Author’s response to reviews

Title: Identification of a rare SEPT9 variant in a family with autosomal dominant Charcot-Marie-Tooth disease

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Author’s response to reviews:

Dr. Sulev Köks
Editor
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Dear Dr. Köks

the decision letter for our manuscript entitled “Identification of a rare SEPT9 variant in a family with autosomal dominant Charcot-Marie-Tooth disease and cognitive deficits” (ID: MGTC-D-19-00504) was received on December 8, 2019 and was very much appreciated. We would like to thank both reviewers for their valuable comments. We have studied these points carefully and have made corresponding revisions that we hope will meet with your approval.

Please find our responses to the reviewers’ comments in a point-by point manner below. Please note that significant changes were made to the title, figure 1 as well as to the authors’ order.

We hope the revised version is now suitable for publication in BMC Medical Genetics.
Reviewer 1:

Comment 1.1: Since the development of cognitive defect may be caused by multiple factors, which include environmental ones, the interpretation of their findings should be performed in a very careful manner. The authors, therefore, should address the following issues in Discussion: Previous studies on in vitro or in vivo model of cognitive defects, particularly those involved SEPT9 variants

Response 1.1: We agree with the reviewer’s concern about multiple factors being involved in cognitive deficits, including environmental and genetic influences. Comparable concerns were raised by the second reviewer. An association of cognitive deficits with the identified variant may be assumed, as both SEPT9 variant-carriers show a similar cognitive impairment phenotype. Moreover, alterations of other septin genes have been associated with neurodegenerative disorders. However, in line with the concern of both reviewers, the association of the reported SEPT9 alteration with cognitive deficits is speculative. We accordingly further discuss this point on p. 8, ll. 22-29:

“To note, known genetic variants associated with cognitive impairment were not excluded in this family. However due to similar cognitive deficits in the here identified SEPT9-variant carriers and the positive family history, cognitive deficits may be an additional clinical feature in the SEPT9-phenotype spectrum. Of interest, alterations of other members of the septin family have been associated with neurodegenerative disorders (20): SEPT1-4 have been linked to Alzheimer’s disease while for SEPT5 an association to Parkinson’s disease has been reported (20). Thus, one might speculate that the SEPT9 alteration could be associated with the cognitive changes in our current study.”

Moreover, we deleted the term “and cognitive deficits” in the manuscript title (p. 1, l. 3).
Reviewer 2:

Comment 2.1: In the introduction the authors should specify that they found a missense variant in SEPT9 gene providing some information on its function.

Response 2.1: We accordingly added information on the SEPT9 gene in the introduction as follows (p. 4, ll. 20-26): “We identified a rare heterozygous missense variant in the SEPT9 gene, a gene previously described as a cause of hereditary neuralgic amyotrophy (HNA) when heterogeneously mutated (5). SEPT9 is part of the SEPT3-group and encodes the ubiquitously expressed Septin 9 protein, which belongs to the conserved septin family of GTPases involved in various cellular processes such as motility and cytokinesis (6). Interestingly, Septin 9 interacts with the cytoskeleton including microtubules and actin and thereby promotes asymmetric neurite outgrowth (7).”

Comment 2.2: In the discussion it would be useful to expand the part on SEPT9 giving more information on the protein, its function, the family to which it belongs, the expression profile and cellular processes in which septins are involved.

Response 2.2: We agree with the reviewer that further information on the specific SEPT9 characteristics would be useful. We therefore expanded the according section in the discussion as follows (p. 8, ll. 3-10): “The ubiquitously expressed septin 9 (SEPT9) is one of the 13 members of septins, a highly conserved family of GTP binding proteins that have been linked to a broad spectrum of cellular functions. The septin family interact with the cytoskeleton, including actin, and function in processes such as cytokinesis, motility, and cell polarity. Other functions include angiogenesis and bacterial autophagy (for review see: (6)). SEPT9 knockout in mice results in embryonic lethality (17). Septins form oligomeric complexes, which can assemble into higher ordered structures such as filaments and rings. A well-known septin complex consists of alternating SEPT2/6/7/9-units (18).”

Comment 2.3.A: The authors should perform trio exome sequencing for two important reasons: a) to exclude other variants associated with the cognitive impairment in this family especially in the subject III.4
Response 2.3.A: The suggested trio exome sequencing experiment would certainly be of interest and would provide particularly additional information about de novo variants in the subject III.4, but we feel that it falls outside the scope of this study as we here demonstrate a clearly autosomal dominant pedigree in a clinically well characterized CMT-family. Subjects III.4 and II.3 share specific CMT-symptoms as well as cognitive impairments. CMT is known to be a syndromic disease. The spectrum of cognitive deficits in the SEPT9 variant carriers were shown to be similar. Together with the overlapping CMT phenotype and the knowledge about other affected and unaffected subjects (here III.2, a non-SEPT9 variant carrier, with no signs of neuropathy or cognitive deficits) it can be assumed that this neuro-cognitive phenotype results out of one genetic hit. However, we agree that multiple combined inherited genes, or environmental factors cannot be fully excluded with our targeted sequencing approach. We therefore have made significant changes to the manuscript to emphasize this point:

1) We excluded “and cognitive deficits” in the manuscript title (page 1, line 3).

2) We changed the abstract (p. 3, ll. 15-16): “We, for the first time, present a SEPT9 variant associated to a distinct CMT phenotype with cognitive deficits and suggest SEPT9 as new sufficient candidate gene in CMT.”

3) We adapted the according part in the discussion (p. 7, ll. 20-21):” In this study, we used a targeted NGS approach to investigate the genetic cause of a distinct CMT1-phenotype with cognitive deficits in a German family.”

4) We added following sentences for clarification (p.8, ll. 22-25): “To note, known genetic causes of cognitive deficits were not excluded in this family. However due to similar cognitive deficits in the SEPT9-variant carriers and the positive family history, cognitive deficits may be an additional clinical feature in the SEPT9-phenotype.”

Comment 2.3.B: The authors should perform trio exome sequencing for two important reasons: b) to exclude variants in cancer predisposition genes. In effect, even if SEPT9 plays a role in the development of some types of cancer, germline variants have never been reported in association with cancer-prone syndromes, to my knowledge.

Response 2.3.B: We agree that our given association is speculative, and deleted our hypothesis of a potential cancer underlying pathomechanism associated with germline SEPT9 variants as it is not the scope of this study: “In addition, SEPT9 has been linked to diverse types of cancer including leukemia, breast and colon cancer. Remarkably, the index patient II.3 suffered from breast cancer and non-Hodgkin lymphoma in the past. High and low expressed septin9 levels were identified in patients with breast cancer, which may lead to a gain of toxic function or to loss of septin9 function [14]”
Comment 2.4: Authors should provide the CADD score of the identified variant

Response 2.4: To address the reviewer’s suggestion we incorporated following sentences (p. 5, ll. 28-30 and p. 7, ll. 13-14):

“For further variant characterisation the Combined Annotation Dependent Depletion (CADD) score (10) and the American College of Medical Genetics (ACMG) criteria (11) were used.”

“The CADD score was 22.7 (CADD GRCh37-v1.4), indicating that the variant belongs to the top 1% of deleterious variants in the human genome.”

Comment 2.5: Authors should provide in the main text the classification of the variant and the criteria used for the classification according to the ACMG guidelines

Response 2.5: We have, accordingly, included the ACMG classification of the variant and the criteria we used in the main text (p.5, ll. 28-30 and p. 7, ll. 16-17):

“For further variant characterisation the Combined Annotation Dependent Depletion (CADD) score (10) and the American College of Medical Genetics (ACMG) criteria (11) were used.”

“The variant was classified as “uncertain significance” (class 3, evidence of pathogenicity: PP1, PP2, PP3, PP4) based on ACMG guidelines.”

Comment 2.6: It would be useful to add the molecular modelling study to show the effect of the missense variant on the protein

Response 2.6: We thank the reviewer for this suggestion. It would have been interesting to explore this aspect. However, the current data on the protein septin 9 does not provide sufficient information on experimental structures to describe the consequences of the identified variant. There are three crystal structures, but they do not represent the entire protein. Although p.Val469Ala is a conservative substitution, Valine is of course sterically more demanding compared to alanine, the substitution thus may lead to steric problems in the interaction with binding partners or other structural elements that we cannot extract from the structure of the available protein data. To address this important point, we have added a sentence about possible changes in the protein structure caused by the exchange from valine to alanine (p. 8, ll. 33-38).
“By an alanine-for-valine substitution in this binding domain, essential protein-protein interactions and functions may be disturbed, particularly as valine unlike alanine contains two non-hydrogen substituent attached to their c-beta carbon which can be important in protein binding (22). Due to the location of p.(Val469Ala) in a central domain of the protein and the potential demolishing impact on oligomerisation we suppose a rather loss of function effect. Further studies are needed to confirm this hypothesis.”

We further adapted the conclusion (p. 9, l. 4):

“(…) and suggest a potential relevant functional implication on protein level.”

Comment 2.7 Authors should provide Sanger sequencing figure of the variant.

Response 2.7: We agree that Sanger sequencing figures would fit nicely in our manuscript. We included electropherograms obtained by Sanger sequencing of subjects II.3 and III.4 and the healthy family member III.2 in figure 1a.

The according figure legend has been revised as follows (p.11, ll. 14-16): “Electropherograms obtained by Sanger sequencing demonstrate the heterozygous missense variant SEPT9:c.1406T>C, p.Val469Ala in subject II.3 and III.4, which was not detected in the healthy subject III.2.”

Further changes: Please note that Dr. Alma Osmanovic and Dr. Christoph Schrader changed positions as equally contributing senior authors.