely agree with the reviewer that DNA methylation alterations observed in patients with KDM5C mutations are unlikely to reflect the direct action of KDM5C, as this protein is not known to possess DNA methyltransferase activity. We have emphasized this fact in the text of the Discussion section (page 19). We have also added to the Discussion information regarding the specificity of loss of DNA methylation in the context of loss of H3K4 demethylase activity (pages 20-21).