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Column: Retrospective Study

Title: The Role of Telomere Shortening in Anticipation of Inflammatory Bowel Disease

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Dear Editors,

Thank you for reviewing our manuscript and for the reviewers' comments concerning our manuscript entitled "The Role of Telomere Shortening in Genetic Anticipation of Inflammatory Bowel Disease" (Manuscript NO.: 55669). All the comments were valuable and helpful for revising and improving our paper. We have revised the manuscript according to the comments and suggestions. Please see the point by point responses to the comments as listed below.
We hope that our revision will be approved.
Thank you again for considering our manuscript for publication in the World Journal of Gastrointestinal Pharmacology and Therapeutics.

Reviewer #1: 02941507

Reviewer Comment:

According to the following publication [Lee JC, Bridger S, McGregor C, Macpherson AJ, Jones JE. Why children with inflammatory bowel disease are diagnosed at a younger age than their affected parent. Gut. 1999; 44:808-11. doi: 10.1136/gut.44.6.808. PMID: 10323881; PMCID: PMC1727524], "There was no evidence of genetic anticipation or genomic imprinting of age at diagnosis in this sample of IBD families. Ascertainment bias is responsible for the age differences at diagnosis between affected parents and children".

Another publication [Faybush EM, Blanchard JF, Rawsthorne P, Bernstein CN. Generational differences in the age at diagnosis with Ibd: genetic anticipation, bias, or temporal effects. Am J Gastroenterol. 2002;97(3):636–640. doi:10.1111/j.1572-02 41.2002.05542.x] also suggested that "There is a tendency for children to be younger than their parents at the time of diagnosis of familial IBD, and that this difference in mean age at diagnosis is almost doubled for grandparent/grandchild pairs. However, we conclude that these differences are most likely due to a bias based on length of follow-up or recent multigenerational temporal changes in the risk of IBD, or both".

Author response:

As the editor corrected noted, genetic anticipation in IBD was questioned by many scientists through the years. It has been suggested that anticipation is the result of ascertainment bias. However, recently, a Bayesian method (corrects for random effects, isolating the confounding effect of changes in secular trends, screening and medical practices, and adjusts for changes in age-specific incidence across birth cohorts), confirmed anticipation among successive generations of Lynch Syndrome families, which likewise had controversy regarding genetic anticipation. It is therefore possible that genetic anticipation in IBD may be demonstrated using an optimal study design and statistical methods and/or evidence of a molecular mechanism.
Please also see section "Discussion", paragraph 1, line 12.

Reviewer Comment:

The authors also stated that "The study represents the first evaluation of telomere length defects in IBD". However, there were actually relevant data published previously [Risques RA, Lai LA, Brentnall TA, Li L, Feng Z, Gallaher J, Mandelson MT, Potter JD, Bronner MP, Rabinovitch PS. Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. Gastroenterology. 2008; 135:410-8. doi: 10.1053/j.gastro.2008.04.008.. PMID: 18519043; PMCID: PMC2574910].

Author response:

We were referring to the first evaluation of telomere length defect in anticipation. We corrected our sentence and clarify "The present study represents the first evaluation of the role of telomere length in genetic anticipation of IBD."
Please see correction made in section "Discussion", paragraph "Strengths and limitations", line 1.
We also made reader aware of your comments regarding the prior research on telomere length in ulcerative colitis.
Please see section "Introduction", paragraph 5, line 5; added reference number 26-28.

Reviewer #2: 03478404

Reviewer Comments:

1. The authors of this limited study concluded that "telomere shortening appeared unlikely to be involved in mechanisms of possible genetic anticipation in IBD". However, they included only three IBD families, 24 patients of European descent (eight parent-child pairs affected by IBD).

Author response:

We recognized the limitation of the study in the paragraph "Discussion". However, we did choose the most likely cases to seek evidence of a molecular basis for genetic anticipation

(multigenerational, large age of onset delta 16.5 years on average) in this proof of concept study.
Please see section "Discussion", paragraph "Strengths and limitations", line 6.

ABSTRACT

Reviewer Comment:

2. Abstract: It should clearly state that only 11 individuals with IBD were included, of the 24 subjects.

Author response:

This is now clearly stated in the abstract (section "Results", line 1 and 2), in the main manuscript body (section "Results", first paragraph, line 2) and in the table title.

Reviewer Comment (Core tip):

3. Core Tip: too long – it should have a maximum of 100 words.

Author response:

Core Tip was shortened to meet the maximum of 100 words requirement. It now reads as follows:
"This is a retrospective study to evaluate the role of telomere shortening in genetic anticipation of inflammatory bowel disease (IBD). Genetic anticipation is a long disputed concept in IBD, and lacks an explanatory mechanism. We analyzed generational changes in telomere length of eight parent-child pairs of three generation IBD families with anticipation and performed whole exome sequencing to identify genetic variants for autosomal inheritance. Neither telomere shortening or autosomal inheritance was associated with anticipation in our three generation IBD families, suggesting other potential mechanisms underlie this phenomenon." [87 words]

INTRODUCTION

Reviewer comment:

4. Introduction: background well presented; length appropriate.

A. Page 4 – „which has an increased risk of colon cancer" - please insert that the overall risk of cancer is increased, not only colon cancer.

Author response:

The correction has been made, so that the revised text now reads "increased risk of malignancies such as IBD (which has an increased risk of cancer)."
Please see section "Introduction", paragraph 6, line 3.

Reviewer comment:

B. Please formulate clearly the „Aim of the study" (or, preferably, primary and secondary objectives).

Author response:

We revised our paragraph as suggested, and now present our primary and secondary objectives. Please see section "Introduction", paragraph 7, line 1 and 3.

MATERIAL AND METHODS

Reviewer comment:

5. Material and Methods:

A. When was this study carried out? The authors wrote "beginning in 1990", but IRB Initial Approval Date appears as 5/18/2010. When did it end?

Author response:

This was a retrospective study using data and specimens already collected as part of Johns Hopkins IBD family study. IRB Initial Approval Date was 1996. The study was registered with clinical trials in 2010. Please see the correct IRB approval uploaded.
The introductory sentence of the paragraph "methods" refers to the year (1990) when these patients started receiving care at Johns Hopkins IBD Center. The genetic analysis and electronic record review were performed in 2019. We have re-arranged our text and included additional information to bring clarity to the study design. Please see section "Material and Methods", paragraph 1 "Study design".

Reviewer comment:

B. Study families: Please replace the term of "indeterminate colitis". It has been replaced many years ago by the term "IBD Unclassified" (IBD-U).

Author response:

We changed the term "indeterminate colitis" to IBD-U throughout the text.
Please see sections "Material and Methods", paragraph 1, line 3 and paragraph 4, line 8; section "Results", paragraph 1, line 4 and line 7; Table 1, row 5 and row 11.

Reviewer comment:

C. *Please specify what criteria/guidelines were used for the diagnosis of CD, UC and IBDU in these three families. Since 1990, many guidelines changed, according to the more recent scientific findings.*

Author response:

We use the NIDDK IBD National Genetic Consortium phenotype operating manual (version May 10, 2006) to classify the disease as Crohn's, ulcerative colitis and IBD-

unclassified. These criteria include clinical presentation, endoscopic, histologic and radiographic findings (mainly upper gastrointestinal study, barium enema and computer tomography) https://repository.niddk.nih.gov/media/studies/ibd/ibd_phenotyping-manual.pdf.
Please see section "Material and Methods", paragraph 4, line 5 through 9.

D. No MRE was performed at all?

Author response:

MRE was not performed on any of the patients included in the study.

Reviewer comment:

E. *This paragraph "Material and Methods" has only two sub-paragraphs: "Study families" and "statistical analysis". "Study families" should be divided in study design (maybe including the questionnaire – even as supplementary material), families, IBD diagnosis, genetic analysis etc.*

Author response:

We re-organized the "materials and methods" which originally had only two subheadings. It now is more clearly divided with five sub-headings: study families (with sub-categories for study design, IBD diagnosis, and genetic analysis) and statistical analysis.
We included the form we used to collected phenotype information as supplementary material.
Please see section "Material and Methods".

RESULTS

Reviewer comment:

A. Clinical characteristics of patient population: Please replace the old term of IC with IBDU. Again – Importance of the criteria used to classify/diagnose CD, UC and IBDU. Please mention.

Author response

We changed the term "IC" to "IBD-U". We offered a description of how the criteria were used to make the diagnosis and include a link in the section "Material and methods" also.

Reviewer comment:

B. Please replace subtitle" Children were younger at diagnosis than their parents" with „Children's age at diagnosis".

Author response:

We replaced the subtitle "Children were younger at diagnosis than their parents" with "Children's age at diagnosis".

Please see note changes in section "Results", paragraph 2, line 1.

Reviewer comment:

C. The following sentence is incomplete; „The mean age of parents at diagnosis was 40+/-17 and the mean age 21+/-13, p<.0014."

Author response:

We completed the sentence "The mean age of parents at diagnosis was 40+/-17 and the mean age *of children at diagnosis* was 21+/-13, p<.0014."
Please see paragraph 2, line 3 and line 4.

Reviewer comment:

D. Comparison of disease extent in parent-child pairs: Data are presented in Table 1. However, we cannot get anything from it, since only old classical therapies were used.

Author response:

Table 1 describes patient characteristics: 1) age at diagnosis 2) the relationship between the affected members, 2) age at disease diagnosis, 3) smoking history 4) presence of NOD2 mutation 4) severity and extent of the disease. Patients included in our study were treated before the era of biologics.
Please also note section "Discussion", paragraph 4, line 3 through 9.

Reviewer comment:

E. Table 1: Patient Characteristics: please remove IC and replace with IBDU. Most patients were treated with steroids and/or 5ASA. Only 3 received thiopurines. No other medication? What about Metotrexate (in Crohn's), Biologics? When were these patients treated? As per the current guidelines, 5ASA is generally not effective in CD, only in very selected patients with very mild inflammation. Those mentioned patients (Table 1) had complicated behaviour: B2 or B3. Why 5ASA then?

Author response:

There are many comments included in E, so we address them now in four groups.

(i) Terminology
We changed the term "IBD Unclassified" to "IBD-U".

(ii) Medications
We selected those families where all three affected generations had access to similar therapy to avoid the confounding factor of different class of therapy on severity of the disease. Therefore, most of the patients were treated with mesalamine, thiopurine and steroids. None of the patients were treated with methotrexate. The patients were treated before biologics were commonly used as part of the IBD therapy (explanation inserted in paragraph "Study design")

(iii) Time of treatment: In the late 1990's.

(iv) 5ASA: Before the top down therapy 5ASA where widely used in most of the IBD patients, steroids and thiopurine been the only medical therapy available before biologics were introduced in the treatment of IBD.

Reviewer comment:

F. Family with UC: The text mentions that "All the affected members of second family underwent total colectomy for disease refractory to medical therapy with the youngest member requiring surgery at only 6 years of age." But, what therapy? Only steroids, 5ASA and thiopurine are mentioned in Table 1(thiopurine in two patients). I would have liked a Discussion about this family, with such a severe UC, however since no proper therapy was administered, what to expect? These are probably very old data therefore we cannot apply them to our daily basis, with so many possibilities of biologic agents used as therapy.

Author response:

With the goal of minimizing the effect of different and newer therapies on disease severity, all the study patients were selected if they had access to similar therapeutic options (e.g. classic therapy). In order to capture three generations, we had to go back to the 1990's when access to biologics was limited. For the purposes of this study, this recruitment approach did not interfere with the validity of the results. Since population genetic has a temporal stability, our results, independent of the time of the recruitment and/or therapy, remain valid today.

Reviewer comment:

G. Testing TL: "No associations were found between TL in lymphocytes and granulocytes and anticipation of the age at onset observed in successive generations". Not useful study for practice.

Author response:

Our study was an exploration of one possible molecular mechanism of genetic anticipation in IBD. While we have shown that genetic anticipation is unlikely to be the result of telomere shortening (and thus did not yield a finding of immediate clinical applicability), our results does point the way to exploration of other mechanisms.

Reviewer comment:

H. Genetic variants detected by WES: "WES did not detect any rare frameshift, nonsense or missense coding variants in genes shared by these three families for either dominant or recessive modes of inheritance." Not bringing any info that should be important.

Author response:

We explored the possibility that these IBD families with Mendelian pattern of inheritance represent a monogenic form of IBD, similar to VEOIBD. The monogenic disorders, e.g. Huntington disease, have clear evidence of genetic anticipation and a well-established molecular mechanism consistent with the trinucleotide repeats--a mechanism that would have been worth exploring in IBD if such.
Significant information can also be learned from these negative results including that IBD, even in these familial cases, remains a disease most likely characterized by a polygenetic susceptibility with microbial and environmental factors playing an important role.

DISCUSSION

Reviewer comment:

7. Discussion paragraph is extremely flimsy, almost unbelievable for a manuscript.

Main limitation: very few patients: 11 with IBD. Three families – 1 with CD, 2 with UC (and IBDU).

Author response:

The discussion is now more robust and includes consideration of the points raised in results comments E-H. Limitation of the study are also now addressed.
Please see the "Discussion" section.

REFERENCES

Reviewer comment:

8. References very old:

nothing from 2020, 2019, just 1 from 2018, none from 2018, 2017.

Most of the other references are quite old.

At the end of the used references, we still find 5 references (still very old), not included (numbered 1 to 5). Maybe the authors did not submit the last version of their paper.

Very superficial.

Author response:

We added one reference from 2018 (reference number 42) in addition to the existent one (reference number 30). We added four references from 2019 (reference number 20, 37, 38, 39) and two references from 2020 (reference number 3 and 25). The most recent reference on genetic anticipation in IBD that we are aware of is Faybush, *et al,* which has been cited (reference number 12). The five references at the end of the manuscript were excluded.

OTHER

Reviewer comment:

9. Copyright License Agreement – not signed and written. Why?

Author response:

The signed Copyright License Agreement is included with the revised version of the manuscript. When we submitted the initial version, the information on the PDF form did not upload.

Reviewer comment:

10. IRB Approval Date: Principal Investigator: Florin Stelaru – does not appear in the manuscript at all. Is this the same study?

Author response:

Dr. Florin Selaru's contribution to this study did not meet the journal's scientific criteria for authorship.

Reviewer comment:

11. Signed Informed Consent Form(s) or Document(s). The name of the PI - Theodore M Bayless does not appear in the manuscript at all. In the end of the paper, we learn that this study was "In Memoriam". The date of expiration on this signed consent form is 1997. We should be very careful with this study.

Author response:

Dr. Bayless was the founder of the family study. He was our mentor. He died in February 2019. In our institution, consent forms are valid for the life of a study, until the study expires, is terminated, or the consent form is revised (at which time the previous version of the consent form is invalid and the new document becomes the document to use for the life

of the study). The IRB stamp on the consent form simply indicates that this particular version of the consent can only be used for one year to enroll new subjects. Each year, the protocol is reviewed by the IRB and renewed, at which time a new stamp is placed for the consent to be used for another year. Once a consent is signed, the authorization to use the samples and data does not expire.

Reviewer comment:

12. The manuscript is not prepared according to the WJG rules. No ORCHID number of authors, no required format, etc.

Author response:

We apologize. The ORCHID numbers are now listed for all authors and the manuscript has been formatted according to journal requirements.

Sincerely,

Brindusa Truta, MD