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Analysis of *CACNA1A* CAG repeat lengths in patients with familial ALS

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ABSTRACT

Intermediate-length ATXN2 CAG repeats are a risk factor for amyotrophic lateral sclerosis (ALS). Here we report on a female patient with heterozygous repeat expansion mutation in the *CACNA1A* gene presenting with a pure ALS syndrome while her father, who also carries that CACNA1A mutation, suffers from a classical spinocerebellar ataxia type 6. Hypothesizing that CACNA1A CAG repeat expansions could be a monogenic cause for familial ALS (fALS), we analyzed the CAG repeat lengths in *CACNA1A* in a large cohort of genetically unexplained patients with fALS. Our results indicate that CAG repeat expansion mutations in *CACNA1A* are not a frequent monogenic cause of fALS but could phenotypically present as ALS in rare instances.

1. Introduction

Spinocerebellar ataxia type 6 (SCA6) is caused by CAG repeat expansion mutations (more than 22 repeats) in the *CACNA1A* gene encoding for the calcium voltage-gated channel subunit alpha1 A. SCA6 accounts for approximately 20% of the families with inherited ataxias in Germany. It most often manifests as a "pure" cerebellar ataxia with variable onset, whereas non-cerebellar symptoms occur much less frequently than in other SCAs (Paulson et al., 2009). Only one previous report describes a patient with SCA6 (14/24 CAG repeats) with concomitant motor neuron loss (Ohara et al., 2000; 2002). While intermediate *ATXN2* trinucleotide repeat expansions are a potentially therapeutically relevant risk factor for amyotrophic lateral sclerosis (ALS) (Becker et al., 2017; Elden et al., 2010), sporadic ALS has previously not been found associated with an altered CAG repeat length in *CACNA1A* (Lee et al., 2011). Here we report a patient with a pure ALS syndrome and CAG repeat expansion in *CACNA1A* and analyze the *CACNA1A* repeat lengths in a large cohort of patients with ALS.

2. Methods

2.1. Patients and ethics

All patients with ALS were diagnosed according to the El Escorial criteria (Brooks et al., 2000). With informed written consent and approval by the national medical ethical review boards in accordance with the Declaration of Helsinki, EDTA blood samples were drawn from index patients with familial ALS (fALS). DNA was extracted from the EDTA blood samples according to standard procedures.

2.2. MR imaging volumetry

A fully automated quantitative 3-D MR imaging (MP-RAGE) analysis by the use of atlas-based volumetry was performed according to previously described standards (Huppertz et al., 2010).

2.3. Video-oculographic analysis

Video-oculographic analysis was performed as previously described (Gorges et al., 2015).

2.4. Analysis of CSF markers

Analysis of neurofilaments (pNfH) was performed as previously described (Steinacker et al., 2015).

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2.5. Genotyping for known ALS genes

Mutations in *SOD1* and *C9orf72* were excluded before exome sequencing of fALS cases as described before (Freischmidt et al., 2015). We performed whole exome sequencing of a total of 160 European index patients with fALS. Sequencing, read mapping, and variant calling were performed on HiSeq2000/2500 systems (Illumina) as described previously (Freischmidt et al., 2015). Patients with mutations in the following genes were excluded from CAG repeat size analysis based on whole exome sequencing: *FUS, TARDBP, TBK1, OPTN, CHCHD10, UBQLN2, SETX, NEFH, VAPB, VCP, ALS2, NEK1, ERBB4, FIG, PFN1, SQSTM1, HNRNPA1,* HNRNPA2B1, *ANG, CCNF, CHMP2, BGLE1, MAPT, MATR3, SIGMAR1, SPG11, and TUBA4A*.

2.6. CAG repeat size determination

Analysis of the *CACNA1A* repeat length was performed by fragment length analysis. The PCR primers were designed using NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are available on request. 10 ng of DNA was used for fragment analysis by application of the OneTaq Quick-Load 2X Master Mix (Biolabs, MA, # M0487L) according to the manufacturer's instructions and an optimized 55°C–65°C touchdown cycling protocol. Before electrophoresis, the amplificates were prepared using 1 μL DNA template, 0.3 μL ROX size standard (Serac, Germany, #LS02) and 15 μL formamide (Sigma, MO, USA, #F9037) and incubated for 2 min at 96°C. Electrophoresis was performed on an ABI PRISM 3130 Genetic Analyzer (Life Technologies, CA) using POP-7 polymer (Life technologies, CA, #4352759) and dye or filter set D. Data analysis was performed using the Peak Scanner v1.0 software.

3. Case report and results

We describe a German female patient with ALS carrying a heterozygous CAG repeat expansion mutation with 22 repeats in the expanded *CACNA1A* gene, and 8 repeats in the other allele. Her father suffers from a classical SCA6 (Fig. 1A) without signs of motor neuron degeneration. The patient with ALS developed a classical, pure ALS syndrome with adult, focal onset of paresis and signs of upper and lower motor neuron degeneration with subsequent characteristic spreading (Fig. 1B). During the course of the disease, pseudobulbar and bulbar motor involvement, emotional lability, weight loss, respiratory insufficiency, and rapid disease progression (Fig. 1C) were observed. Remarkably, at the early disease stage, paresis of hand and finger flexors outweighed the extensor palsy of the hand and fingers. Electromyography was compatible with motor neuron disease (MND) showing acute and chronic denervation at the cervical level and chronic denervation at the thoracic and

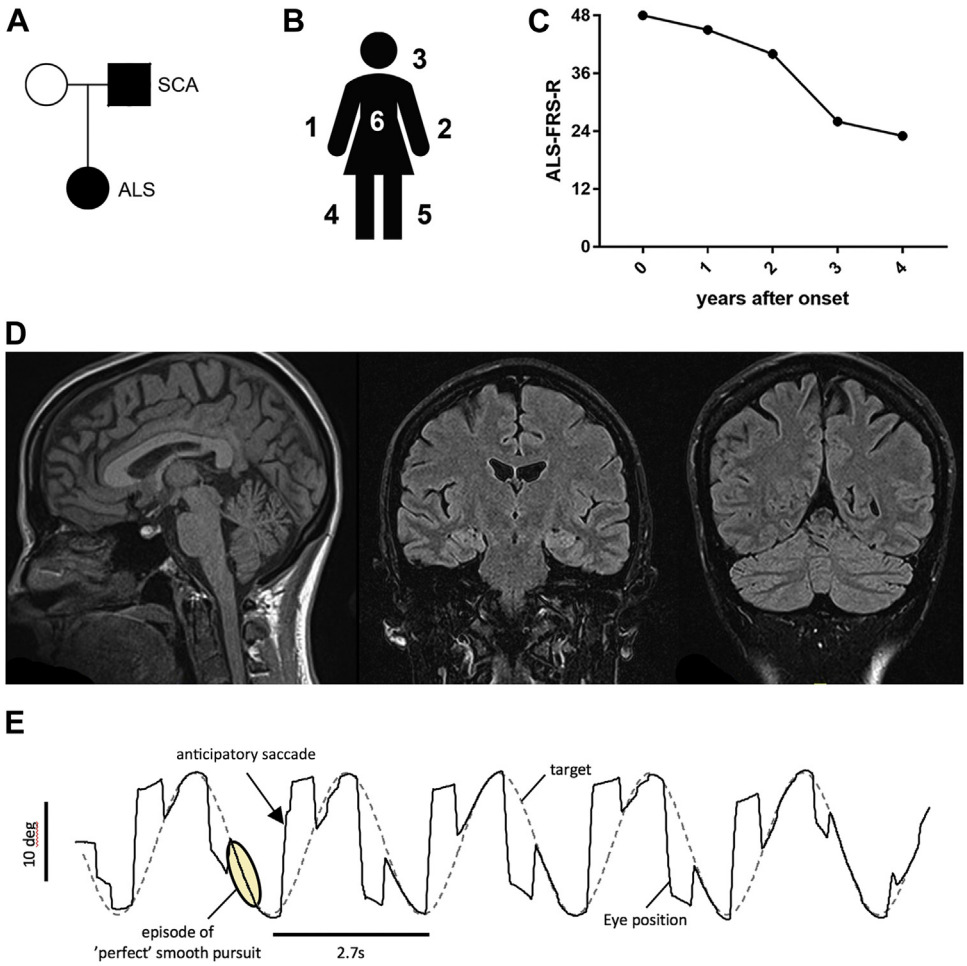

Fig. 1. Disease course and diagnostics in the second year after onset. (A) Pedigree of the affected family. (B) Spreading of palsy (always upper motor neuron and lower motor neuron affected). (C) Course of the ALS-FRS-R score. (D) Cranial MR imaging showed no atrophy of cerebellar structures. (E) Horizontal eye position (solid trace) for the patient tracking a sinusoidally oscillating spot (dashed trace). Anticipatory saccades ("intrusions") frequently interrupted episodes of 'perfect' smooth pursuit. Abbreviations: ALS, amyotrophic lateral sclerosis; ALS-FRS-R, revised ALS functional rating scale; SCA, spinocerebellar ataxia.

lumbar level. The electroneurography was normal. Within 4 years, the disease progressed to a severe tetraparesis, neck weakness, and anarthria. There was no clinical indication of cerebellar symptoms or cognitive impairment as indicated by a normal Edinburgh Cognitive and Behavioral score of 128/136. Cranial 1.5 T MR imaging including volumetric brain analysis did not show pathological findings (Fig. 1D). In particular, the cerebellum had a volume of 126.6 mL which was not different from an age-matched in-house control database. MRI of the spinal cord excluded myelopathy and signs of radicular compression. Video-oculographic recording of eye movement control indicated executive dysfunction, that is, high number of saccadic intrusions, multiple antisaccade errors, and low frequency of voluntary gaze shifts. There were no signs of cerebellar involvement as revealed by both episodes of perfect smooth pursuit eye movement (Fig. 1E) and by the absence of any pathological nystagmus. CSF analysis revealed an elevation of pNfH levels compatible with MND (981 pg/mL; cutoff value at 560 pg/mL; Steinacker et al., 2015). Screening for mutations in the most frequently affected genes SOD1 and C9Orf72 resulted negative.

Given the pure ALS phenotype of our patient, we hypothesized that CAG repeat expansions in *CACNA1A* might be a rare monogenic cause of ALS. Consequently, we determined the *CACNA1A* trinucleotide repeat length in 160 index patients with fALS and 15 technical healthy controls. In these 160 patients, mutations in known ALS genes had been excluded before (see methods) using whole exome sequencing, Sanger sequencing, and Southern blotting for *C9orf72,* to enrich for potential novel genetic contributors. We did not find intermediate (18–22 repeats) or pathological (>22 repeats) length CAG expansions in *CACNA1A* in the 160 patients with fALS. The mean length (±SD) was 11,51 ± 1,78 (fALS). The range of repeat lengths was 7–14 CAG repeats (Fig. 2). In addition, we analyzed the exome data for missense and loss of function mutations in *CACNA1A*. We detected no loss of function mutation and 5 missense variants with an allele frequency below 1/10,000 and in our fALS cohort of 160 patients (allele frequency 1.56% in fALS versus 1.40% in gnomAD data set, http://gnomad.broadinstitute.org/, Lek et al., 2016). Contingency analysis by Fisher's exact test showed no enrichment of *CACNA1A* missense variants in the fALS cohort (p-value = 0.8).

4. Discussion

Here, we described a patient carrying a CAG repeat expansion mutation in *CACNA1A* presenting with a classical ALS phenotype. The only unusual clinical aspect was a predominance of finger and hand flexor over extensor paresis, which might reflect an unusual spreading pattern earlier affecting polysynaptic corticospinal connections (Braak et al., 2013). Apart from this finding, the patient shows a disease course undistinguishable from sporadic ALS. The observed pattern of oculomotor dysfunctions is characteristically observed in ALS (Gorges et al., 2015) but is inconsistent with impaired eye movement control in SCA6 due to cerebellar deficits (Falcon et al., 2016). Because DNA of this patient was not subjected to exome sequencing co-occurrent (de novo) mutations in less frequently affected or yet undiscovered ALS genes cannot be completely excluded.

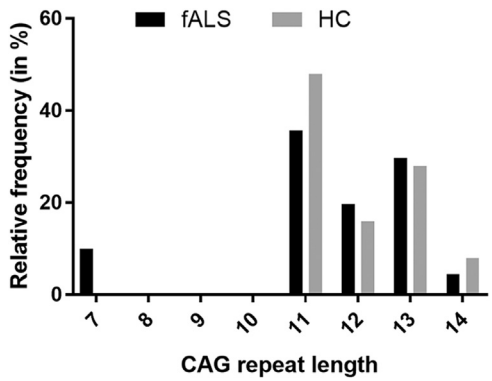

Fig. 2. CAG repeat length analysis of *CACNA1A* in patients with fALS. Distribution of CAG repeat lengths in *CACNA1A* in patients with familial amyotrophic lateral sclerosis (fALS) and healthy controls (HC).

To assess if CAG repeat expansion mutations in *CACNA1A* are a monogenic cause of ALS, we additionally analyzed the CAG repeat length in a large cohort of genetically unexplained patients with fALS. We did not find intermediate or pathological length CAG expansions in *CACNA1A* in 160 genetically unexplained patients with fALS. We conclude that *CACNA1A* CAG expansion mutations are not a frequent cause of ALS. This is in line with a previous study in patients with sporadic ALS (Lee et al., 2011), in which an altered distribution of CACNA1A repeat length was excluded as a risk factor for ALS. Nevertheless, it seems plausible to speculate that *CACNA1A* CAG repeat expansion mutations with pathological repeat length may predispose for motor neuron degeneration under certain, very rare conditions, possibly requiring a specific constellation of genetic co-factors. Suggesting a possible link between ataxia-causing mutations and motoneuron degeneration, co-occurring MND has been reported in SCA2, SCA3, and SCA8 (Kim et al., 2013; Paulson, 2009). Moreover, intermediate-length polyQ mutations in *ATXN1* and *ATXN2* have been associated with sporadic ALS (Lattante et al., 2017; Lee et al., 2011). Furthermore, oligogenic causation by co-occurrence of *CACNA1A* CAG repeat length expansion mutations and mutations in known ALS genes cannot be ruled out because patients with fALS with known mutations were excluded from this study. However, the molecular mechanism possibly causing motor neuron degeneration in *CACNA1A* CAG repeat expansion carriers remains unclear (Ohara et al., 2002).

Finally, although unlikely in view of the rareness of both diseases, accidental co-occurrence of sporadic ALS and (presymptomatic) SCA6 cannot be completely ruled out in this case.

Disclosure statement

The authors report no actual or potential conflict of interest.

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All authors approve all procedures and the article. The study was approved by the Ethical Committee of University of Ulm University ("German Network for Motor Neuron Diseases (MND-NET): A Registry and Trace Study", permission nr. 19/12). All participants provided written informed consent and all the methods were carried out in accordance with the approved guidelines.

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