Author's response to reviews

Title: A missense founder mutation in VLDLR is associated with Dysequilibrium Syndrome without the quadrupedal locomotion

Authors:

Bassam R Ali (bassam.ali@uaeu.ac.ae)
Jennifer L Silhavy (jsilhavy@ucsd.edu)
Joseph G Gleeson (jogleeson@ucsd.edu)
Lihadh Al-Gazali (l.algazali@uaeu.ac.ae)

Version: 3 Date: 17 June 2012

Author's response to reviews:

Dear Professor Scaglia,

Subject: 1448978059687292 - A missense founder mutation in VLDLR is associated with Dysequilibrium Syndrome without the quadrupedal locomotion.

We would like to thank you and all the reviewers for your effort in evaluating and improving our manuscript. We addressed all the comments and below you will find the point-by-point response to the comments.

We hope that you will find the manuscript is now acceptable for publication in BMC Medical Genetics and we are looking forward to hearing from you.

Best Regards,
Lihadh Al-Gazali

Reviewer 1:

Major Compulsory Revisions

1) The title indicates that a founder mutation has been identified. If the authors have identified a shared haplotype to support this claim, they are encouraged to report it. Otherwise, the title should reflect the lack of certainty — potential founder mutation?.

Our response: We thank R1 for this question, which is addressed in the revised manuscript. We tested for a common founder haplotype surrounding the mutation by designing a panel of 9 informative markers to genotype in both families. We knew that the SNP markers were informative in Family 1 because we have previously generated genome-wide linkage analysis on the family, and we evaluated that data to select the markers. These informative markers, analyzed in both families, demonstrated a ~1.5 million base pair haplotype that is identical in both families, and surrounded by recombinant markers, as expected. Thus we can conclude that this represents a founder mutation. We clarify this in these points in the revised manuscript and we include the genotyping data in a new
2) The authors need to make explicit and discuss how the patients described in this manuscript expand the phenotype.

Our response: We have removed this statement from the manuscript.

3) The abstract states that ?massive parallel sequencing in patients from two unrelated consanguineous families? was used. However two methods were described in the Materials and Methods section: homozygosity mapping followed by candidate gene analysis and sequencing was used to identify the mutation in family 1; exome sequencing was used for family 2. The abstract should be changed to reflect methods used.

Our Response: R1 is correct that massive parallel sequencing was applied just in one family (Family 2), since we already had the mutation identified in the Family 1. We have corrected this point in the revised manuscript.

4) In the Materials and Methods section, the individual(s) from family 1 who were genotyped and sequenced should be indicated.

Our Response: To clarify this point, we have added this information to the manuscript, but we thought that it was better suited to the Results section. We now indicate that all of the members of Family 1, generation III and IV underwent SNP genotyping, and individual IV-4 was genotyped. We also indicate which members were used to generate haplotype data in the Results section.

5) In the Materials and Methods section DNA methodologies, the authors indicate that for family 2, variants identified in the exome were ?intersected with identity-by-descent blocks identified by HomozygosityMapper?. Clarification of whether the exome variants were used as input into the software or prior SNP genotyping performed is needed.

Our Response: This is a good question. For Family 2, we did not generate SNP genotyping. We only generated exome sequencing data from one affected, which produces a list of about 20,000 genetic variants located across the genome. This data can, at some level, be treated like SNP data to evaluate blocks of homozygosity using a program like HomozygosityMapper. Therefore, we analyzed the genetic variants from the exome data for blocks of homozygosity, and identified the mutation within one of these blocks.

6) Mutation nomenclature used is inconsistent and does not reflect current guidelines (http://www.hgvs.org/mutnomen/). The mutation found should be described as c.2117G&T at the nucleotide level. Other mutations have also been incorrectly described, such as c.1342C.T. Some mutations described at the protein level are lacking the ?p.?

Our Response: We have corrected the mistakes in nomenclature as suggested.

The RefSeq accession number used to describe the mutation should be listed. The methods section describes sequencing a 17 exon gene yet the longest
VLDLR transcript is 19 exons (NM_003383.3). This may be a typographical error but if not, the authors should confirm that the previously reported mutations listed in the paper are consistent with this transcript.

Our Response: We apologize for this typographic error. The accession number should have listed NP_003374.3, which is now listed in the Results section.

Minor Essential Revisions
1) References should reflect the BMC Medical Genetics reference style.
   Our Response: We reformatted the references according to BMC Genetics style.
2) All genes should be italicized.
   Our Response: All the gene names have been checked and italicized.
3) In the background section of the Abstract, ?disequilibrium? should be replaced with ?dysequilibrium?.
   Our Response: Disequilibirium has been replaced with dysequilibrium as suggested, to match the spelling in OMIM.
4) On page 6, under the heading ?Identification of a pathogenic missense mutation?, the term ?freeze? half way down the paragraph to describe the hg19 build should be replaced with ?build?. In the same paragraph, the term ?transversion? is incorrectly used as it applies only to the nucleotide change and not the amino acid substitution. Suggested change ?This alteration resulted in a c.2117G>T nucleotide change and a p.C706F amino acid substitution?.
   Our Response: The requested changes have been carried out.
5) Throughout there are grammatical errors. While the message is conveyed, it would be improved, by having someone for whom English is his/her first language edit it.
   Perhaps Joe could look into this.
   Our Response: The manuscript was read and edited by a native English Speaker.
6) On page 6 in the ?Discussion?, the opening brackets are missing for references 6, 10, 11.
   Our Response: Opening bracket has been added.
7) In the final paragraph of the ?Discussion?, the word ?seizures? is incorrectly spelled (seizers).
   Our Response: Misspelling has been corrected.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore) None.
Level of interest: An article of importance in its field Quality of written English: Needs some language corrections before being published.

Our Response: We have edited the version to fit with standard written English.

Reviewer # 2

Major Compulsory Revisions
1. In the title and conclusion of the main text, the authors state that the mutation is a founder mutation. There is no molecular evidence presented regarding this conclusion? for instance microsatellite or SNP markers were not used to show an area of IBD shared between the two families. The authors must either change the interpretation of the data (as they state in the abstract ?possible founder effect?) or provide the evidence.

Our Response: This is the same point raised by reviewer 1. We provide new data to support the founder effect (new table 2)

Minor Essential Revisions
Abstract:
1. Page 2, Line 7: Change sentence ?The quadrupedal locomotion ? in all three genes? to ?Quadrupedal locomotion in this syndrome has been reported in association with mutations in all three genes.

Our Response: The suggested change has been carried out


Our Response: The suggested change has been carried out

3. Page 2, Line 18: Remove the last sentence of the results section as it is redundant ? also stated in the conclusion.

Our Response: The suggested change has been carried out

4. Page 2, Line 20: As there has been no significant expansion of the phenotypic spectrum by the patients reported here, the authors should change the conclusion to instead reflect the expanded mutation spectrum associated with VLDLR, and add the word homozygous (?identified the first homozygous missense mutation?), as there has been at least one missense mutation previously described (Boycott et al., 2009).

Our Response: The suggested changes have been carried out

Background
5. Page 3, Line 5, Background: references [1-3] should be [1,3,8].

Our response:
Reference [2] has been moved to the end of the first sentence of this paragraph and became reference [1] and reference [8] moved to become [4]. The other references have been changed accordingly [1 is now 2; 4 is now 5; 5 is now 6, 6 is now 7 and 7 is now 8].

6. Page 3, Line 6, Background: references [8-9] should only be [9].
Our Response: We have removed reference 8 as suggested

7. Page 3, Lines 10-12: remove sentence ?Patients with those disorders?.the first few years of life? as this sentence is simply repeating the information provided in the prior sentence.
Our Response: Sentence removed as suggested

Our Response: The suggested changes have been carried out

9. Page 3, line 19: remove the space between p.I779fsX3 Materials and Methods: no comments DNA
Our Response: The space has been removed as suggested

Methodologies: no comments

Results:

10. Page 5, Line 16: Family 1: Given that short stature is characteristic, it would be helpful to include the height percentile.
Our response: The height percentiles have been added as requested

11. Page 5, Line 19: Family 1: The authors should provide more detailed description of the cerebellar hypoplasia (areas involved) and not just refer to it as ?characteristic? ? to what?
Our Response: We have added additional text to the manuscript to describe the severe cerebellar hypoplasia of the vermis, with less severely affected cerebellar hemisphere, and nearly absent cerebellar folia, as well as the square-shaped pons, as have been reported in other patients with Dysequilibrium syndrome.

Our Response: Left sided squint has been changed to left sided strabismus

13. Page 6, Line 1: Family 2: The authors should provide more detailed description of the cerebellar hypoplasia (areas involved).
Our Response: We have now added some additional text, but did not want to repeat the same text as we added for Family 1. Rather we mention that the findings were very similar to those of Family 1. At R2’s request, we would be happy to include images from control brain for comparison.
Identification of a pathogenic missense mutation: no comments

Discussion:
14. Page 6, Line 25: The authors should remove number sign before OMIM # 224050.
Our Response: The number sign has been removed as suggested

15. Page 6, Line 26: The authors should change upper case to lower case C for Cerebellar hypoplasia.
Our Response: The suggested change has been carried out

Our Response: The missing bracket has been added

17. Page 6, Line 30: The authors should change ?In this manuscript, we report? to ?In this report, we describe??
Our Response: The suggested change has been carried out

18. Page 7, Line 15: The authors should remove ?Some of the features?are variable.? as this sentence does not contribute to the paper.
Our Response: The sentence has been removed as suggested

Conclusions:
19. Page 8, Line 20: The authors should change the sentence to reflect that the spectrum of VLDLR mutations has been expanded, not the phenotypic spectrum.
The patients described in this report have a very typical phenotype for VLDLR-associated cerebellar hypoplasia, and the authors state this in the discussion. In addition, as previously noted, founder effect should be removed unless supported by additional analysis or can be changed to ?possible? founder effect.
Our Response: The suggested change has been carried out

Bibliography:
20. Please change the bibliography style to match that of the BMC Medical Genetics Journal:
Authors: Title. BMC Med Genet [year], [volume number]:[article number].
Our Response: The references has been formatted to match BMC Medical Genetics style

Table:
21. Page 13, Table 1: Please indicate the number of patients that have been identified in each publication (i.e. affected with each mutation) 22. Page 13,
Table 1: ?Absent inferior vermis? might be better described as ?Hypoplastic inferior vermis?.

23. Page 13, Table 1: Use of +/- should be used in some of the categories that are variable in presentation? e.g. seizures? not all patients in a particular report will have had this feature.

Our Response: We have modified the table as suggested

Figure Legends:

24. Legends for Fig 2 and 3: Instead of ?affecteds?, please change this term to ?affected individuals? or ?affected persons?.

Our Response: The suggested change has been carried out.

Pedigree:

25. Page 15, Pedigree 2: As patient III-12 was not examined and not clearly affected, please indicate in some other way than a completely shaded circle.

Discretionary Revisions

26. Page 8, Paragraph 2: The paragraph ?Kaya et al?. does not really strengthen or add to the paper and could be removed and the authors consider a paragraph outlining the utility of identity by descent mapping with candidate gene sequencing and/or exome sequencing to rapidly come to a diagnosis in such families. This addition would strengthen the paper to emphasize the utility of these methods for rare diseases.

Our Response: we elected to keep this part of the discussion

Quality of written English: Needs some language corrections before being published

Our Response: We have edited the text to make it more nature English style.