Author's response to reviews

Title: Identification of novel mutation in cathepsin C gene causing Papillon-Lefevre Syndrome in Mexican patients.

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Author's response to reviews: see over
Dear Editor,

We received your letter, and carefully modified the manuscript to address the reviewers’ comments. Below you will find the point-by-point answers to the referees.

The novel loss-of-function mutation c.203T>G (dbSNP, rs199474831), and the enzymatic activity and gene expression level of CTSC in PLS patients are now discussed in more detail. These changes, together with the association of HLA-DRB1*11 with the disease, improved the clarity and relevance of the manuscript.

After these constructive revisions, we hope you will find the paper suitable for publication in your journal.

Faithfully yours,

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ANSWERS TO REFEREES

We thank the reviewers for a thorough and critical examination of our manuscript and deeply appreciate their constructive comments. The corrections were made, and now the manuscript is more focused and clear.

Thank you very much.

REFEREE 2:

Major Concern:
Cases 2P and 4P were found to be compound heterozygous for mutation 203 and 458 (T/G and C/T) and no other causal mutation has been identified in these cases?

Answer:
Sequencing included exons and splice junctions of the CTSC gene of all patients and their relatives, and only c.203T>G and c.458C>T were found. The 458 (T/G) mutation is defined as a polymorphism (symptomless mutation) in the previous literature (Ref 50 in this manuscript; Allende et al 2006; Human Mutation) and (Ref 49 in this manuscript; Lefèvre et al; 200; Journal of Investigative Dermatology). In these studies, this 458 (T/G) conservative mutation was reported as a polymorphism, along with other causal mutations in the affected individuals. Moreover, there were normal controls identified with this mutation in homozygous state and the mutation was
present in high frequency in normal population. Allende et al 2006 had thus suggested that not all mutations in Papillon-Lefèvre Syndrome should be considered as a cause of disease, whether case studies or general population screening is performed. The study by Hart et al 2000 (Ref 48 in this manuscript) was the only one that documented 458T>G variation as a causal mutation in heterozygous state with 199-222del. However, I am not sure about the control data, if any available for this mutation in study by Hart et al?

**Answer:**

We do not know how c.458C>T affects the protein function. However, in our study, this change correlated with a reduced enzymatic activity observed in patients and their relatives. In fact, c.458C>T genotypes’ frequencies in our population were 18.0% for C/T, and 82.0% for C/C. Moreover, p.Thr153Ile substitutions do not fall in a highly conserved residue, and PolyPhen-2 software predicted a benign mutation. However, Hart et al. reported this change as causal mutation in compound heterozygous PLS patients (Ref. 47 of the manuscript), as do we.

**Discretionary Revisions**

1= Study to determine founder effect for 203T>G mutation will add interest in this manuscript.

**Answer:**

As mentioned in discussion section, the founder effect can be determined by haplotype analysis of DNA markers in CTSC gene region. We are applying for grants to accomplish this analysis, but we believe that the information presented in the manuscript is relevant enough for publication.

**Minor Essential Revisions**

Check following lines/words

Page 3

1= Para 1: 2nd last line: “Among others conditions”
2= Para 2: line 1: more than 60 mutations have been reported; (there are 77 mutations reported so far according to hgmd)
3= Para 3: revision of sentences (CTSC, also known as -------to ----- monomers) as to be more clarified/simple for general readership
4= Para 3: “The enzyme has been involved on the cleavage”

Page 4

5= Para 2 (last line): “such severe periodontitis”
6= Para 3 (last line): mention as “of nine PLS patients”

Page 5

7= Cell isolation: “LPS”-free (full for abbreviation once)

**Answer:**

These seven comments were corrected in the manuscript as suggested.
section. I would suggest being consistent with patients number. If remaining 11 patients are not available then there is no reason to mention 20 patients in this study. Also authors should make clear in text that 9 patients belong to seven different cases/families.

Answer: The number of diagnosed and studied patients was clarified in the manuscript. The finding of 20 PLS patients highlight the prevalence of this syndrome in our region. Therefore, is relevant to keep this information.

2) Authors presented the clinical features in much detail in text. Authors should summarize these features in a table form.

Answer: The clinical information was summarized as suggested.

3) Authors investigated 7 families having in total 9 patients and all patients interestingly having same disease-causing mutation 203C/T (7 homozygous and 2 heterozygous). This apparently suggests a founder mutation and potentially a common ancestral haplotype among these Mexican families. I would suggest to analyze few STR markers around CTSC gene in these families. This would generate real message of this study.

Answer: As mentioned in discussion section, the founder effect can be determined by haplotype analysis of DNA markers in CTSC gene region. We are applying for grants to accomplish this analysis, but we believe, that the information presented in the manuscript is relevant enough for publication.

4) Authors presented “Cases 2P and 4P are found compound heterozygous for mutations 203T/G and 458C/T”, although 458C/T is a neutral polymorphism. How authors argue for disease causation in these 2 patients?

Answer: We do not know how c.458C>T affects the protein function. However, in our study, this change correlated with a reduced enzymatic activity observed in patients and their relatives.

In fact, c.458C>T genotypes frequencies in our population were 18.0% for C/T, and 82.0% for C/C. Moreover, p.Thr153Ile substitutions do not fall in a highly conserved residue, and PolyPhen-2 software predicted a probably benign mutation. However, Hart et al. reported this change as causal mutation in compound heterozygous PLS patients (Ref. 47 of the manuscript), as do we.

5) Most sections of the methods are written in much detail. Authors should shorten these sections (with proper references).

Answer: This part was summarized as suggested.

6) Results are mostly repeated in “Discussion”. Discussion should be more compact and meaningful.

Answer: Discussion section was modified to improve clarity and relevance.
7) Can authors explain how 203T/G is loss of function mutation?
Answer:
We found that c.203T>G mutation associates with the diminished enzymatic activity in our PLS patients. This enzymatic deficiency was not due to diminished CTSC gene expression levels, as demonstrated by quantitative analysis using qPCR. These results strongly suggest that c.203T>G mutation explains the enzymatic deficiency. c.203T>G mutation results in a change of Leucine for Arginine at residue 68 (p.Leu68Arg). Leucine 68 is a highly conserved residue located in the exclusion domain, and the presence of Arginine at this site may interfere with the insertion of the dipeptide substrate, blocking the enzymatic activity.

8) Did authors check splice junctions of CTSC gene for mutations in these patients? If yes then they should mention in manuscript that coding and splice junctions of gene were screened in patients.
Answer:
Yes, coding sequences and splice junctions of CTSC gene were sequenced for all patients and their relatives. This information is now included in the manuscript.

9) Number of controls (n=8) for the SSCP analysis is not sufficient.
Answer:
SSCP analysis of eight healthy individuals was used just as normal electrophoretic mobility pattern of PCR products, and not for statistical analysis against PLS patients and their relatives. This explanation is now included in the manuscript.

Minor revisions:
1) Since this study does not contain a large patient cohort, I would suggest to modify the title of the paper like “identification of novel mutation in CTSC gene causing PLS in Mexican patients” or Mutational analysis of CTSC gene in nine Mexican patients with PLS.
Answer:
The title was modified as suggested.

2) Gene name should be italics throughout the manuscript.
Answer:
This observation was corrected in the manuscript.

3) Author affiliations should be in English for general readers.
Answer:
Author affiliations are now in English.

4) DNA isolation method should go first and then PCR-SSCP/Sequencing.
Answer:
DNA isolation method is now before PCR-SSCP technique.

5) 5’ and 3’ ends of primer sequences are not shown.
Answer:
This observation was corrected in the manuscript.
6) Mutations at protein and nucleotide should be represented according to standard format http://www.hgvs.org/mutnomen/.
   Answer: Nomenclature of genes and proteins were corrected in the manuscript.

7) Did the authors look at disease gene mutation databases to see if the presumed pathogenic change or changes have been described previously?
   Answer: c.203T>G mutation was searched in gene databases, and has not been reported.

8) Did the authors look at bioinformatics tools (PANTHER, Polyphen-2, SIFT etc) whether mutation in CTSC is predicted deleterious or benign?
   Answer: The analysis of c.203T>G mutation with the PolyPhen-2 software is now described in the manuscript. This analysis predicted a probably damaging mutation with a score of 1.00.

9) Previously how many CTSC mutations are known causing PLS? Here authors should cite Human gene mutation database (HGMD) for quick reference to have number of mutations in this gene.
   Answer: The updated information of CTSC gene variants in HGMD is now included in the manuscript.

10) Make sure that reference section is written on journal format.
    Answer: References are now with the journal’s EndNote template.

11) Manuscript should be reviewed by expert in language.
    Answer: Manuscript was carefully revised by an expert in English.