

January 24, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1599 For Revision).

Title: Aggressive Juvenile Polyposis in Children with Chromosome 10q23 Deletions

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 1599

The manuscript has been improved according to the suggestions of reviewers:

They must suggest how to performed the post-colectomy endoscopic surveillance.

This was addressed as we suggest yearly endoscopic surveillance to include both upper and lower endoscopy.

They must explain more about the type of surgery (open or laparoscopic) and associated complications.

Further details about the surgery (laparoscopic) and complications (excessive stool) were described.

In the genetic testing flowchart, they need to explain the "OFC" abbreviation.

OFC= occipital-frontal circumference

-Paragraph 2 begins with saying there are 3 genes that cause JP, which is fairly controversial. Authors in the past have been careful to distinguish between JP and Cowdens syndrome when discussing PTEN germline mutations, while the current study says that this is one of the 3 genes "associated" with JP. This is misleading if there is no effort made to distinguish between the genes known to predispose to JP (SMAD4 and BMPR1A) and one that may also be lost with BMPR1A in a subset of patients who may have features of BRR and JP (PTEN). Those patients with overlapping deletions of PTEN and BMPR1A are a special group of JP patients, as the paper implies.

This was addressed with slight changes for clarification. There are three genes that can cause juvenile polyps in general, not necessarily juvenile polyposis syndrome (JPS). This was clarified with the changes as seen in the manuscript, describing the genes that may be responsible for juvenile polyps both with JPS and with PTEN hamartoma tumor syndrome including Bannayan-Riley Ruvalcaba and Cowdens syndrome.

-Paragraph 3 should specify that the chromosome microarrays are CGH arrays.

At many facilities, chromosome microarray can be done by either CGH chip or SNP chip. Both kinds of chips can detect copy number variants. We happened to use CGH chip to do this patient. If this were done by SNP chip or CGH+SNP combo chip, it shouldn't be called CGH arrays. We therefore elected to leave the term chromosome microarray to include both CGH or SNP.

-In Discussion paragraph 3, the authors state that "Clearly" the patient's medical issues are likely due to the 10q22-23 deletion; how can they know that this is the case, especially when the

patient also harbors a large deletion on chromosome 1p31?

I changed several words to address this as seen in the manuscript. I took out the word 'clearly' as this is debatable. We still believe it is very highly likely that his gastrointestinal problems (polyposis and colectomy) are related to his chromosome 10q23 deletion but hopefully changing a few words makes this more acceptable to all.

-The algorithm developed is interesting, and makes sense for those with macrocephaly on the right side of the figure (although "microarray" should be further specified as CGH microarray). On the left side, the recommendation to do BMPR1A sequencing then deletion/duplication analysis (prior to SMAD4) in those without symptoms of HHT is not something that is likely to be done. It does serve to highlight the known fact that the subset of JP patients with HHT signs will likely have SMAD4 mutations, but should not change the current practice of sequencing both genes in those without, then doing deletion/duplication analysis if sequencing is negative (because such a small subset has these changes).

We have elected to leave the algorithm as is. There are several potential ways to do genetic testing for juvenile polyps. In the past the two genes (SMAD4 and BMPR1A) were likely sequenced and if normal, they were tested for deletion/duplication (taken together, about 55% of JPS patients will have a change found in one of the two genes: a sequence change in one of the two genes in 40-45% and a del/dup change in 10-15%. Commercial labs may test by sequencing both and then doing del/dup on both, or they may do del/dup and sequencing of SMAD4 and del dup of BMPR1A first and then if normal, sequencing of BMPR1A. However, the proposed algorithm gives direction on testing based on clinical findings such as nosebleeds, telangiectasia and macrocephaly and is a logical, economical step by step method for genetic testing in the case of juvenile polyps.

-Page 8-9, paragraph about the algorithm (page 8 line 23 and further)

The algorithm should also include SMAD4 immunohistochemistry on juvenile polyps from JPS patients. It has been shown that loss of SMAD4 expression in juvenile polyps strongly correlates with an underlying SMAD4 germline mutation and immunohistochemistry can in this way guide the choice of subsequent genetic testing; i.e. start with analysis of SMAD4 gene if loss of SMAD4 expression is found (Reference: SMAD4 immunohistochemistry reflects genetic status in juvenile polyposis syndrome. Langeveld D et al, Clin Cancer Res. 2010 Aug 15;16(16):4126-34.)

I added a sentence about SMAD4 immunohistochemistry to the manuscript. Since this is not routinely done in most labs I did not add it to the algorithm but felt it was important to include and cite the paper.

-The final paragraph states that pts. with these deletions harbor a greater risk of cancer than those with either mutation alone; this may indeed be the case, but the small numbers of cases make it difficult to be sure.

We agree there are fairly small numbers but believe the numbers are significant enough to provoke cancer surveillance in these children.

-Page 4, line 15-18:

"Many of these patients were originally tested by chromosome analysis or more recently by chromosome microarray due to congenital anomalies, macrocephaly and/or developmental delay, but who also developed aggressive juvenile polyposis and in some cases required colectomy." This sentence is not clear, please revise.

Sentence revised for clarity as follows "Many of these patients were originally tested by chromosome analysis or more recently by chromosome microarray due to congenital anomalies, macrocephaly and/or developmental delay. Many of them also developed aggressive juvenile polyposis and in some cases required colectomy."

-Page 8, line 10-12

Please add " and high recurrence rates of polyps in the remnant rectum and the pouch" to the following sentence:

"Additionally, post-colectomy endoscopic surveillance is also warranted by the presence of upper intestinal polyps in a majority of reported cases."

This phrase was added as requested.

-Page 10 and 12

Reference 8 and 27 are the same.

Fixed.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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