Author’s response to reviews

Title: De novo truncating variant in WHSC1 gene leading to mild Wolf-Hirschhorn syndrome phenotype: a case report

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Reviewer reports:

Nobuhiko Okamoto (Reviewer 1): The authors reported a boy with de novo truncating variant in WHSC1 with similar but mild clinical features comparing with typical 4p16.3 deletion related WHS. This is an interesting report covering WHSC1 mutations.

1. Please show the SD score or percentile of occipitofrontal circumference.
   Growth parameters should be included in the Table.
   Response: We have revised and added the SD score information of occipitofrontal circumference for our patient. We have added birthweight information in the table and calculated percentile of birth weight.

2. How about the endocrinological data including GH?
Response: Growth hormone level was normal in our patient. Since there is no obvious report of abnormal hormone level in previously reported patients with truncating variants in WHSC1, indicating endocrinological effects have much less effect than expected.

3. Figure of NSD2 with its domains and positions of reported variants will be a help for readers.

Response: We have revised the figure and added a sketch map of domains and positions of reported variants.

   "de novo" "de novo"

Page numbers of the references lack coherence.

Please check other minor errors.

Response: We have checked our manuscript and revised all minor errors.

Thank you for your previous comments and suggestions. We have revised our manuscript accordingly.

Marcella Zollino (Reviewer 2): Yanrui et al report on an additional case of a de novo truncating variant in WHSC1 gene leading to a clinical presentation in the spectrum of WHS.

The report is interesting and it deserves publication, however several amendments must be provided before.

In introducing them, this reviewer would like to discuss about the nosology of WHS, to allow proper diagnosis in the current era of whole genomic investigations, that can be offered outside a specific clinical suspicion, as in the present report.

Typical WHS, even in the mild form of its clinical phenotype, is largely assumed to be a multigenic disorder. Minimal diagnostic criteria include the typical facial appearance, intellectual disability, growth delay AND seizures (Zollino et al, 2008; Zollino and Doronzio, 2018). WHSC1 is the major, but not the unique gene for the facial dysmorphisms (Zollino et al, 2008), growth delay and, as confirmed by the recent reports of intragenic loss-of-function variants, for intellectual disability. However other genes, different from WHSC1, are responsible for seizures, in a comorbidity model of pathogenesis, most likely, including LETM1 and additional genes residing on 4pter distally to it (Zollino et al, Epilepsia, 2014). Thus, haploinsufficiency limited to WHSC1 can not cause WHS, even in its mild presentation.
The WHS critical region was redefined after the initial report by Wright et al. (1997), and it was referred to as WHSCR-2 (Zollino et al, Am J Hum Genet, 2003). Of importance, WHSCR-2 shares the WHSC1 gene with the previous defined region, but, in addition, it includes LETM1. Neither WHSCR nor WHSCR-2 were established as genetic cause of WHS, but they just allowed for search of candidate genes. As a matter of fact, in considering the multigenic pathogenesis of both the facial dysmorphism and the seizure disorder, expanding the WHS critical region on 4pter beyond both WHSCR and WHSCR-2 is more appropriate, as suggested (Zollino et al, Epilepsia, 2014).

All these aspects are in general considered by the Authors, but they need to be presented in a more clear manner, following all the above considerations. References need revision as well.

Major criticism are in order.

1) Title. "… leading to atypical Wolf-Hirschhorn phenotype" is more appropriate

Response: We have revised the title accordingly.

2) Abstract (Background), lines 29-30 and background, lines 47-53. Please modify the text according to the following suggestions: WHS is a contiguous gene syndrome caused by partial 4p deletion highly variable in size in individual patients. The core WHS phenotype is defined by the association of growth delay, the typical facial characteristics, intellectual disability and seizures. Depending mostly on the extent of the 4p deletion, additional clinical signs include major malformations, as midline defects, congenital heart defects, renal and skeletal anomalies (Zollino et al, Am J Med Genet C Semin, 2008)

Response: We have revised these contents in the background and abstract accordingly.

3) Please avoid using "mutation" throughout the paper; pathogenic variant is more appropriate, as usually used by the authors

Response: We have revised through the manuscript, using ‘pathogenic variants’ instead of ‘mutation’.

4) Background, lines 54-56 (about WHS critical region): please add the reference about WHSCR-2 (Zollino et al, Am J Hum Genet, 2003). Importantly, the sentence "Monosomy of WHSCR covering WHS candidate gene 1 and 2 (WHSC1 and WHSC2) was established as a genetic cause of WHS” must be omitted since it is incorrect.

Response: We have revised these contents accordingly.
5) Discussion, lines 114 and 115. The statement is incorrect. Please modify into "Minimal diagnostic criteria for WHS have been proposed by Zollino et al including typical facial appearance, mental retardation, congenital hypotonia, growth delay and seizures" (Avoid using mild). With respect to this point, the right reference is Zollino et al, Am J Med Genet C Semin Med Genet. 2008 Nov 15;148C(4):257-69.

Response: We have revised these contents accordingly.

6) Discussion, lines 133-135. In cases of intragenic WHSC1 loss-of-function variants minimal diagnostic criteria for the diagnosis of WHS are not satisfied, due to the absence of seizures. This sentence is nosologically incorrect. Please modify it according to all the above considerations.

Response: We have revised this statement to make it clear that patients with WHSC1 loss-of-function variants failed to meet the minimal diagnostic criteria due to the absence of seizures but manifest atypical Wolf-Hirschhorn phenotype.

7) Absence of seizures in association with haploinsufficiency limited to WHSC1 deserves to be highlighted more emphatically. That is relevant for prognostic evaluation and for surveillance as well. Importantly, seizures act as independent prognostic factors for the final degree of intellectual disability (Zollino et al, 2008).

Response: We have revised the paragraph in the discussion part to emphases absence of seizures in association with haploinsufficiency limited to WHSC1. None of the five patients reviewed with de novo truncating WHSC1 variants had epileptic symptoms and have less severe intellectual disability compared with typical 4p deletion WHS case, consistent with the previous study that suggest other genes are responsible of seizure disorder other than WHSC1.

Thank you for your previous comments and suggestions. We have revised our manuscript based on these comments accordingly. You suggestion that haploinsufficiency of WHSC1 related with atypical WHS syndrome not mild syndrome is critical and help to improve the quality of our manuscript in a great extent. We have also revised our reference based on your suggestion.