ANSWERING REVIEWERS

March 14th, 2014.

Dear Editor,

Please find enclosed the edited manuscript in Word format, file name:7133 edited R1.

Title: PRSS1 and *SPINK1* mutations in idiopathic early onset chronic pancreatitis and idiopathic recurrent acute pancreatitis in Mexico City

Author: Mario Pelaez-Luna, Guillermo Robles-Diaz, Samuel Canizales-Quinteros, Maria T Tusié-Luna

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7133

The manuscript has been reivsed, modified and improved according to the suggestions of reviewers. We resubmit it for its reviwe and consideration for its publication in the World Journal of Gastroenterology

Sincerely yours,

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REVIEWER 1.

Your work has relevance because it brings new information about a population that has not yet been studied and describes the occurrence of new mutations. The study of patient's relatives contributes to understanding the impact of the occurrence of mutations and helps to investigate the presence of other cofactors that may have collaborated to the development of pancreatitis. You have obtained sufficient number of patients because the inclusion criteria were restricted to the defined characteristics of an uncommon disease. It allowed to select patients most likely to have these genetic mutations. Direct sequencing was used for the genetic study and is the most adequate technique for the proposed research. The presentation of the results and discussion is well prepared and allows you to reach the conclusions.

I would add a few suggestions that could contribute to presenting what is currently being studied. In the fourth paragraph of the Introduction you report that mutations of SPINK1 gene may have a role in the phenotypic presentation of the pancreatitis. I suggest you to describe some examples and include the reference.

1. **I suggest you to mention the N34S mutation in the sixth paragraph of the Introduction, because it is the most often found in the SPINK1 gene in patients with chronic pancreatitis.**

As advised by the reviewer, we added the N34S mutationin the introduction and reads as follows:

…SPINK1 needs the presence of other pancreatitis-associated factors in order to the individuals to express the disease, as seen with the p.N34S mutation one of the most commonly found but yet with no apparent functional effect…

2. In the 10th. paragraph of the Discussion you refer to the paper presented by Bernardino et al.
This was the only work that studied the SPINK1 and PRSS1 genes in Brazil. I would like to remind that our group published a study in 2009 (da Costa MZ, et al. Pancreatology), however it refers to another gene, the CFTR. For this reason I would suggest this sentence to be clear that you refer to SPINK1 and PRSS1 genes.
We modified the text as suggested, an in its current version it clearly states the genes that have been studied in Latin America (Brazil), thus we decided to add the Pancreatology 2009 reference by Da Cozta et al, in order to update and present a more accurate picture of the available information in Latin American countries. Now it reads:

Information of CP related mutations in Hispanic population is scarce; only two studies from Brazil have reported on chronic pancreatitis related mutations specially PRSS1, SPINK1 and CFTR in Latin America.

I congratulate you for choosing this subject and for the presentation of your work. The study of genetics in pancreatic diseases is crucial to better understand the pathogenesis of these diseases, but it still has been developed by few researchers.
We thank the reviewer for the encouraging words.!!!!!

Reviewer 2

ESPS Manuscript number: 7133 Cationic trypsinogen (PRSS1) and serine protease inhibitor Kazal 1 (SPINK1) mutations in subjects with idiopathic early onset chronic or idiopathic recurrent acute pancreatitis in Mexico City. Pelaez-Luna et al. In this manuscript 19 individuals with pancreatitis were examined for mutations in PRSS1 (exon 2 and 3) and SPINK1 (exon3).

1. The number of individuals tested is relatively small and genetic testing was restricted to 3 exons of two genes. Testing for CTRC, CPA1 and CFTR was not done.

We agree that this is a small sample. Prevalence of Chronic pancreatitis in our country calculated by a prior study from our group a (Pancreas 1990; 5:479-483) has been appraised in between 3 to 5 per 100, 000 inhabitants, with 66% of alcoholic origin and 29% idiopathic (no AIP or genes were studied at that time) and from these, in a small proportion the onset was before age 35.
We chose to study only those with early onset CP and idiopathic RAP before age 35 and sequenced only the exons where the most common mutations had been described, since we considered it is this specific group of patients that have a high pre test probability for finding mutations.

We are prospectively registering new cases. We have plans to retest and sequence the whole PRSS1, SPINK1, CTRC, CPA1 and CFTR in a larger population in the future.

2. The consequence of the newly identified mutations remains unclear.
No functional data were obtained for PRSS1 p.V39E (not p.V39A ?), p.N42S and SPINK1 p.V46D.
All three variants seem to be private variants detected in single individuals only.
In collaboration with other research groups, functional analyses should be performed for these 3 variants.

I believe, that the group of Prof Sahin-Tóth in Boston as well as the group of Claude Férec in Brest or even other groups might be willing to collaborate and to test these variants.

Functional data will greatly improves the conclusion of the manuscript.

We agree that functional studies would significantly improve the manuscript. However due to technical problems in the laboratory, we lost our samples. We are currently locating and recalling the participants to draw blood again, but has been difficult since most of them live far away and samples might have been obtained during their follow up visit, which will take a long time. Again, We are prospectively registering new cases. We have plans to retest and sequence the whole PRSS1, SPINK1, CTRC, CPA1 and CFTR in a larger population in the future

3. Replication of PCR in the SPINK1 p.V46D index case and paternity test in the parents might be done.

All sequences were run on the F and R directions twice. We considered and discussed the option and need to perform a paternity test at the time we found the new mutation, however this was not part of the study´s informed consent and we decided no to discuss such results with the parents due to potential ethical and perhaps family problems.

4. The main text as well as references might be shortened.
The manuscript is very long for what it has to say.

We edited, revised and rephrased the manuscript and the references, shortening its length.

Minor points:

1. **"early onset" pancreatitis: 4 affected subjects are older than 40 years. Early onset might not be really appropriate**.
   Probably the information in table 1 as it was presented was confusing. All patients presented pancreas related symptoms and were diagnosed with chronic pancreatitis or recurrent idiopathic acute pancreatitis before age 35. We have modified table 1 and corrected the method section in order to make clarify this confusion.
   Now, according to other reviewer's suggestions, table 1 includes information only from individuals carrying mutations.

2. **The figures in the table differ from the figures in the abstract (range 13-40 vs. range 15-48 years)**
   We corrected this mistake.

3. **Figures: Both can be omitted**. **The information given by pherograms is relatively low**.
   Figures were submitted in order to provide a clear example of how the N34S mutation does not produce disease by-itself and also represent the family tree of a new mutation. However, according to the reviewer

suggestion, we agree that for expert readers they provide limited information, thus we decided to eliminate them from the manuscript. Should the editor and other reviewers consider them necessary, we would resubmit them.

4.Table: The table should be restricted to the individuals with positive findings (1, 6, 8, 15, and 19).
We have modified the table, accordingly**.**

5. References: The manuscript has more references as individuals investigated. This should be an original paper not a review. References might be limited to the essential literature i.e. 20-30 references at max.

References are not unified in style (e.g. ref. 24) or cite wrong names (Audrézet ref. 26). Sharer (ref. 41) might be cited together with Cohn and colleagues earlier in the main body.

Some references cannot be correct: e.g. ref. 41 (Sharer 1998) does not deal with SPINK1 mutations, which were described two years later. Also Sibert (ref. 48) contains data of the pre-genetic era.
We have revised, corrected, edited and deleted some of the references in order to decrease the number, preserving those more recent and essential ones according to the reviewer suggestion.

REVIEWER 3.

Manuscript: ID:02548398 ESPS Manuscript NO: 7133 Title: Cationic trypsinogen (PRSS1) and serine protease inhibitor Kazal 1 (SPINK1) mutations in subjects with idiopathic early onset chronic or idiopathic recurrent acute pancreatitis in Mexico City. Authors: Mario Pelaez-Luna, Guillermo Robles-Diaz, Samuel Canizales-Quinteros, Maria T Tsuie-Luna Review: Summary: The authors present data of 19 patients with early onset chronic pancreatitis or idiopathic recurrent pancreatitis from Mexico in which sequencing of PRSS1 and SPINK1 was performed. Two new PRSS1 and 3 SPINK1 variants (one new) were found.

Major points:
1.The approach for the identification of chronic pancreatitis (CP) variants is not sufficient. Although, it might not change the picture dramatically I would suggest to sequence all exons of PRSS1, SPINK1, CTRC, CPA1 in these patients and in the 50 controls.
Maybe it will even be possible to investigate more controls.
We agree that preforming a full gene sequencing that includes all 4 genes is advisable and a better approach, we discuss this issue and highlight it as a limitation of our study.
Regrettably, due to technical problems in the lab, we lost our samples. We are currently locating and recalling the participants to draw blood again, but has been difficult since more of them live far away and we might be able to obtain

the new samples during their follow up visit; however we are actively and prospectively registering and recruiting new cases with the intention to sequence the whole PRSS1, SPINK1, CTRC, CPA1 and CFTR in a larger population.

2. **The data presented so far are interesting. Since, the PRSS1 variants are new, functional analyses of these variants might help to understand their role in CP better. The same might be true for the new SPINK1 variant. With this approach the paper could be improved from my point of view**.
We agree that functional studies would significantly improve the manuscript however as we commented previously, due to the lack of samples at present time we wont be able to run functional analyses. We are prospectively registering new cases. We have plans to retest and sequence the whole PRSS1, SPINK1, CTRC, CPA1 and CFTR in a larger population in the future and run functional analysis either here or through an international collaboration. We estimate that it will take as at least the whole year to complete this study.

3. In parts (Introduction, Discussion) it seems that the paper needs to be rewritten to make the points made clearer and to give it a red line.
We have revised, repharesed and edited both sections as advised, hoping the current version be sharper.

Minor points:
The references are quite a huge number, maybe a reduction is possible.
References have been revised, selected and decreased according to reviewers advise.

The number of patients is rather low to give a solid picture of the mutation distribution in Mexico, but the newly described variants make the manuscript interesting (if they have functional consequences).
How have the controls been recruited?
We chose to study only those with early onset CP and idiopathic recurrent acute pancreatitis which onset was before age 35, since we considered they have a high pre test probability for mutations. Also we decided to only sequence the exons where the most common mutations had been described to have a higher chance to find mutations and compare our population to what has been reported.
We just started a prospective study with the intention to sequence the whole PRSS1, SPINK1, CTRC, CPA1 and CFTR genes in a larger population.
Controls were selected from and obtained from stored serum of unrelated healthy individuals obtained randomly among blood donors.