Reviewer's report

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

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Reviewer: Alfonso Fasano

Reviewer's report:

Targeted next-generation sequencing (NGS) approaches (gene panels) as well as next-generation whole exome sequencing are becoming more widespread in routine molecular diagnostics for patients with ataxia. Since NGS at present is not suitable to detect trinucleotide repeat expansions, a pre-NGS testing for common polyglutamine expansion SCAs seems mandatory. The authors claim that non-coding expansions SCAs and other ataxias known to be associated with repeat expansions such as fragile X-associated tremor ataxia syndrome (FXTAS) should also be taken into account before applying NGS-based diagnostics. The study aims to provide more information about the frequency and phenotypic characteristics of rare repeat expansion disorders associated with ataxia in order to find an optimal diagnostic strategy.

The authors tested a cohort of 441 patients with "cerebellar ataxia, dysarthria and other unspecific symptoms" who were referred to a German center. More details about eligibility criteria are not provided. All patients showed alleles in the normal range for Polyglutamine expansions SCAs as SCA1-3, SCA6, SCA7 and SCA17 and were screened for non-coding expansion SCAs (SCA8, SCA10, SCA12 and SCA36), and for FXTAS, another ataxia known to be associated with repeat expansions. Patients were also tested for the pathogenic hexanucleotide repeat in the C9ORF72 gene, rarely reported in ataxia syndromes.

No expanded repeats for SCA10, SCA12 or SCA36 were identified in the analyzed patients. Five patients showed SCA8 CTA/CTG combined alleles (83-129) that are discussed to be potentially pathogenic. One 51-year-old male patient with dementia symptoms (unclear if had ataxia) was diagnosed with a large GGGGCC repeat expansion in C9ORF72. Analysis of the FMR1 gene revealed one patient with a premutation (>50 CGG repeats) and seven patients with alleles in the grey zone (41 to 54 CGG repeats). The authors conclude that the results support the assumption that smaller FMR1 gene expansions could be associated with the risk of developing neurological signs. No diagnostic strategy based in the results is described.
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.
   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).

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.
   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)
   e. Row 127: Authors should provide the list of primer sequences for follow-up studies (reproducibility) as supplementary 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
   g. Table 2: what is the gender of case VII?
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)

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.

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.

The raised mRNA (toxic gain-of-function mechanism) in gray zone cases was previously described, but in this study the mRNA levels were not assessed by RT-PCR. Thus, the study adds only by describing the clinical characteristics of the gray zone cases, not by adding evidence to the raised mRNA mechanism.

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

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.

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

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.

f. 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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript
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