Author's response to reviews

Title: Clinical and Molecular Characterization of Ataxia with Oculomotor Apraxia (AOA) Patients In Saudi Arabia

Authors:

Saeed Bohlega (boholega@kfshrc.edu.sa)
Jameela Shinwari (jshinwari@kfshrc.edu.sa)
Latifa Al Sharif (AlSharifL@kfshrc.edu.sa)
Dania Khalil (DANIAKHALIL@internal.kfshrc.edu.sa)
Thamer Alkhairallah (TKhairallah@Kfshrc.edu.sa)
Nada Al Tassan (naltassan@kfshrc.edu.sa)

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Author's response to reviews: see over
Thank you for considering the revised version of the above referenced manuscript for publication in your respective journal. We are thankful to the referees and the Editor for pointing out some important modifications needed in the report. We have thoughtfully taken into account all comments. The explanation of what we have changed in response to the reviewers concerns is given point by point below. The revised manuscript with all the modifications highlighted is uploaded. We hope that all these changes fulfill the requirements to make the manuscript acceptable for publication. The project was approved by KFSH&RC the project RAC# is 2050036.

Looking forward to hearing from you soon.

Sincerely yours,

Nada A AL Tassan, PhD
Department of Genetics
King Faisal Specialist Hospital and Research Center.
P.O box 3354 Riyadh 11211
Tel: +966 1 4647272 ext:39609
Fax: +966 1 4424585
naltassan@kfshrc.edu.sa
MS: 8115768994308955 - Clinical and Molecular Characterization of Ataxia with Oculomotor Apraxia Patients In Saudi Arabia

Reply to reviewers:

Reviewer 1
1- The authors might consider describing the phenotypes of the patients in some more detail - for instance, were oculocutaneous telangiectasias present in any of them? Were there white matter T2 hyperintensities on MRI scanning? Did they perform a colony sensitivity assay (radiation sensitivity) in lymphoblasts from any of these patients?

- None of the patients in this study had any oculocutaneous telangiectasias, No white matter T2 hyperintensities observed on MRI. Unfortunately we were not able to perform any colony sensitivity assay on these patients as the consent form only cover mutation screening.

2-A number of minor typographical/spelling errors should be corrected.
- All errors were corrected.

Reviewer 2
1. Molecular genetic studies have allowed the characterization of a novel group of recessive ataxias defined by the association of cerebellar atrophy and peripheral sensorimotor neuropathy. This group includes ataxia with oculomotor apraxia form 1 (AOA1), ataxia with oculomotor apraxia form 2 (AOA2), ataxia-telangiectasia-like disease (ATLD) and spinocerebellar ataxia with neuropathy (SCAN1). Therefore, ataxia with oculomotor apraxia is a group of recessive ataxias and it is not a single clinical entity. Consequently, talking about AOA as a rare autosomal recessive disease is not correct.

Based, exclusively, on clinical terms is not possible to state that a patient is affected with AOA1, as the clinical features of all the diseases belonging to the group of recessive ataxias with oculomotor apraxia overlap in an extensive manner. Only genetic molecular studies can provide the doctor and the patient with accurate diagnosis for these diseases. In conclusion, a patient will be diagnosed with AOA1 if his Aprataxin gene is found mutated.

Taking this in consideration the authors must correct and/or precise the sentences/paragraphs highlighted bellow and should also avoid the use of the term "subtypes" concerning AOA1 and AOA2, but rather use "form" or "type".

- Corrections were made and the association of AOA1 in patients with MRE11 was omitted and a general term of Ataxia with oculomotor apraxia was used.

- Abstract (Objectives) line 2: “Ataxia with Oculomotor Apraxia (AOA) is a rare autosomal recessive disorder with two subtypes...”
- Changed to: Autosomal recessive ataxias represent a group of clinically overlapping disorders (Abstract page 2 lines 5 and 6).
- Abstract (Objectives) lines 8 and 9: “… from four Saudi families with either Ataxia...”
- Changed to: “from 4 Saudi families with ataxia and oculomotor apraxia”. (Abstract lines 14-15).
- Introduction, lines 1-3: “Ataxia with Oculomotor Apraxia (AOA) is one of the autosomal...”
- “Ataxia with Oculomotor Apraxia (AOA) is an autosomal recessive cerebellar ataxia” (Introduction page 4 line 3).

And
“In addition patients with Ataxia-Telangiectasia-Like Disorder (ATLD also known as MRE11 ataxia MIM# 604391) present with early onset ataxia and oculomotor apraxia” (was added to introduction page 4 lines 12-14).

- Introduction, line 33: “These findings support the genetic heterogeneity of the disorder”
- The sentence was omitted.
- Discussion, line 16: “...with two or three patients with AOA1 were enrolled in this study...”
- Changed to: “Three Saudi families from consanguineous marriages with early onset of ataxia and oculomotor apraxia were enrolled in this study” (Discussion page 10 line 22-23).
- Discussion, lines 20 and 21: “…comprehensive screening by direct sequencing of the APTX gene did not identify any mutations in the three Saudi families diagnosed with AOA1”
- The term AOA1 was omitted (Discussion page 11 lines 4-5).

2. Ataxia-telangiectasia-like disease (ATLD) patients carry mutations in MRE11, AOA1 patients carry mutations in APTX. Even if clinically both disorders share several features they are different entities easily identified by molecular genetic studies.

Following this line of reasoning the authors must clarify the statements referred bellow:
- Abstract (Results), lines 16 and 17: “...reported missense mutation W210C in MRE11 gene was identified in two families with AOA1”
- Changed to; “The previously reported missense mutation W210C in MRE11 gene was identified in two families with autosomal recessive ataxia and oculomotor apraxia” (Abstract page 2 lines 22-23).
- Results (Mutations in MRE11 gene) lines 16 and 17: “...This mutation was also identified in affected individuals from family B with AOA1 phenotype”
- The sentence was changed to; “This mutation was also identified in affected individuals from family B” (results page 10 lines2-3).

3. New data on Aprataxin function have been recently published. The authors should add those data:
- Introduction, lines 18-20: “... it was suggested that it may play a role in the repair...”
- The sentence was omitted and the following was added; “Aprataxin is a nuclear protein of three domains; a forkhead-associated (FHA) domain that mediates protein-protein interaction with molecules that respond to DNA damage such as binding to DNA single strand break repair scaffold protein (XRCC1) and binding to DNA double strand break repair scaffold protein (XRCC4). Aprataxin also contains a histidine triad (HIT) domain and a COOH terminal zinc finger domain, the HIT domain is similar to Hint, a universal conserved AMP-lysine hydrolase, studies showed that Aprataxin has an active site dependent AMP lysine and GMP lysine hydrolase activity that also depends on the zinc finger for protein stability and on the FHA domain for enzyme activity” (Introduction page 5, lines 1-8).

4. The authors must take in account what was said in all the above comments and make the necessary alterations in:
- Discussion, last paragraph: “We screened nine affected...”
- Changed to; “We screened nine affected individuals from 4 families with ataxia and oculomotor apraxia for mutations in the reported genes APTX, SETX and MRE11 and identified a novel truncating mutation in SETX gene in one family and a previously reported missense mutation in MRE11 gene in two families” (Discussion page11 lines 21-22 and page 12 lines 1-2).

5. The inclusion of family C in this study should be explained/discussed, knowing that the two patients belonging to this family do not present with cerebellar atrophy.
- The absence of cerebellar atrophy was noted in the text; “Screening all these genes in affected individuals of family C failed to detect any pathogenic segregating mutation, the two affected siblings in this family had ataxia and oculomotor apraxia with no cerebellar atrophy and normal tendon reflexes” (Discussion, page 10 lines 16-18).

6. The provided supplementary Table 1 does not correspond to what is stated in the text on the Materials and Methods section (Amplification and sequence ..., line 44), therefore it was not
evaluated.
- Table was modified to match the text.

MINOR ESSENTIAL REVISIONS
- Abstract (Methods) line 12: “Comprehensive sequencing of all coding exons of genes related to this disorder.”
Please, rephrase the sentence naming the studied genes. - Abstract (Conclusion) line 20: “...development of this disorder...” Please, correct the statement.
- Genes Names were included.
- The sentence in line 20 was omitted and conclusion was changed to; “Mutations in APTX, SETX and MRE11 are common in patients with autosomal recessive ataxia and oculomotor apraxia. The results of the comprehensive screening of these genes in 4 Saudi families identified mutations in SETX and MRE11 genes but failed to identify mutations in APTX gene” (Abstract page 3 lines 1-5).
- Introduction, lines 11 and 12: “... they are genetically heterogeneous.” Please, rephrase the sentence. - Materials and Methods, line 20: “derealization” Please, clarify the expression.
- The term derealization was omitted.
- Figure 2: labels (i) and (ii) are missing - Figure 3: labels (i), (ii) and (iii) are missing.
- All figures labels were added.

DISCRETIONARY REVISIONS
It would be very interesting if the authors could add to Table 1 more biochemical data, such as albumin and cholesterol values as well as the mutations found and respective genes.
- Mutations were added to the table. We didn’t have full biochemical data on all patients therefore we can’t add the albumin and cholesterol values.

MINOR ISSUES NOT FOR PUBLICATION - List of authors, line 3: see spelling of “Khalil D” and “Alkhairallah T”
- Spelling of Author names was corrected.
- Introduction, lines 13, 21; Results, line 5; Discussion, line 26: see spelling of “missense”
- Corrected.
- Introduction, line 23: see spelling “autosomal” - Introduction, line 26: see spelling “arginine”
- Corrected.
- Materials and Methods (Families and samples), line 7: see spacing “Table 1 and...”
- Corrected.
- Materials and Methods (Amplification and sequence ...), line 43: see spelling ”intrinsic”
- Corrected.
- Results, line 8: see spacing “Y359, H1049, ...”
- Corrected.

Reviewer 3
- Introduction - end of paragraph 2. Refs 6 and 7 refer to the cloning of the aptx gene. They do not refer to repair of DNA breaks.
There are many references on DNA repair in AOA2 cells eg. Clements et al 2004; Gueven et al 2004 etc. which should be used.
- The sentence was modified and the above mentioned references were included” “Aprataxin is a nuclear protein of three domains; a forkhead-associated (FHA) domain that mediates protein-protein interaction with molecules that respond to DNA damage such as binding to DNA single strand break repair scaffold protein (XRCC1) and binding to DNA double strand break repair scaffold protein (XRCC4). Aprataxin also contains a histidine triad (HIT) domain and a COOH terminal zinc finger domain [7-8, 10-11], the HIT domain is similar to Hint, a universal conserved AMP-lysine hydrolase, studies showed that Aprataxin has an active site dependent AMP lysine and GMP lysine hydrolase activity that also depends on the zinc finger for protein stability and on the FHA domain for enzyme activity [11]” (Introduction page 5 lines 1-8).
4. The conclusions in the last paragraph of Discussion are not warranted. They refer to lack of mutations in aptx in Saudi families with AOA1. It is possible that they failed to detect mutations or that these patients are part of another syndrome, not AOA1, but with clinical overlap. These possibilities should be acknowledged. It is difficult to see how another gene defect could be responsible for a disorder identical to AOA1. More likely, it is a different disorder and perhaps the clinical phenotype should be more carefully analysed.

- The sentence was modified to; “Lack of mutations in APTX, SETX and MRE11 genes in the third family diagnosed with ataxia and oculomotor apraxia and no cerebellar atrophy suggests the involvement of another mechanism for the development of this disorder” (Conclusion Page 12 lines 8-11).

Also the following paragraph was added to the discussion; “Screening all these genes in affected individuals of family C failed to detect any pathogenic segregating mutation, however, we didn’t check for regulatory mutations or large deletions. Moreover, the two affected siblings in this family had ataxia and oculomotor apraxia with no cerebellar atrophy and normal tendon reflexes and they may belong to a similar overlapping form of ataxia” (Discussion page 11 lines 16-19).

5. There are immunerable misspellings and poor construction of English throughout the manuscript. This needs to be addressed.

- Spelling mistakes were corrected.