Author’s response to reviews

Title: Large intragenic deletion of CDC73 (exons 4-10) in a three-generation hyperparathyroidism jaw tumor (HPT-JT) syndrome family

Authors:

Vito Guarnieri (v.guarnieri@operapadrepio.it)
Raewyn Seaberg (Raewyn.Seaberg@mail.utoronto.ca)
Catherine Kelly (catherine.kelly@wchospital.ca)
Jean Davidson (jeandavidson@mac.com)
Simon Raphael (Simon.Raphael@nygh.on.ca)
Andrew Shuen (andrew.shuen@sickkids.ca)
Filomena Baorda (filomenabaorda@yahoo.it)
Orazio Palumbo (o.palumbo@operapadrepio.it)
Alfredo Scillitani (alscill@tin.it)
Geoffrey Hendy (geoffrey.hendy@mcgill.ca)
David Cole (davidec.cole@utoronto.ca)

Version: 1 Date: 02 Jun 2017

Author’s response to reviews:

Reviewer reports:

William F. Simonds (Reviewer 1): MGTC-D-16-00236

In this manuscript, Guarnieri et al conduct germline CDC73/HRPT2 mutation analysis in a family with hyperparathyroidism-jaw tumor syndrome (HPT-JT) in which the proband was found to have parathyroid cancer. The authors confirm carrier status of the proband's daughter, and uncover a positive history of HPT in the proband's father. A novel deletion of exons 4 to 10 of the CDC73/HRPT2 gene was detected in the three affected family members. In addition, a novel single base insertion in the 5'UTR that co-segregated with the large intragenic deletion was identified. The 5'UTR single-base insertion was shown to significantly impair the expression of the parafibromin protein in in vitro assays.
This work emphasizes the importance of screening for large deletions in CDC73/HRPT2 in kindreds with possible HPT-JT or even familial isolated HPT, even when initial PCR-based screening for CDC73/HRPT2 germline mutation is negative, when there is a high index of clinical suspicion.

Minor suggestions:

a) In the abstract (p. 3 line 12) "co-segregated" is misspelled.

- We thank the Reviewer; we have corrected the typo.

b) Please add a reference (e.g. on page 6, line 3), for the benefit of the less specialized reader, regarding the association of uterine neoplasia and dysplasia with HPT-JT and CDC73/HRPT2 mutation

- We agree with the Reviewer. We have inserted the suggested reference in the text (p 6, line 3, and Ref 20).

Jessica Mester (Reviewer 2): This article was nicely written and contained valuable information about an interesting and rare disorder.

- We are grateful to the Reviewer for the kind remarks.

I would ask the authors to address a few points below, and I look forward to seeing this article in publication.

1. Based on the co-segregation of the 5’ UTR variant and large deletion, these variants are occurring on the same allele (in cis) in this family. Given that the large deletion is likely to result in nonsense-mediated decay (NMD), do the authors believe the 5’ UTR variant would ultimately have any impact in this situation? If the answer is "no" given the predicted NMD it would be beneficial to the reader for the authors to state as such. I do appreciate that the authors did not ascribe any clinical significance to the presence of this variant or try to suggest that its presence modified phenotype in any way.

- We thank the Reviewer for helpful suggestions. Recently, the 5’UTR variant we report here has been uploaded into the ClinVar database, and given an rs# (886043365) and Allele identifier
At the moment, there is no further information to be found (ExAC or gnomAD). Minor allele frequencies (MAFs) in different populations and associations with clinically relevant phenotypes are not currently available. We have revised our conclusions on the effect of the 5’UTR variant accordingly (page 10, lines 16-18).

2. I appreciate the authors' noting the presence of polymorphic variants within the 5’ UTR region discussed. I'd also direct the authors to review the variants identified in this region in the ExAC (http://exac.broadinstitute.org/) and gnomAD (http://gnomad.broadinstitute.org/) browsers, which include population data with much higher allele counts than the resources noted on page 10, lines 5-7, and consider incorporating that population data into their discussion.

- See response to point #1.

3. In looking at the conservation of the region where the insertion occurs, I found myself disagreeing with the authors' assertion that the region was "absolutely conserved from humans to rodents" (page 10 line 8). Per the UCSC genome browser, which calculates conservation using PhyloP, conservation appeared poor at a few nucleotide positions in the region of interest, appearing very poor at c.-5 even among apes and rodents. I'd ask the authors to soften their language with respect to the conservation of this region.

- We agree with the Reviewer. We have characterized the phylogenetic conservation of flanking sequence as “limited” (page 10, lines 5-8).

All the corrections were highlighted in yellow in order to facilitate the revision process. We are grateful for the consideration of our manuscript and we hope to have addressed all the concerns raised by the Reviewers into this revised manuscript.