Reviewer's report

Title: A probable case of enhancer adoption in a patient with t(2;8)(p16.1;q23.3) and clinical presentation resembling Trichorhinophalangeal syndrome

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Reviewer: Hermann-Josef Lüdecke

Reviewer's report:

The paper by M. Crippa and co-authors describes a female patient with an unusual presentation of tricho-rhino-phalangeal syndrome type I, TRPS I, because she does not have the pathognomonic abnormalities of the hands, the cone-shaped epiphyses of the phalanges. Unfortunately, the patient refused having a photograph of her published, as a proof of the suspected clinical diagnosis of TRPS I for the presented case. Did she also refuse to have a hand X-ray published?

A standard karyotype revealed a balanced translocation t(2;8)(p16.1;q23.3), the breakpoints of which were mapped and precisely determined by FISH and sequencing of the junction fragments, respectively. Array-CGH and sequencing of the TRPS1 coding region, the patient did not disclose any inactivating deletion or mutation of the TRPS1 gene. The authors determined that the translocation disrupts a large intergenic non-coding RNA upstream of the TRPS1 gene, and that the translocation puts a conserved enhancer element from chromosome 2 in the vicinity of the TRPS1 gene.

These basic analyses, including the in silico analyses of the breakpoint regions are well done.

Major comments:

However, the TRPS1 gene expression analysis by quantitative real-time RT-PCR is not suitable to obtain valid data. The level of TRPS1 expression in peripheral leukocytes varies significantly between individuals. Thus, it is impossible to detect differences between patients and healthy individuals. The authors recognize and admit this in the last sentence of the paragraph 'Gene expression analysis' and their supplementary figure 2. They couldn't find any difference between the patient's TRPS1 expression level and those of ten controls. This means that the authors have no data supporting their proposal of an impairment of the TRPS1 expression level by enhancer adoption, as the likely pathogenic mechanism.

TRPS is always caused by a reduction of the expression level of wild-type TRPS1 - mainly by mutation or deletion of one allele. No convincing data have ever been published that an increase of TRPS1 expression can also cause TRPS. E.g., even the recent publication by David and colleagues obtained conflicting expression data with different methods.

One and maybe the only trustable method to determine differences in expression...
levels - if the coding sequence of both alleles is intact - is the detection of allelic differences by SNaPshot analyses. For this purpose, one needs expressed SNPs. TRPS1 has some highly informative SNPs in its 3'UTR. The SNPs in exon 2 of TRPS1 are not suitable for this analysis. Since the first description of the TRPS1 gene, it is known that this non-conserved exon is only present in small amount of transcripts.

Reporting cases with unusual clinical presentation of a known condition due to unusual gene mutations is always worthwhile. However, speculations about disease causing mechanisms should only be made if sufficient experimental evidence can be provided to support the suggested mechanisms.

Thus, for the current manuscript by Crippa et al, I suggest that the authors either (I) remove all speculations and inconclusive gene expression data from the title, the results and discussion, and publish an interesting case report or (II) improve their expression analyses towards conclusiveness, and discuss true results. - I am almost convinced that they will find a reduced expression of the TRPS1 allele, which is in phase with the translocation, due to a removal of distant promoter or enhancer elements.

Minor comments:
The authors claim that the translocation occurred de novo in the patient but have neither done any analyses on the parents nor do they provide any evidence that the parents were indeed healthy. Thus, they should remove the description 'de novo.'

Table 1 lists clinical features of TRPS. Unfortunately, no references are given. Some of the clinical signs are incorrectly assigned to one or the other type of TRPS. E.g., ureteral reflux is quite common to all types of TRPS (but not always addressed in publications), and ID is certainly not a general sign in TRPS, but is more frequent in patients with TRPS I or TRPS II and deletions that involve more than the TRPS1 gene, alone. This is always the case for the contiguous gene syndrome TRPS II, and within this group the severity of ID correlates to some extent with the size of the individual deletion. Thus, this table must be revised or, alternatively, can be removed completely, because the absence of the pathognomonic cone-shaped epiphyses of phalanges in the presented case is the only true difference to classical TRPS. And the clinical report describes the patient’s phenotype in sufficient depth.

**Level of interest:** An article of importance 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.