Author's response to reviews

Title: Familial imbalance in 16p13.11 leads to a dosage compensation rearrangement in an unaffected carrier

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Version: 2
Date: 24 July 2014

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

Below is the response to the reviewers’ comments for the manuscript entitled “Familial imbalance in 16p13.11 leads to a dosage compensation rearrangement in an unaffected carrier” which was sent for publication in BMC Medical Genetics. The original manuscript has been changed according to the following points:

**Major Compulsory changes:**

**Pathogenicity of the 16p13.11 CNV.** Referee 1 suggests to highlight and to reference the role of this duplication in disease, whereas Referee 2 requests the manuscript to address the main subject which is the familial rearrangement. The Background and Discussion have been reformatted according to the abstract and the title, to give the main message of the manuscript a principal role. The revised clinical significance of the CNV and the genomic architecture of the chromosomal region including additional references suggested by both reviewers have been left as a secondary topic.

**Orientation of the duplication.** As observed by Referee 2, the word “tandem” used in page 7 to designate the duplication in the patient is misleading, since we actually don’t know the orientation of the duplicated fragment in the region. So, we have removed it from the text. We actually meant that the extra gene dosage observed by aCGH is located on that same region, as verified by metaphase and interphase FISH. A two-color FISH experiment would indeed test for the orientation of the extra fragment, but we think this would be irrelevant for the proposed mechanism, which would be still based on interchromosomal NAHR. Moreover, regarding the different distance between both interphasic signals in the patient and his mother, we checked back other interphase FISH images of the patient’s mother and verified that the distance between duplication signals shown in Figure 1f is in the upper edge, being on average similar to that of the patient. We think that the difference between both images is not significant, and uncondensed interphase chromatin in the absence of a reference signal is not suitable to determine genomic sizes. We propose an alternative Figure 1f
which is attached to this revision. According to this reviewer, red number 16 has also been deleted from Figure 1e to clarify the image.

**Phenotype/genotype of the grandmother.** Referee 2 asks for the phenotype of the patient’s grandmother. This information has now been included in the clinical report since it had also been provided by the patient’s mother. The probability of the patient’s grandmother having a germline mosaicism for deletion 16p13 exists indeed and it has also been added to the discussion. We agree that “preferential proliferation” gives the postzygotic hypothesis an excessive speculative character. We actually mean that selected cells with only the genomic compensation would have been confined to the embryo, as in the postzygotic model proposed by Carelle-Calmels et al., 2009. The Discussion and Figure 3 have been edited according to this idea.

**Minor Essential revisions:**

**Clinical report.** As stated by Referee 2, phenotypic description is not the main subject of the paper. Nevertheless, although the developmental milestones will not be detailed, the word “apparently” has been replaced by a more outright statement attesting for the whole evaluation. “Retardation” has also been replaced by “delay”.

**Coverage of the region by aCGH.** Referee 1 notes the low density of oligos in region 16p12.3-p13.11 as compared to other regions in 16p. When this custom array was designed, this region was not so well clinically characterized and hence was only represented in the aCGH backbone. As newly stated in the methods section, further versions of the array design will include this and other genomic regions as they are being described.

**Quantification of the STR PCR.** Some of the STR markers could only determine trisomic dosage in the children, maternal grandfather and maternal uncle in a semiquantitative manner, therefore only supporting results of other techniques. This idea was not properly expressed in the manuscript as implied by Referee 1 and it has been changed.

**Repetitive structure.** As asked by Referee 1, a repetitive structure may characterize the LCRs but not the short arm of chromosome 16. For that reason, this attribute has been unlinked to the whole region.
**Cell types and genotypes.** Referee 1 asks whether testing of two different cell types is enough to state that all cell lineages with different genotypes would have been confined to extraembryonic tissue. The answer is no. This part has been changed to consider other possibilities as well.

**Genetic counseling.** A too categorical statement was made about the segregation of the rearranged chromosomes, as observed by Referee 1. A more realistic statement has been rewritten in this part of the Discussion.

**Citations.** References provided by Referee 2 to describe the genomics and phenotype of CNVs in chromosome 16p and the role of NDE1 have been included in the discussion.

**Figure 1 legend.** A mistake acknowledged by Referee 2 has been corrected.

**Linguistic/syntactic changes.** All suggestions provided by Referee 2 have been incorporated in the manuscript. “Associated with” has been replaced by “along with” to avoid confusion, since there is no causal relationship between both findings. The unnecessary speculation of a ‘deliberate’ postzygoti compensation mechanism has been removed from the text. Language has been reviewed as suggested by Referee 1.

Sincerely,

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