Author’s response to reviews

Title: Frequency of SCA8, SCA10, SCA12, SCA36, FXTAS and C9orf72 repeat expansions in SCA patients negative for the most common SCA subtypes

Authors:
Gülsah Aydin (Guelsah.Aydin@uni-wh.de)
Gabriele Dekomien (gabriele.dekomien@rub.de)
Sabine Hoffjan (sabine.hoffjan@rub.de)
Wanda Gerding (wanda.gerding@rub.de)
Jörg Epplen (joerg.t.epplen@rub.de)
Larissa Arning (larissa.arning@rub.de)

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Author’s response to reviews:

Dear Editor

Many thanks for your response to our submission and we are happy to respond to the reviewer’s comments, which we outline below. We would like to thank them for their helpful feedback which has further improved the clarity of the manuscript.

We hope that you will now find it suitable for publication

Yours sincerely

Larissa Arning (on behalf of the authors)
Reviewer reports:

Alfonso Fasano (Reviewer 1)

Comments:

1. Introduction:

a. Row 78: Authors should cite examples and reference in the sentence "SCA forms that are caused by rare conventional mutations", such as Durr A, Lancet Neurol 2010.

Response: Done

b. Row 95: Authors failed to cite previous studies investigating frequency of non-coding expansion SCAs in Europe (Brusco A, J Neurol 2002) and other locations (Choubtum L, BMC Neurology 2015).

Response: Done

2. Methods

a. Row 105: Authors describe the setting (Germany) and relevant dates, genotyping methods and platforms used, but do not state the specific laboratory/centre where genotyping was done.

Response: Done

b. Authors do not describe eligibility criteria in detail. Studies assessing frequency of rare SCAs should establish exclusion criteria (eg. positive history of alcohol abuse, chronic treatment with anticonvulsant drugs, laboratory tests) as to minimize false positive results of rare ataxias.

c. In addition, the study did not enroll controls. In SCA 8, expanded alleles have also been found among healthy subjects; therefore inclusion of controls is of utmost importance.
d. Mean age at onset of the first neurological symptoms related to ataxia is not described. Presence of family history in the population studied is not described (familial cases/sporadic cases)

Response: We agree and address these issues now in a limitation section at the end of the discussion.

e. Row 127: Authors should provide the list of primer sequences for follow-up studies (reproducibility) as supplementary material.

Response: All primer sequences are available upon request or can be found in the cited literature. However, if explicitly desired, this information could be provided as additional material.

f. Table 1: please use NA instead of ?; one case has "atrophy of the cerebellum": any sign?; one case has "movement disorder": which one? one case has paraparesis and no ataxia? please elaborate

Response: We now use NA instead of ?. The information of the clinical features is as provided from the referring physician, unfortunately, in this case “movement disorder” is not further defined. After careful elaboration we decided to take out patient six from the dataset. Patient 6 represents an exception – at the time of examination, this young girl showed progressive spastic paraparesis with dysarthria, rather symptoms of hereditary spastic paraparesis. However, all analyzed forms of HSP (3,4,7,11,13) as well FRDA turned out to be negative, so that she was also referred to ataxia diagnostics.

g. Table 2: what is the gender of case VII?

Response: It is a male, we added this information.
3. Discussion

a. Row 182: "our results substantiate the assumption that SCA10 is a rare cause of ataxia in ethnic populations other than Mexican". SCA 10 is found in regions of Latin America, particularly in Mexico and Brazil, where it is the second most common SCA (Teive HA, Parkinsonism Relat Disord. 2011) (Durr A, Lancet Neurol 2010)

Response: The statement has been refined.

b. Row 197: SCA8: Authors provide clinical characteristics of six patients with 83 or more repeat sizes in the SCA8 gene, but do not compare with the phenotype of patients previously reported in the literature (family history, age of onset, severity of symptoms). Authors correctly point out that diagnostic testing results for SCA8 should be interpreted with caution, since there are reports on the presence of pathogenic repeat lengths in healthy control cohorts and of patients with other identified genetic causes for ataxia.

Response: We added the available information on the age at onset in the table. Furthermore, we now state that the common initial symptoms of SCA8 are scanning dysarthria with gait instability with disease onset typically occurring in adulthood and that because of the reduced penetrance of the SCA8 repeat expansion, the most common presentation is a single affected person in a family.

c. Row 219: " FXTAS: our results add to the growing body of evidence that gray zone alleles are associated with specific phenotypes associated with the toxic gain-of- function effect of raised mRNA". Authors show clinical characteristics of the 7 patients (4F, 3M) with alleles in the gray zone in Table 2. All patients present with cerebellar symptoms, one of the 7 patients had gait disturbance associated with erectile dysfunction and micturition disturbance, no parkinsonism is described. Development of FXTAS and/or parkinsonism were previously noted in gray zone cases (Hagerman RJ, Nat Rev Neurol. 2016) therefore the study results add to reinforce this association, but are not surprising.

Response: The statement has been refined.

Authors do not discuss the higher number of female patients in the gray zone.

Thank you for mentioning this interesting point, we now discuss this issue.
“Previous published reports of FXTAS have also suggested that women are far less frequently affected than males, possibly related to the presence of the second X chromosome and random X inactivation and/or a sex-specific protective effect, perhaps related to estrogen”……

“Therefore, our results add to the growing body of evidence that gray zone alleles are associated with neurological symptoms. It is also interesting to note that we found a higher share of women than men amongst the patients with FMR1 premutation or grey zone alleles. Possibly women are underdiagnosed in the current diagnostic practice for FXTAS, especially when they present with a clinical course that is typical of males with FXTAS.”

d. Row 252: C9ORF72: The patient diagnosed with a large GGGGCC repeat expansion in the cohort was a 51-year-old man with unclear dementia syndrome and psychiatric problems. Since dementia is the dominant symptom in this patient, the authors concluded this case does not broaden the phenotypic spectrum of pathogenic C9orf72 repeat expansions. It is unclear in the manuscript whether the patient with C9ORF72 mutation had an ataxic syndrome, highlighting the importance of defined inclusion/exclusion criteria. The results should emphasize that none (or one) of the ataxia patients presented with C9ORF72 mutation, therefore being a rare cause of ataxia.

Response: We now state that the patient had no ataxia and conclude that the results do not support genetic testing for C9orf72 expansion in ataxia patients.

In fact, it is not even clear why Authors decided to C9ORF72 in the first place.

Response: We now state that the spectrum of neurological conditions associated with the repeat expansion in C9orf72 is very broad, including rarely cerebellar ataxias. In order to check if C9orf72 expansions also contribute to the spectrum of neurological conditions found in our cohort, we included the screening for C9orf72 expansions in our analyses.

e. Row 260: "the phenotypic spectrum of C9orf72 expansions extends to other neurodegenerative syndromes such as PD, progressive muscular atrophy (PMA), primary lateral sclerosis (PLS), Huntington disease (HD), ...": HD is not caused by C9orf72, Authors should say HD-like.

Response: The statement has been refined.
e. Limitations:

Authors should depict the clinical relevance of their findings more clearly. Authors do not discuss study limitations regarding inclusion/exclusion criteria and generalizability of the findings (German population, prevalence estimations vary considerably between countries). Authors discuss that it should be critically considered whether SCA8 diagnostic should be a fixed component of SCA routine diagnostics but do not propose a diagnostic strategy for Spinocerebellar ataxias.

Response: We agree and address these issues now in a limitation section at the end of the discussion. Additionally, we added that diagnostic testing for SCA8 should be considered when the family history gives the impression that the symptoms are sporadic or inherited in an autosomal recessive manner. However, diagnostic testing results for SCA8 should be interpreted with caution, especially when used for genetic counseling.

Antonio Elia, MD (Reviewer 2):

Abstract:

In the "background" the C9ORF72 gene is not reported. The authors should list here all the genes that they will test in this study.

Response: Done

In the "methods" the cohort should be clearly defined: is it a consecutive cohort of patients? Is it defined on clinical base or on genetic base?

Response: Done

In the "results" SCA8 patients are 5, but in the text and table they are 6

Response: Done, see also answer to Reviewer 1.

In the "conclusions" I suggest to add also a comment on SCA8 and C9ORF72.

Response: Done
Introduction:

Among the many different genetic causes of ataxia the authors choose to test the cohort for SCA 8-10-12-26, FXTAS and C9ORF72. They should indicate more clearly the reasons of these choices.

Response: The intention was to screen for rare repeat expansion disorders associated with ataxia, but it is also clear that this selection could be extended.

Methods:

"The cohort is clinically heterogeneous ..." The authors should clearly indicate how the cohort was defined. Is it a consecutive series of patients affected by any form of ataxia? Is it the whole cohort of patients who were found to be not carriers for common types of SCA?

Response: The statement has been refined.

Results:

"Patients that appeared homozygous for one allele in the normal fragment range were reanalyzed… " This sentence should be put in methods.

Response: This sentence is also written in the methods section.

I suggest to add more clinical features of the SCA8 patients (age at onset, symptom of presentation, clinical progression).

Response: We added the available information on the age at onset in the table. Unfortunately, no more clinical features are available. We address this issues now also in a limitation section at the end of the discussion

One female patient was found to be affected by FXTAS and 4 other female patients were found to carry an allele in the grey zone of FXTAS. I suggest to add more clinical features of these patients, are they affected also by primary ovarian insufficiency?
Response: Unfortunately, no more clinical features are available.

I suggest also to add a clinical description of phenotype of the patient with C9ORF72 mutation.

Response: Unfortunately, no more clinical features are available.

Discussion:

"The symptoms of the corresponding patients comprise various neurological symptoms (Table 1) …" I suggest to add a comparison with previously reported SCA8 patients

Response: We added the available information on the age at onset in the table. Furthermore, we now state that the common initial symptoms of SCA8 are scanning dysarthria with gait instability with disease onset typically occurring in adulthood and that because of the reduced penetrance of the SCA8 repeat expansion, the most common presentation is a single affected person in a family.

In line 262 I suggest to change "Huntington disease (HD)" with "Huntington disease phenocopies".

Response: Done, see also answer to Reviewer 1.

Tables and figure: A description/legend for figure is lacking.

Response: Done.

Hsiu-Chuan Wu, MD, PhD (Reviewer 3):

This manuscript conveys limited novelty as a considerable number of previous reports have addressed similar conclusion. The merit of this report mainly resides in a substantial number of the cohort which could add up to future systemic reviews. The description is generally easy to follow. There are, however, several concerns that might require further revision and clarification from the authors.
1. Abstract

* The authors are advised to specify the cohort is composed of 'unrelated' patients in Methods in Abstract, not until that in the main manuscript.

Response: Done.

2. Introduction

* Several sentences in Introduction are far too long and complicated.

Response: We endeavored to shorten several sentences.

* The full description of abbreviations should be provided when the abbreviation is first mentioned. Then the authors are advised to use the abbreviation throughout instead of repeating the full descriptions again and again.

Response: Done.

* The authors are advised to avoid descriptions in parentheses except abbreviations when first mentioned.

Response: Done.

* Line 87: not suitable 'to detect' ==> should be 'for detecting'

Response: Done.

* The rationale for analysing the hexanucleotide repeats in C9orf72 gene is vague here. As the authors only specify mutations in this gene are associated with FTD and ALD, not ataxia. Although increasing evidence has suggested a possible link between C9orf72 repeat expansion
and cerebellar ataxia, the authors might want to intrigue the readers by shortly describing that in the Intro, not until the Discussion.

Response: We now state that the spectrum of neurological conditions associated with the repeat expansion in C9orf72 is very broad, including rarely cerebellar ataxias. In order to check if C9orf72 expansions also contribute to the spectrum of neurological conditions found in our cohort, we included the screening for C9orf72 expansions in our analyses.

3. Methods

* The authors might want to clarify what 'other unspecific symptoms' means when describing the clinical presentations of their cohort.

Response: The statement has been refined. We now state that other neurologic symptoms of varying duration are meant.

4. Results

* Typo:

SCA 8 ==> should be 'SCA8'

Response: Done.

TP PCR ==> should be 'TP-PCR' (there are few of them throughout the manuscript)

Response: Done.

Line 143: As for SCA8 ==> I suppose it should be SCA10

Response: We rewrote the sentence for the purposes of clarity.

* The authors used 'SCA8' to describe this gene in Result section, whereas using ATXN8OS instead of the 'SCA8' (gene) in the Conclusion section when describing the RAN translation. The authors are advised to use one consistent gene name throughout.

Response: Okay, but this part of the discussion section has been deleted (see answer below).
Although 6 patients had repeats greater than 83, the authors suggested none of them showed a pathogenic pattern. However in Result section in Abstract, the authors described these repeats are discussed to be (?) potentially pathogenic. The authors seemed to contradict themselves in deciding whether their result in SCA8 was pathogenic or not. Even in Discussion, they only mentioned that the established SCA8 pathogenic threshold is questionable. The authors might want to carefully clarify the definition of pathogenic pattern of SCA8 repeat expansion before drawing a clear conclusion from their results.

Response: The statement has been refined.

* Figure legend of figure 1 is missing.

Response: Done.

* The authors are advised to organise the order of genes mentioned in Result section in Abstract in line with that in the main manuscript. Although this does not affect the conclusion at all, it indicates that the authors did not put sufficient effort into preparing their manuscript.

Response: Done.

5. Discussion

* In order to improve the readability, the authors are advised to organise the order of genes mentioned in Discussion section in Abstract in line with that in the main manuscript. Alternatively, they can discuss the positive results first and then all negative results.

Response: It was considered preferable to keep the order.

* Line 212, do the authors mean that SCA8 patients usually have other identified genetic causes for ataxia? Could the authors specify what these other genetic causes are and cite relevant references?

“The reduced penetrance, together with reports on the presence of pathogenic repeat lengths in healthy control cohorts and in patients with other identified genetic causes for ataxia”

This sentence means that expanded SCA8 repeats are also found in healthy controls as well as in patients who also carry another SCA mutation which is responsible for their symptoms.
* C9orf72 vs C9ORF72. Italic vs normal font. The font should be italic for the name of genes and normal for that of proteins. The authors are advised to put more efforts in correcting these editing errors in their revision.

Response: Done.

* Line 259: Beside ==> should be 'Besides', and there are several 'beside' in other parts of the manuscript. Done

Response: Done.

6. Conclusion

* Far too long. It should be rewritten in order to be concise and clear. The authors tried to discuss the necessity of including SCA8 in the routine diagnostics for patients with ataxia but did not actually come to a conclusion. They then carried on discussing how RAN translation could be one of the molecular mechanisms for SCA8 repeat expansion. The authors have never mentioned RAN translation in their Discussion, why do they draw a conclusion here this mechanism might play a role in the inconsistent phenotypes of SCA8 repeat expansion? All these discussions should be shorten and moved to Discussion section.

Response: We appreciate this comment very much. We now restructured the conclusion section to clarify the aim of our study

* The authors mentioned that RAN translation has been reported in other nucleotide expansion disorders: DM1, FXTAS and C9ORF72. First of all C9orf72 is not a disorder, it's a name of a protein or gene, depending on the font. Secondly, RAN translation has been reported in C9orf72-related ALS/FTD, not all disorders related to mutations of this gene.

Response: This point can be omitted. The respective section has been deleted.
7. General concerns:

* A considerable number of sentences in the manuscripts are too long and complicated. It is therefore strongly recommended that this manuscript is checked by an English proofreading expert before submitting the revision. This is to ensure an accuracy in some texts and correct grammar usages.

* Editing error: typos, a consistent principle of using abbreviations, italic font for gene names, orders of genes mentioned in Result (both in Abstract and main manuscript) and Discussion

Response: We endeavored to shorten several sentences.

Thank you for this recommendation, we have now edited several sentences and hope that the manuscript is now clearer and easier to understand.