Peer Review File

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Reviewer A

This case report is about very early onset CRC in a 24 years old male. Family history of late-onset CRC in paternal grandparents. Routine genetic investigation of gene panel negative. WGS report a germline duplication on chrom1, similar to what was published before as a probable cause of inherited CRC. This duplication was inherited from the maternal grandfather and there was no history of CRC. Conclusion was that this excluded a high risk because of this mutation. Besides was also reported a VUS in CHECK2.

I think this was an interesting case. Conclusion reasonable still since there was no family history associated with it.

Reply from the authors: We thank Reviewer A for having read our manuscript and providing valuable comments for improvement.

However, I am curious on other candidate variants found in the patient? If WGS was done there are numerous deleterious variants found in any person and also those are of interest and could perhaps be included and discussed? In particular if they were not inherited from any of the parents. Any new gene variant is a strong candidate to cause this early CRC.

Reply from the authors: We have gone through the WGS data from the proband again and identified 11 variants using a gene panel of 390 known or suspected cancer predisposing genes and an allele frequency below 1%. Besides the already mentioned *CHEK2* variant, we observed a relatively rare *MSH2* synonymous variant, which based on in silico analysis has no effect on splicing and are classified as likely benign 11 times in ClinVar. However, we observed this variant in homozygote form. All other finding are regarded as being too common based on allele frequency or not relevant for CRC. We have now included the gene list as Table 1 in the manuscript, and discussed the findings (page 5, line 105−113). We are unfortunately unable to examine if any variants were *de novo* since the parents only agreed to data analysis of the chromosome 1 duplication (page 5, line 117−119).

Reviewer B

General comments

The current case report demonstrated a colorectal cancer (CRC) case with young onset (24 years old). The authors analyzed genetic background and speculated the causality. They detected a duplication of chromosome 1 (g.117487504_117687735dup) and a CHEK2 (c.1522C>G, p.Leu508Val). Both variants are, so far, evaluated to be VUSs. They quoted the previous report demonstrating the similar duplication of chromosome 1 detected in the another family with colorectal cancer, suggesting the pathogenicity of the duplication.

To the reviewer, the speculation is interesting, however, it seems to be too weak to draw the conclusion on the cause of CRC with young onset.

Reply from the authors: We thank Reviewer B for having read our manuscript and providing valuable comments for improvement.
Regarding the conclusion we have tried to address causality from different angles. One is the duplication which fell on account on the segregation analysis, whereas the polygenic risk score did not find a specifically high PRS for our patient. We have tried to draw a conclusion based on this: we have not found a monogenic cause of this patient's cancer, but we cannot rule out that both the duplication and PRS play a role in this specific patients risk (in unison with environmental factors, life style factors etc.) (page 6, line 134–147, page 7, line 148–157).

Exact comments
The point is if the duplication of chromosome 1 recognized in the current CRC patient is pathogenic and a cause of development of the CRC.

1) The only the positive information suggesting the pathogenicity of this duplication is the previous literature [9].

Reply from the authors: We agree – the publication by Franch-Expósito et al. was the initial cause of our interest and why the finding was reported.

2) The authors should present with functional assay of the variant (Chr. 1 duplication).

Reply from the authors: It is unclear to us what the reviewer is thinking of besides transcript expression? The germline duplication on chromosome 1 is spanning 200 kb and includes the *CD101, TTF2, MIR942,* and *TRIM45* genes, as well as parts of the *PTGFRN* and *VTCN1* genes. By WGS analysis the duplication was shown to be located in tandem (see Figure 1).

3) In line 95-95, the authors described the duplication was covering the genetic site including TTF2, MIR942, and TRIM45. So, how was the expression level of these genes in the current CRC? Were these up-regulated?

Reply from the authors: This is a very interesting point. However, we do not have access to CRC tissue from the proband and are therefore unable to examine the expression levels of *TTF2, MIR942* and *TRIM45*. Interestingly, this was examined in the publication by Franch-Expósito et al. and they show that *TTF2* and *MIR942* transcripts were overexpressed, while no significant change was observed for *TRIM45* (described on page 6, line 141–145). Whether this is the case in our carriers is unknown.

4) Fig. 1A: Family tree and the segregation analysis did not support much on the speculation.

Reply from the authors: We do not completely agree with this comment. Had the duplication been inherited from the patient's maternal grandmother and from her mother (the patient's

maternal great grandmother) we would say that the variant segregate with disease in the family – as these have had a tubular adenoma with high grade dysplasia and colon cancer aged 67 years, respectively. This is why the family pedigree and segregation analysis are included. However, we agree that would only have been able to say; that the duplication segregate with disease (had it done so) and not been able to say anything about penetrance etc.

5) L111-112: The normal range of polygenic risk score does not support much on the speculation.

Reply from the authors: We agree – the patient falls completely within the average range. However, we think it is an important finding to report since PRS have been found to predict the risk of CRC in patients/family members. In case the PRS had come out with a high score, we would have had another potential explanation of the patients very early onset colon cancer.