Author's response to reviews

Title: Molecular and Neurological Characterizations of three Saudi Families with Lipoid Proteinosis

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Author's response to reviews:

24 December 2010
Dear Editor:
RE: MS 2038918614461278, “Molecular and Neurological Characterizations of three Saudi families with Lipoid Proteinosis”

We have carefully assessed the comments of your two reviewers and attempted to respond below to each comment. In each case, the reviewer’s comment is in bold, and our response is immediately below.

Reviewer 1

Genetic results: Family 1; The stop codon created by the two base deletion ( AA ) occurs not in the next codon, but in the second codon downstream (….GCATAA…).

The reviewer is correct that the missing AA resulted in a frame shift creating an aberrant codon 435 (GCA) and a stop codon (TAA) at codon 436. This is now stated correctly in the Results and in Discussion.

Family 2; Exon 7 is also present in ECM1c, a transcript which has an extra exon 5a within intron 5 and known to be expressed in the basal layer of the epidermis of human skin (Mongiat M., et al., J Biol Chem.2003, 278:17491-17499).

This is now stated correctly in the second paragraph of the Results section with the suggested reference.
Discussion: Particular attention should be paid to the correct referencing of publications:

1) The human ECM1 gene was identified independently by two research groups: ref. 11 and Johnson MR et al., Matrix Biol. 1997, 16: 289-292.


3) ECM1 is known to interact with several proteins in the extracellular matrix of the human skin: perlecan, collagen IV, lam 332, fibulin1C/D, fibulin-3,PLSCR1 and MMP-9. Either they are referenced individually or one refers to a review article (cfr. Sercu S., et al., The importance of the extracellular matrix protein1 (ECM1) as basement membrane protein in maintaining skin function. Textbook of Aging Skin (eds. MA.Farage; K.W.Miller, H.I.Maibach) p.77-91, Springer-Verlag Berlin,Heidelberg 2010.

4) Identification of the ECM1 c transcript by the research group of Dr. R.Iozzo should be referred to (see above).


The cited references have been added to the manuscript.

Clinical description: It is always important information for future readers to have a detailed dermatologic description. Therefore, could you for example specify the localization of the pigmentation, which joints are affected etc….

Additional dermatologic details have been added to a new paragraph 5 and to paragraph 8 of the Results section as requested.

Table-1: change II-5 in II-6 ? (Sub ID).

The Reviewer is correct that termed Patient II-5 in Family 2 is, in fact, Patient II-6. This patient is now labeled correctly in the manuscript as Patient II-6.

Fig 1: missing labels a, b and c.

These labels have now been added to Figure 1.

Fig 1: Family-3 ; II-1 is F (circle) and II-3 is M (square).

The Reviewer is correct. The pedigree in Family 3 has been altered to indicate that Patient II-1 is female and that Patients II-2 and II-3 are male.

Fig.3: (d) : arrow is missing in the photograph.
The appropriate arrow has been added to Figure 3d.

Discretionary Revisions

Genetic Results: Family 2; add to the text that the ECM1 chromatograms of patients I-1 and II-4 are not shown, or otherwise add the chromatograms to figure 1.

The following sentence has been added to the appropriate place in the legend for Figure 1: “Similar heterozygous results were found in father (II-1) and an unaffected sibling (II-4) but are not shown.”

Family 3; change sentence into: ……from the transcripts ECM1a/c and ECM1b …… and …. related by a founder effect…….

The suggested changes have been made.

Minor issues not for publication

Background:….. neurologic and neuroradiologic characteristics (features)…..

This change has been made to the Background section of the Abstract.

Results: …. two novel mutations in family 1 ……

This change has been made to the Results section of the Abstract

Conclusions: …… less common than….

This change has been made to the Conclusions section of the Abstract.

Methods: …. delete point in Declaration of Helsinki

This has been done.

Conclusion: … less common than ….

This change has been made.

Figure legends: fig. 1: …… intron 8

This change has been made.

Reviewer 2

1. This manuscript is not clearly written. Failed to present what they have done precisely and briefly.

For example, this study included seven affected individuals from three unrelated Saudi Arabian families (Table 1). The study was approved by the institute IRB and all participants signed an informed consent. The study adhered to the tenets of the Declaration of Helsinki.

You can put them together in another order to make it brief, like this:
This Study was approved by the institute of IRB and adhered to the tenets of the declaration of Helsinki. 7 participants from unrelated Saudi Arabian (table 1) were enrolled and all signed the informed consent.

The suggested change has been made to the first paragraph of the Methods section.

2. Misusing the sub-title, did not understand the organization of the paper.
For Example, under “Method”, one sub-title “Study participants” is listed but you put everything under it. You can either put everything together under “Method” or list variant sub-title for each part.

The sub-title “Study participants” has been removed from the Methods section.

Suggestion: To help reduce the possibility of such problems, we strongly encourage the author to take at least one of the following steps:
1. Have your manuscript reviewed for clarity by a colleague whose native language is English.
2. Use one of the many English language editing services that are available.
This has been done.

Associate Editor Comments:
- It is unclear from the methods section whether RNA or DNA was analyzed.
- If RNA was analyzed: have the mutations been verified in genomic DNA of the patients and parents?
To clarify this issue, a statement was added to the Methods section saying “Blood samples were collected from all participants and DNA was extracted and stored at -20°C until needed for genetic testing.”
- In the Genetic Results section please indicate the position of the mutations as relative to the A of the start codon: c.1300-1301delAA should probably read: c.1102-1103delAA
g.8544G>A should probably read c.806G>A and indicate which reference sequence has been used, in this case probably: NM_004425 You can look up the recommendations for mutation nomenclature in: Hum Genet (2001) 109:121?124
We are somewhat confused by the initial comment above because we believe that the labeling of the mutation for Family 1 is correct (c.1300-1301delAA) and follows standard recommendations for human mutation nomenclature as given in the reference cited above by the Editor.
For Family 2, “g.8544G>A” was replaced with “c.806G>A” in order to be consistent. Nucleotide numbering for all mutations is now in reference to the NCBI sequence (NG_012062), and this is mentioned in the Methods section.
-In the Genetic Results section for family 3 please indicate what the predicted effect on the protein would be.

We have re-worded the appropriate portion of the manuscript, and the current manuscript now states in paragraph 3 of the Results section that the 1163 base pair deletion in Family 3 “… results in complete loss of exons 9 and 10 from transcripts ECM1a/c and ECM1b and is predicted to have a deleterious effect on protein structure and function [2].” We used PolyPhen to predict the possible impact of the novel single amino acid substitution in Family 2 on the structure and function of the ECM1 protein using physical and comparative considerations. Unfortunately, Polyphen is not applicable to a 1163 bp deletion, but we agree with Hamada et al 2002 (reference #2 above) that it seems intuitive that an 1100 base pair deletion will affect ECM1 protein structure and function.

We appreciate all the effort that the editorial staff and the reviewers have put into assessing our manuscript. We agree that this is now a stronger report with the suggested changes.

Take care. We look forward to hearing from you.

Sincerely,

Mustafa A. Salih, MD
Khaled K. Abu-Amero, PhD
Thomas M. Bosley, MD