Peer Review File

Article information: https://dx.doi.org/10.21037/tp-23-60

Reviewer A

Comment 1: The authors present interesting data that contributes to the literature regarding the possible association of nuchal-type fibroma with Gardner syndrome (or at least polyposis syndromes) and possible underlying genetic mechanisms. I think the key findings were germline mutations in POLD1 or APC in 3 patients with nuchal-type fibromas. This suggests a possible link between nuchal fibroma and polyposis syndromes. I would make this the focus of the paper. I'm not sure the 2 patients with DT add to the literature.

Reply 1: We very much acknowledge that you consider our study interesting. We have included your suggestions that have contributed to improve our manuscript and to clarify its interpretation.

Comment 2: Line 24 is rather generic. I'm not sure I agree that (all) fibromas are precursors to DT. I would give more intro with literature support to the possible relationship between nuchal-type fibromas, Gardner fibromas, Gardner syndrome, and reports of nuchal-type fibromas recurring as DT (eg. Diwan et al The American Journal of Surgical Pathology 24(11): 1563–1567, 2000 or Kostakis et al in vivo 34: 2217-2223 (2020) doi:10.21873/invivo.12032). I am interested in more detail regarding patient #4 who is said to have recurred as DT after presenting as nuchal-type (same site? time to recurrence?).

Reply 2: Thank you very much for pointing this issue. We agree with your comment and we have included in the manuscript the papers you have suggested to further define the concepts of nuchal-type fibroma, DT and their association with Gardner syndrome. See page 1 lines 24-25 and pages 2-3, lines 53-74.

In the case of patient #4, after a partial surgical resection of the lesion (nuchal-type fibroma) in the cervical area, she had a local relapse as DT at two years. It is described in page 6, lines 174-176.

Comment 3: The authors state in line 80 and 81 that they were studying "APC germline mutations in pediatric patients diagnosed with DTs in a Gardner syndrome context". This is confusing since 3 of the patients have nuchal-type fibroma not DT. And since Gardner syndrome is a variant of FAP it would be relevant to know if these patients had colorectal polyps or not. Or at least were they scoped after the described mutations were found?

Reply 3: Thank you for this observation. We have changed this paragraph to clarify the objective of this study. See page 4, lines 108-110.

All patients in whom genetic alterations have been identified are being followed up by the gastroenterologist. According to current ESPGHAN recommendations, colonic surveillance should begin between the ages 12 to 14 years. However, colonoscopy should be performed at any age in the event of rectal bleeding or mucous discharge (Hyer *et al.* J Pediatr Gastroenterol Nutr. 2019). At present time, in patients in whom colonoscopy has been performed, no colorectal polyps have been observed. We have added this information in the manuscript. See page 8, lines 233-240.

Comment 4: Again, I think you have interesting data here that adds to a small literature on the possible relationship between nuchal-type fibroma, DT, and Gardner syndrome. I think you could make this case more concise and clear.

Reply 4: We very much acknowledge your recommendations. We have clarified the relationship between nuchal-type fibroma, DT and Gardner syndrome. We have modified the lines 244-247 in page 8 and page 20, table 2.

Reviewer B

Comment 1: In it, the authors describe the germline mutations in a series of 5 children with either desmoid tumors or nuchal type fibromas. Overall, I found the manuscript well organized and thoughtful as well as contributory to our current understanding of the germline landscape in these pathologic entities. Moreover, the manuscript rightly suggests from this very limited case series that attention should be paid to other germline abnormalities in the case that APC mut testing is negative. I think a few minor revisions will help strengthen the paper and a comment and optional possible major revision if the data is available.

Reply 1: Thank you very much for considering our manuscript well organized and thoughtful. We have included your comments in our manuscript that have helped to make it more complete.

Comment 2: The authors include nuchal fibromas with desmoid tumors. I think a clear discussion and distinction of the putative difference of nuchal fibromas and desmoid tumor is important in the discussion. What is the relationship? Are they distinct pathologic entities?

Reply 2: Thank you very much for pointing this issue. We have included more information about desmoid tumors and fibromas in the introduction. See pages 2-3 and lines 53-74.

Nuchal-type fibromas and DTs are distinct pathologic entities (Soft tissue and bone tumours. 5th ed. WHO 2020). However, both types of pathologies can be appear in pediatric age and may be associated with a Gardner syndrome. Some of

Gardner-associated fibromas relapses can appear as DTs and they can be precursor lesions to the DTs. These lesions occur before the development of intestinal polyps. It is important, therefore, to identify Gardner syndrome in early ages to prevent colorectal cancer. We have added this information in the discussion. See page 8-9, lines 261-276.

Comment 3: The authors spend a long paragraph discussion treatment options, trials etc in DTs. This is really out of place, not helpful, nor complete and does not contribute to the strength of the manuscript overall.

Reply 3: Thank you for your recommendation. We have modified and shortened this paragraph. See page 11, lines 339-344.

Comment 4: Optional- Do the authors have sequencing data on the actual tumors to pair with the germline results. For example- how many had CTNNB1 somatic mutations? This would really strengthen the findings and impact, but may not be feasible.

Reply 4: Thank you very much for pointing this. We agree with your suggestion. Indeed, it would be very interesting to have the data of *CTNNB1* gene in somatic tumor samples but this has not been performed because we do not have available DNA from tumor tissues.

Reviewer C

Recommendation: Recommend publishing with minor revisions.

Comment 1: Alba-Pavón et al report on germline genetic testing in pediatric patients with desmoid tumors. While germline pathogenic variants in APC are a well-known hereditary risk factor for desmoid tumors, it has been suggested that there may be other hereditary cancer syndromes that contribute to development of this tumor type more rarely. The authors also provide a useful summary of other cases of desmoid tumors/fibromas reported in individuals with germline pathogenic variants in APC. This is a well-written written manuscript, with a strong introduction and appropriate level of detail. Below, we provide some suggestions to strengthen the manuscript.

Reply 1: We very much acknowledge that you consider our study well written and with an appropriate level of detail. We have incorporated your comments to improve our manuscript.

Minor:

Comment 2: Readers less familiar with familial adenomatous polyposis and other

hereditary cancer predisposition syndromes might benefit from slightly more background information, including the incidence of hereditary cancer predisposition syndromes (and/or FAP specifically) in patients with desmoid tumors, as well as information about the lifetime risk of developing desmoid tumors in those with FAP. Also suggest adding to introduction that there are surveillance/prophylactic surgical options to help decease the risk of colorectal cancer (line 56), highlighting the importance of identifying this hereditary cancer predisposition syndrome in those with desmoid tumors as the presenting feature.

Reply 2: Thank you for your recommendations. We agree with your comment and we have completed this information and more details in the introduction, page 2, lines 53-54 and page 3, lines 79-81.

Comment 3: Please include a citation to support that 20% of all clinically diagnosed FAP patients do not exhibit any pathogenic variant in APC. With newer technologies (including ability to detect low-level mosaicism, for example), I believe this number may be lower in more recent publications.

Reply 3: Thank you for pointing this. We have modified this sentence and have included that some patients with FAP have mosaic *APC* variants, which is estimated to be underdiagnosed. See page 3, lines 93-96.

Comment 4: Suggest adding "pathogenic" (or "likely pathogenic") or "VUS" where appropriate-rather than just using the term "variant"-for example, in the captions for Figure 1B and 2 and in line 143.

Reply 4: Thank you for your observation. We have included the classification of the variants where appropriate. See page 17, Figure 1B; page 18 Figure 2; page 6, lines 172 and 194; page 7, line 208.

Comment 5: It would strengthen the evidence presented in lines 154-157 to note that other frameshift variants in this region have been reported as pathogenic.

Reply 5: Thank you for pointing this. We have added more evidences about other frameshift variants in this region in page 6, lines 190-192.

Comment 6: In reference to Table 2 data (lines 200-201), suggest noting in text that the incidence of these findings is limited by outcome data. For example, all patients would be expected to develop colon polyposis if long-term outcomes were available.

Reply 6: We agree with this comment and we have added this information in the page 8, lines 249-251.

Comment 7: In the section about POLD1 germline variants, the ClinVar classification is mentioned for the second variant but not the first. The first variant is classified by many labs in ClinVar as benign/likely benign (and as VUS by some). Suggest adding this information to the paragraph about c.2275G>A and adjusting manuscript accordingly. Specifically, the conclusion that development of desmoid tumors may potentially being associated with other (non-APC) hereditary predisposition syndromes is not necessarily supported by the results, particularly as one of the variants has been reported by numerous labs as benign/likely benign in ClinVar.

Reply 7: Thank you very much for this observation. We have included the ClinVar classification of the *POLD1* c.2275G>A variant in page 7, lines 213-215. In addition, Rosner and colleagues have described this variant in Ashkenazi Jews individuals and they has been proposed as a low-to-moderate risk variant. See page 10, lines 306-312.

Comment 8: There are several typos throughout the manuscript (including lines 45 "around the 40 years", lines 100, 195, 203 and 212 "MUTHY", lines 173 and 187 "patient had not family history", and in Supplementary table S1 there are a few instances where benign is spelled "Bening", and Table 2 "tyroid").

Reply 8: Thank you for this observation. We have corrected these typos in page 2, line 46; page 4, line 130; page 7, lines 209 and 225; page 8, lines 242 and 253; page 9, lines 278.

Comment 9: Suggest consistency between "mutation" versus pathogenic variant" terminology, with pathogenic variant being preferred.

Reply 9: We acknowledge your recommendation. We have unified this terminology along the manuscript.

Comment 10: If available, in Figure 1 it would be helpful to add whether family history included colon polyps vs colon polyposis, as the former are very common while polyposis in particular is suspicious for FAP

Reply 10: Thank you for pointing this. This information is available and we have included it in the Figure 1, page 17.

Reviewer D

Comments 20.03-23 Astrid Stormorken
Review Analysis of germline variants in pediatric patients diagnosed with…
1…with desmoid tumors
25…associated with

30…in the APC
31…nucheal type fibromas
34…and genetic
45…peaks of….around 40 years
48…three groups located intra-abdominal
50…and can arise in many patients in the context of
56…predispose to
58…in the APC gene
62…Soft-tissue manifestations in early age may
65…and codes for
68…All these facts occur in
73…associated with
100…MUTYH
101…genetic tests were performed
117…Varsome and LOVD
118…pathogenicity prediction tools
128…fibromas…fibromas also had
130…was 11.4 years (range:
135…The pedigrees of patients with a family history are shown..
137…Tumors progressed
141…in the germline
145…underwent partial excision of the lesion
147…low dose chemotherapy
149…tumor progressed after
150…without response, so oral
154…in the APC
155…in a change of the reading frame in residue 1538
156…in residue 1565
159…An APC
160…a cervical DT in childhood
165…Dts treated with radiotherapy
166…The APC
169…Two variants of uncertain significance (VUS)
172…A POLD c…was found in patient 1
173…had no family
174…He had a…The lipoma was
177,,,The POLD1
178…MAF explain
179…Jews with
180…other extracolonic
182…does not cause structural damage
186…with a nuchal
187…had no family…The POLD1
189…The POLD1

195…MUTYH
198…pathogenic germline variant
199…had a family history of tumors
200…two patients
201…in the APC
203…No pathogenic germline variants in MUTYH…have been described in patients
208…tissue lesions…fibromas
209…in childhood
212…MUTYH
214…germline variants in…fibromas
219…associated with…DTS and Gardner fibromas
221…the APC…in the germline
222…with a Gardner…did not respond to treatment
223…and target
230…in the germline
234…VUSes in the germline of two…POLD1
238…has previously been
240…a POLD1
241…a family
243…The POLD1 c….has been proposed to be
247…VUSes
250…a family
252…or have a
254…have been
255…associated with
260…VUSes
263…to identify
264…characterize…associated with
266…We have shown…associated with
266-268…a little unclear rewrite
270…The two patients…still have neoplastic lesions
271…that do not
273…effective treatment for
274…inhibitors for patients
278…In the
279…trial disease control
280…12 months has been demonstrated in
282…impact of cryoablation
283…not respond to
288…the risk of
291…for patients with progressing tumors

REFERENCES: you have to check the whole list thoroughly! Example: ref no 10 and

21 is the same. Then adjust the numbers accordingly
Figure 1: Pedigrees of patients
Table 2: references? Some information on fibroma 3 is missing
Table S1: check spelling benign has become bening 3 places

Reply 1: Thank you very much for all your comments. We have corrected them in the manuscript in the corresponding lines, figure 1 and tables 2 and S1.