

## SHORT COMMUNICATION

# SCA42 mutation analysis in a case series of Japanese patients with spinocerebellar ataxia

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Spinocerebellar ataxia (SCA) is a group of dominantly inherited heterogeneous disorders in which 43 subtypes have been identified to date. Recently, Japanese and French families with SCA type 42 (SCA42) were found to have a missense mutation (c.5144G>A; R1715H) in *CACNA1G*. We performed genetic analysis of 84 unrelated families to find the prevalence of SCA42 in Japan. Two families were found to have the previously reported missense mutation. Clinical presentations of the affected members of these families were similar to those of the previously reported French and Japanese families. Our study demonstrates that SCA42 exists in small numbers in Japan, and further supports the idea that SCA42 is a slowly progressive, pure cerebellar ataxia.

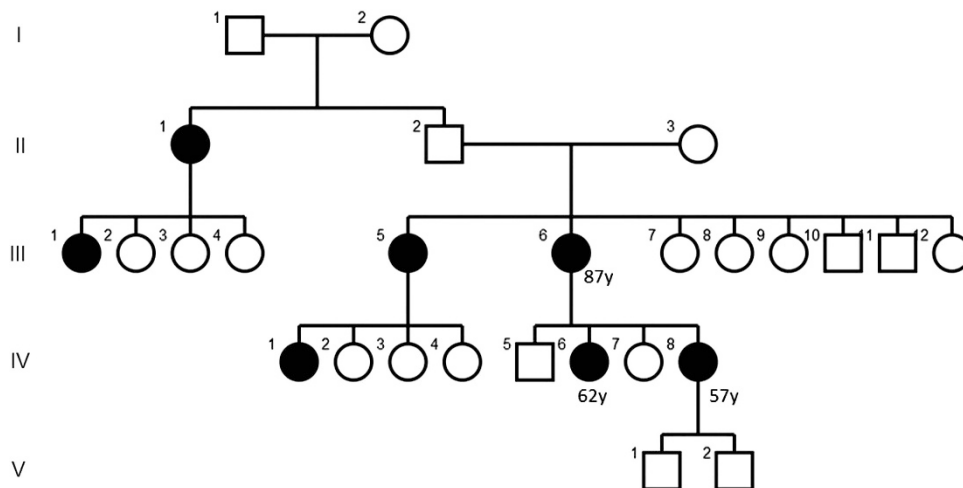
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### INTRODUCTION

Spinocerebellar ataxia (SCA) is a genetically inherited heterogeneous neurodegenerative disorder that affects the cerebellum, brainstem, spinal cord and cranial nerve nuclei, resulting in progressive cerebellar ataxia of the gait and limbs. It is variably associated with nystagmus, dysarthria, intention tremor and ophthalmoparesis. Disease onset is usually between 30 and 50 years of age, although early onset in childhood and onset after 60 years have been reported. The clinical diagnosis of SCA subtypes is complicated by their overlapping of their

phenotypic variation. To date, 43 different genetic loci for SCA have been reported and a causative gene has been identified for most. In 2015, SCA type 42 (SCA42) was reported to be caused by a voltage-gated calcium channel 1G (*CACNA1G*) mutation. Japanese<sup>1</sup> and French<sup>2</sup> families in two separate studies were found to have a heterozygous missense mutation in *CACNA1G* (c.5144G>A; R1715H).

In the present study, we examined the prevalence and incidence of SCA42 in Japan. We performed genetic analyses on individuals whose SCA subtype was unknown.



**Figure 1** Pedigree of family 2. Males are represented as squares. Females are represented as circles. Black-filled circles represent affected individuals, while open circles indicate unaffected members. Genetic analysis was performed in the proband (III-6), and her two daughters (IV-6, IV-8).

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**Table 1 Clinical features of affected family members**

Characteristic	III-6	IV-6	IV-8
<i>Age, years</i>			
At examination	87	62	57
At symptom onset	35	36	35
Symptoms at onset	Difficulty walking	Difficulty walking	Difficulty walking
SARA score	24	18	9
Ocular overshoot	+	+	+
Saccadic pursuit	+	-	+
Smooth pursuit	Defective	Slightly defective	Defective
Gaze nystagmus	-	-	-
Dysarthria	++	+	++
<i>Reflexes of</i>			
Upper limb	Slight hyporeflexia	Slight hyporeflexia	Normal
Lower limb	Slight hyporeflexia	Slight hyporeflexia	Normal
Gait ataxia	+	+	++
<i>Ataxia of</i>			
Upper limb	++	+	+
Lower limb	++	++	++
Muscle tone	Normal	Normal	Normal
Involuntary movement	-	-	-
Neuropathy	-	-	-
Parkinsonism	-	-	-
Dementia	-	-	-
MRI findings	Atrophy of cerebellum Cerebellar and brainstem infarction	Atrophy of cerebellum	Atrophy of cerebellum
Other complications	Hearing impaired hypertension	None	None

## SUBJECTS AND METHODS

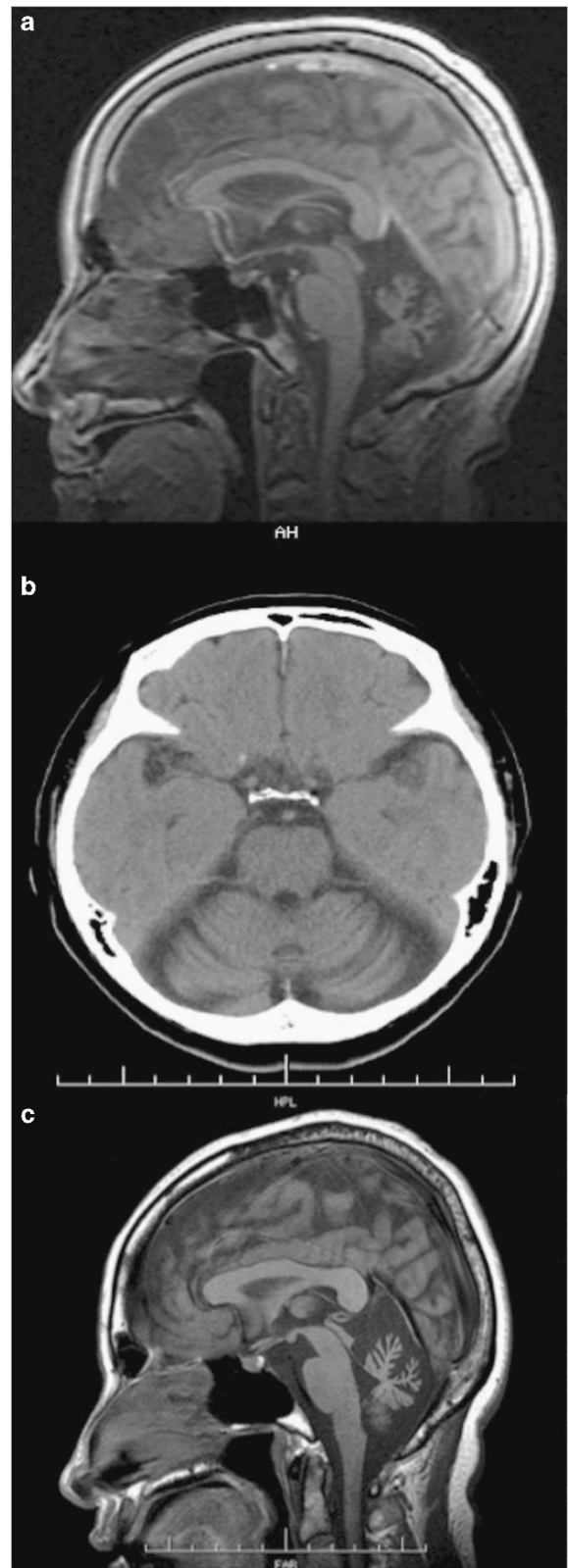
Genetic analyses were performed on 84 unrelated Japanese families with SCA, who presented to our hospital between April 1999 and September 2016. Previously, we published a similar study on patients who presented until March 1999.<sup>3</sup> In all cases, progressive cerebellar ataxia was the cardinal clinical manifestation. The majority of patients presented with an adult onset phenotype. Brain magnetic resonance imaging (MRI) showed cerebellar atrophy or pontocerebellar atrophy. All procedures were approved by the Hokkaido University Ethics Committee, and informed consent was obtained from all participants (healthy/asymptomatic and diseased).

Genomic DNA was extracted from blood lymphocytes by standard methods. Abnormal expansions of tri-, penta- and hexa-nucleotide repeats or reported mutations in SCA1, SCA2, Machado–Joseph disease/SCA3, SCA6, SCA7, SCA8, dentatorubal-pallidoluytion atrophy, SCA14, SCA17, SCA31 and SCA36 genes were excluded in all 84 probands. Direct sequencing of all exons of *CACNA1G* for SCA42 was performed (OMIM #616795). After treatment with shrimp alkaline phosphatase and exonuclease I, polymerase chain reaction samples were sequenced directly (BigDye Terminator, version 3.1, and 3130 GeneticAnalyzer; Applied Biosystems, Tokyo, Japan).

## RESULTS

Eighty-four SCA families were examined for *CACNA1G* mutations in all 38 exons, and two families were positive. Both families had the previously reported pathogenic missense mutation (c.5144 G>A; R1715H),<sup>1,2</sup> which was also confirmed by restriction fragment length polymorphism analysis (Supplementary data). No other mutations were found. Family 1 had previously been reported as having a novel SCA with a possible linkage to the SCA12 locus (5q32).<sup>4</sup> Affected members of this family presented with a pure cerebellar phenotype.<sup>4</sup> In our current analysis, all affected members and one asymptomatic carrier (at the time of presentation; previously reported as II-6) were found to have the pathogenic mutation.

The pedigree of the other family, family 2, is shown (Figure 1). Two members (IV-6 and IV-8) still visit a nearby hospital, as their mother (III-6) had previously. All three developed gait disturbance, mild-to-moderate speech difficulties, and smooth pursuit defects in their mid-30s. IV-6 and III-6 eventually became wheelchair bound.



**Figure 2** Neuroradiological findings (a): Mid-sagittal (T1WI) MRI of the brain of patient III-6 from family 2. (b): Mid-axial CT scan of the brain of patient IV-6 from family 2. (c): Mid-sagittal (T1WI) MRI of the brain of patient IV-8 from family 2.

They also had the pathogenic mutation. Their clinical features are shown in Table 1. Neuroradiological examinations revealed atrophy confined only to the cerebellum in all three individuals (Figure 2); therefore, they were also diagnosed with pure cerebellar ataxia.

## DISCUSSION

We identified two Japanese families with a previously reported pathologic missense mutation. This is in addition to the Japanese family reported by Morino *et al.*,<sup>1</sup> indicating that SCA42 is present in small numbers in Japan. In contrast to Morino, who examined families in Hiroshima (southwest Japan), our patients were from Hokkaido (northernmost prefecture of Japan). Analyses of other regions in Japan are warranted to determine the prevalence of this particular subtype and its impact.

To date, this disease has only been reported in France and Japan, and this report would be the third. It is unknown why it is specific to these countries, and it is possible that SCA42 is present in other locations, but has not been reported. Further epidemiological studies are warranted to better understand this rare subtype.

Our study supports the clinical presentations seen in the previously reported SCA42 families: slowly progressive, pure cerebellar ataxia accompanied with dysarthria, gait disturbance and abnormal eye movements. Many patients with SCA subtypes tend to present with nystagmus, especially those with pure cerebellar ataxia. SCA6 patients present with a variety of nystagmus types, while downbeat positioning nystagmus is rare in SCA31 patients.<sup>5</sup> Nystagmus did not present in our patients, although saccadic pursuits were common.

Age of onset varied from 9–78 years in Coutelier's patients and 20–70 years in Morino's patients.<sup>1,2</sup> In contrast, the age of onset in our patients was 13–38 years (average, 31.8 years), which may provide clues to the unknown current status of the asymptomatic carrier (II-6) of family 1,<sup>4</sup> who was 34 years of age at presentation and found to have this mutation in SCA42.

The c.5144G>A mutation is located in the S4 segment, which is the voltage sensor of Cav3.1.<sup>1</sup> Electrophysiological studies showed that activation of mutant Cav3.1 transfected into HEK293T cells shifted toward more positive membrane potentials, while the inactivation curves of Ca<sup>2+</sup> were also shifted positively.<sup>1,2</sup> This mutation leads to a change in calcium channels, but specific details remain

unknown. Further studies should assess why this mutation leads to this specific subtype.

The clinical presentations of SCA subtypes are similar, making a succinct diagnosis difficult. Genetic testing has proven to be invaluable in such patients. In family 1, previous genome-wide analysis suggested linkage to the SCA12 locus. However, subtype-specific genetic analysis identified a mutation at a different locus in the asymptomatic carrier.

In summary, we identified two additional Japanese families with SCA42. The combination of clinical assessments and genetic analyses is ideal for making a subtype diagnosis. Since there is no cure for SCA, identification of the causative genes for the remaining disease loci is important for designing treatment options to improve the function and quality of life of these patients.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)