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CORRESPONDENCE

‘Cortical cerebellar atrophy’ dwindles away in the era of next-generation sequencing

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Cortical cerebellar atrophy (CCA) denotes a non-hereditary degenerative ataxia of unknown etiology.[1] This entity is sometimes referred to as late-onset cortical cerebellar atrophy, idiopathic sporadic cerebellar ataxia or sporadic adult-onset ataxia of unknown etiology.[1–3] The diagnosis of CCA needs to fulfill the following criteria: progressive ataxia; disease onset after 20 years of age; no acute or subacute disease onset; informative and negative family history, or no evidence of a causative gene mutation; no established symptomatic cause; and no possible or probable multiple system atrophy.[1,2] In short, the diagnosis of CCA should be made by ruling out acquired and genetic causes of ataxia, as well as multiple system atrophy.

According to the Japanese Registry for ‘intractable diseases’ run by the Ministry of Health, Labour, and Welfare, Japan, the total number of patients with spinocerebellar ataxia excluding multiple system atrophy was 25 477 in the fiscal year of 2012. On the basis of the data published by Tsuji *et al.*,[4] the estimated number of CCA is ∼10 000 at present. However, it is very unlikely that these patients met the diagnostic criteria shown above, because family history was often not fully informative, and genetic testing was not required to enter the registry. Therefore, it is believed that CCA is a heterogeneous group of ataxic disorders. Previous reports indicated that ∼2–20% of patients with a diagnosis of CCA were confirmed by genetic testing to have mutations in one of the causative genes for autosomal dominant cerebellar ataxias.[2,3] Actually, 11 patients (15.1%) in our cohort of 73 ataxic patients without an apparent family history had known gene mutations (SCA6: 3; SCA31: 4; MJD/SCA3: 2; SCA2: 1; and DRPLA: 1). Furthermore, using next-generation sequencing, it is now possible to identify very rare disease-causing variants.[5–9]

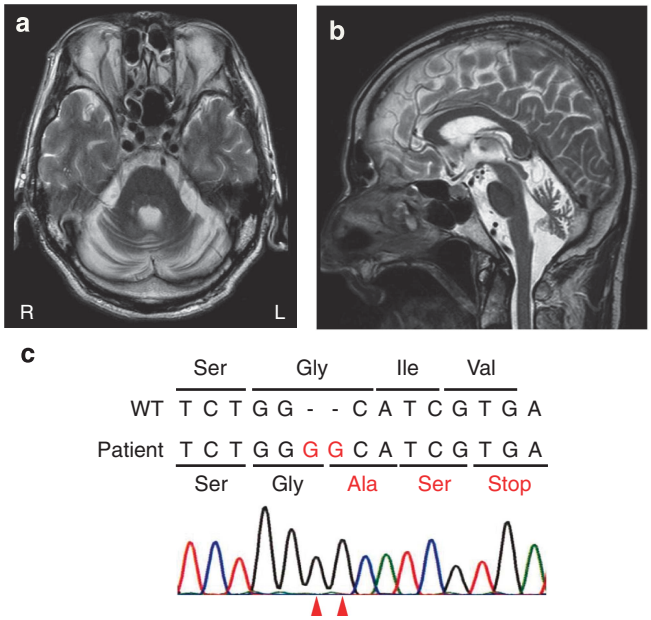

Figure 1 Magnetic resonance images at the age of 64 years show cerebellar atrophy without brainstem or middle cerebellar peduncle atrophy. The hot cross bun sign is not observed (**a**: TR (repetition time) 4427.49/TE (echo time) 100; **b**: TR 4961.44/TE 90). A homozygous frame-shift mutation (*c.493_494dup: p.Ile166Alafs*3*) in *ANO10* is indicated (**c**). Red arrowheads indicate GG duplication in the patient.

Recently, we identified a novel *ANO10* mutation in a CCA patient using next-generation sequencing. He was a 66-year-old Japanese male who developed gait unsteadiness and dysarthria at age 41 years. His mother and father died at age 94 and 78, respectively, but reliable information on their consanguinity was not obtained. Both of them were reported not to have gait ataxia or dysarthria during their lifetime. He had two siblings and two children, all of them being not affected. Since he noticed gait and speech disturbance, his ataxic symptoms have progressed very gradually. He resigned his job as a school teacher at age 55 years because of disabling ataxia. At present, he is wheel-chair bound and cannot stand up without holding onto something because of severe truncal ataxia. Neurological examinations revealed increased deep tendon reflexes with a bilateral positive Babinski sign, in addition to cerebellar ataxia. Intellectual impairment, parkinsonism or involuntary movements were not observed. Muscular wasting and decreased vibration sense on lower extremities were noted. Magnetic resonance (MR) images at age 64 years indicated cerebellar atrophy, but not brainstem or cerebral atrophy (Figures 1a and b). Abnormal

high-signal intensity lesions were not observed in either supratentorial or subtentorial regions. Genetic testing using genomic DNA obtained from peripheral blood leukocytes did not reveal any known mutations for autosomal dominant cerebellar ataxias (SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA12, SCA17, DRPLA and SCA31).

This patient attracted our attention because the consanguinity of his parents was not fully informative, leaving the possibility that he had one of the rare autosomal recessive cerebellar ataxias. To explore further for the genetic cause of his ataxia, we performed whole-exome sequencing. Genomic DNA was captured using a SureSelect Human All Exon v5 kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on a HiSeq2000 with 101 bp paired-end reads (Illumina, San Diego, CA, USA). Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina). The reads were aligned to GRCh37 with Novoalign (http://www.novocraft.com/). PCR duplicates were removed using Picard (http://picard.sourceforge.net/). Variants were called by the Genome Analysis Toolkit (http://www.broadinstitute.org/gatk/) and annotated using ANNOVAR (http://www.openbioinformatics.org/annovar/) after excluding the common variants registered in the common dbSNP135 database (minor allele frequency $\geqslant 0.01$). The mean depth of coverage on whole-exome sequencing was $92.6\times$, and over 93.1% of the total coding sequence of RefSeq genes achieved a $20\times$ read depth. We obtained rare protein-altering and splice-site variant calls after filtering against 575 in-house control exomes. A novel homozygous frame-shift mutation (*c.493_494dup: p.Ile166Alafs*3*) in *ANO10*, confirmed by Sanger sequencing, was identified (Figure 1c). His condition was diagnosed as spinocerebellar ataxia autosomal recessive type 10 (SCAR10, OMIM 613728). This is the sixth mutation identified in *ANO10* so far,[8–10] and this case is the second in Japan following the patient reported by Maruyama *et al.*[9] Our case was clinically quite similar to their case,[9] with both exhibiting the pure cerebellar phenotype of late onset.

We speculated that he might have typical CCA since he visited our hospital at age 46 years. He fulfilled the criteria for CCA clinically, and genetic testing ruled out well-known autosomal dominant cerebellar ataxias with the repeat expansions and SCA31. Now the situation has changed. Whole-exome sequencing has enabled the identification of rare disease-causing mutations even in apparently sporadic patients.[7] Therefore, we need to call the depth of genetic testing into question more carefully when we discuss whether a patient fulfills the CCA criteria. As the cost performance of next-generation sequencing is improving, the precise definition of CCA regarding the genetic testing is becoming increasingly difficult. A significant number of patients with CCA will be proven to have static mutations for rare autosomal dominant cerebellar ataxias or autosomal recessive cerebellar ataxias by this technique in the near future, if they are tested. Now, we should recognize more strictly that a provisional diagnosis of 'CCA' includes many different genetic causes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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