学位論文　博士（医学）甲

# RFC1 repeat expansion in Japanese patients with late-onset cerebellar ataxia
# （日本人の遅発性小脳失調症患者における RFC1リピート延長について）

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BRIEF COMMUNICATION

# *RFC1* repeat expansion in Japanese patients with late-onset cerebellar ataxia

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**Abstract**

Recently, the expansion of an intronic AAGGG repeat in the replication factor C subunit 1 (*RFC1*) gene was reported to cause cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS). In Europeans, the expansion accounted for 22% of sporadic patients with late-onset ataxia. We genotyped 37 Japanese patients comprising 25 familial (autosomal recessive or undecided transmission) and 12 sporadic ones with late-onset ataxia. We found intronic repeat expansions in *RFC1* in three (12%) of the familial patients and one (8.5%) of the sporadic ones. Although our cohort study was small, the disease frequency in Japanese patients with CANVAS might be lower than that in European ones. In addition, we found biallelic ACAGG repeat expansion in one patient, indicating ACAGG repeat expansion might cause CANVAS. Clinically, we found one patient with sleep apnea syndrome, which has not been reported previously. Thus, this study might expand the clinical and genetic spectrum of CANVAS.

## Introduction

Recently, a biallelic expansion of an intronic AAGGG repeat in the replication factor C subunit 1 (*RFC1*) gene was reported to cause cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS), and to be a common cause of late-onset ataxia [1–3]. CANVAS is an autosomal recessive, adult-onset, and slowly progressive neurodegenerative disorder associated with chronic cough and occasionally autonomic dysfunction. To date, most patients confirmed clinically and genetically have been reported in European populations [1–3]. In Japan, there has been one reported case diagnosed genetically [4] and two clinically [5, 6]. Herein, we further reveal the clinical and genetic features of CANVAS in Japanese late-onset ataxia patients.

## Clinical and genetic study

This study was approved by our institutional review board, and written informed consent was obtained from all the participants.

We screened 37 patients with late-onset cerebellar ataxia (age of onset: from 48 to 80 years) comprising 25 patients with autosomal recessive or undecided hereditary transmission from 23 families and 12 sporadic ones. The term "familial cases" used in this study refers specifically to either patients with consanguineous parents, or patients with affected siblings. In total, we identified ten families with consanguineous marriages and 13 families with affected siblings born to non-consanguineous parents. Genomic DNA was isolated from peripheral blood leukocytes using standard methods. All patients were negative for SCA1, SCA2, MJD, SCA6,

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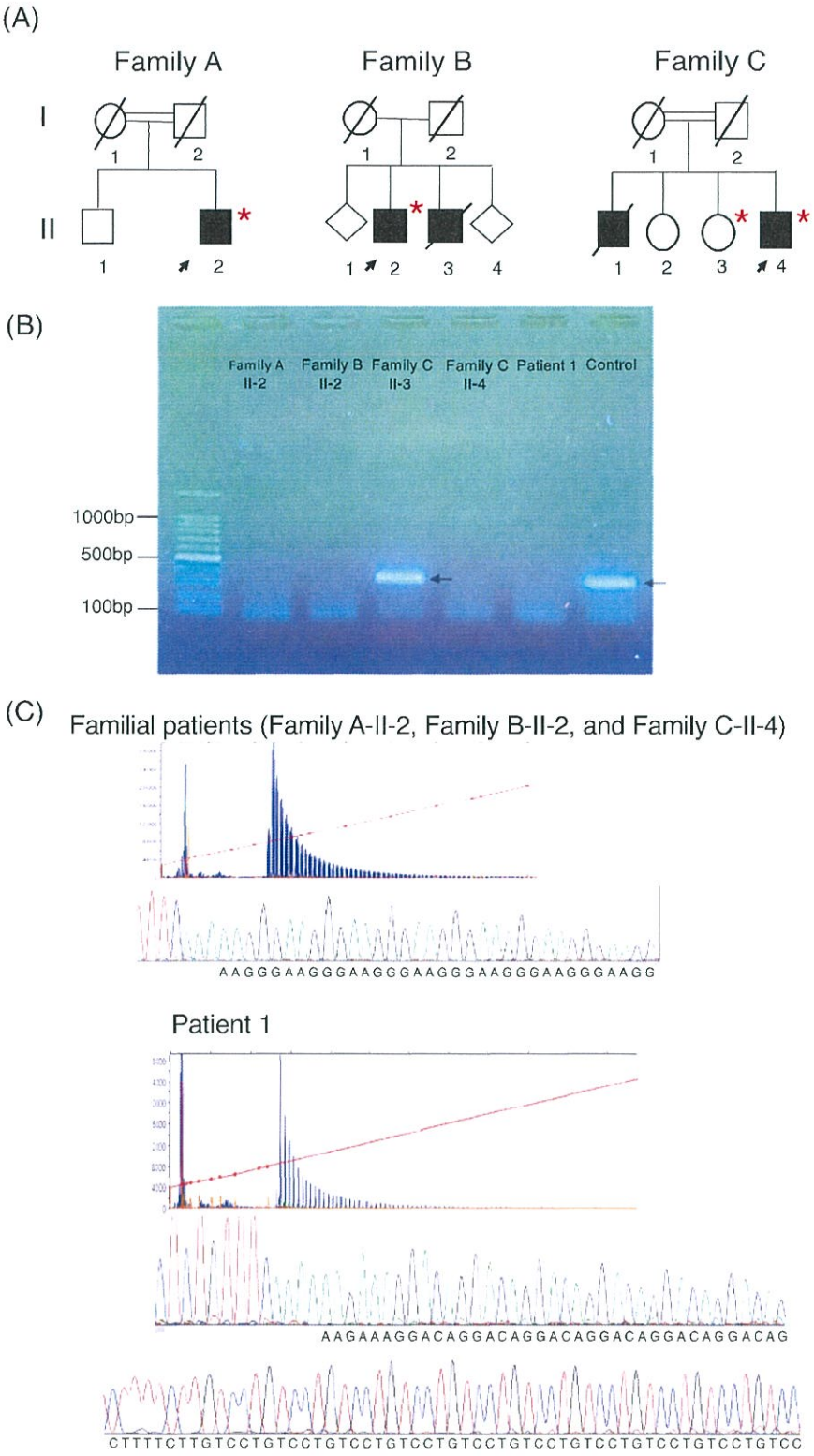


**Fig. 1** **a** Pedigree chart. The squares indicate males and the circles females. CANVAS patients are indicated by filled symbols. The asterisks indicate genotyped individuals. **b** Short-range PCR. Genomic DNA from patients (Family A-II-2, Family B-II-2, Family C-II-3, Family C-II-4, and Patient 1) and one unaffected negative control was analyzed for the presence of a non-expanded *RFC1* allele. PCR analysis of the *RFC1* repeat tract failed to reveal the control 348 bp reference product in patients (Family A-II-2, Family B-II-2, Family C-II-4, and Patient 1) using standard conditions. The unaffected negative control and one healthy family member with a heterozygous expanded RFC1 allele (Family C-II-3) had the 348 bp PCR product as expected (arrows). **c** RP-PCR and Sanger sequencing. RP-PCR targeting the AAGGG repeated unit was performed in three familial patients (Family A-II-2, Family B-II-2, and Family C-II-4). The results gave saw-toothed ladders. Sanger sequencing of long-range PCR products revealed the AAGGG pentanucleotide repeat unit in these patients. RP-PCR targeting the ACAGG repeated unit revealed a saw-toothed ladder in Patient 1. Sanger sequencing of long-range PCR products revealed the ACAGG pentanucleotide repeat in Patient 1, and reconfirmed the complementary TGTCC pentanucleotide repeat observed on sequencing with a reverse primer

or SCA31 on molecular testing. The pedigrees of three families with CANVAS diagnosed clinically are shown in Fig. 1a.

Standard flanking PCR, long-range PCR, repeat-primed PCR (RP-PCR), and Southern blotting were conducted according to the previous report [1]. First, we performed

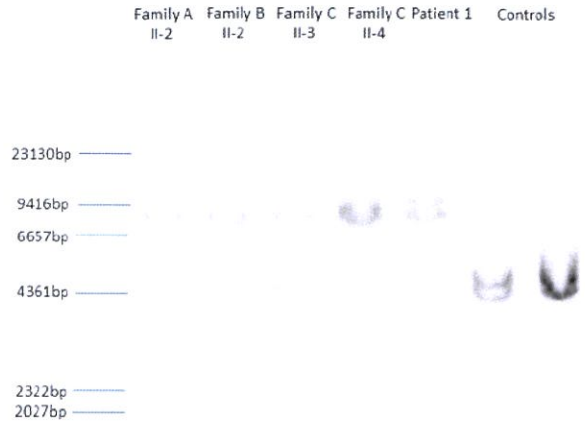


**Fig. 2** Southern blot of genomic DNA. CANVAS patients showed two discrete or overlapping bands approximately ranging from 8 to 11 kbp. The unaffected negative controls showed two discrete or overlapping bands within normal range (about 5 kbp). The unaffected sibling (Family C-II-3) carried one expanded and one normal allele

standard flanking PCR, which amplifies across the repeat tract, to rapidly screen for the presence of a non-expanded allele at the *RFC1* locus. The amplification of a 348 bp fragment indicates at least one allele is not expanded. Conversely, the complete absence of a PCR product provides indirect evidence of a repeat expansion affecting both alleles of the *RFC1* locus. Analysis of all samples revealed the complete absence of a PCR product in only four subjects, who happened to be previously clinically diagnosed CANVAS patients (three familial and one sporadic). On the other hand, the other patients (22 familial and 11 sporadic) who showed the presence of a non-expanded allele did not have any clinical findings suggestive of CANVAS except for cerebellar ataxia. It should be noted that one unaffected sibling (Family C-II-3) had the 348 bp PCR product, suggesting the presence of at least one allele which could be amplified by PCR (Fig. 1b).

Second, long-range PCR was performed for the four samples screened out by short-range PCR. Sanger sequencing revealed that the three familial patients had biallelic AAGGG repeat expansions. Interestingly, the one sporadic patient showed a biallelic ACAGG repeat expansion sequenced from both sides of the repeat (Fig. 1c).

Third, we performed RP-PCR for the three familial patients with primers targeting the mutant AAGGG pentanucleotide unit. We also performed RP-PCR for the sporadic patient with primers targeting the mutant ACAGG pentanucleotide unit. We reconfirmed the presence of AAGGG repeat expansion in all the three familial patients, and the presence of ACAGG repeat expansion in the sporadic patient (Fig. 1c).

Finally, we performed Southern blot hybridization to confirm the length of the repeat expansion. Biallelic expansions could be visualized as two distinct bands in individuals carrying expansions of different sizes, or one thick band if the expanded alleles were of similar size. The four patients who exhibited an AAGGG repeat or ACAGG repeat expansion on Sanger sequencing were confirmed by the presence of biallelic large expansions. The unaffected sibling (Family C-II-3) who showed a 348 bp PCR fragment on flanking PCR carried one expanded and one normal allele as expected (Fig. 2).

Table 1 shows the clinical features of the three patients with AAGGG repeat expansions and the one with ACAGG repeat expansions in the *RFC1* gene. The clinical features of the four patients were typical of CANVAS, including cerebellar ataxia, bilateral vestibulopathy, and sensory neuropathy. Vestibular function was evaluated by the caloric test. Family A-II-2 had obstructive and central mixed sleep apnea (Supplementary Fig. 1), and Family C-II-4 showed exaggerated deep tendon reflexes in the upper and lower extremities, and bilateral Babinski signs.

## Discussion

The genetic basis of CANVAS has now been determined and validated in two studies [1, 2]. In Europeans, the expansion frequency is 22% in sporadic patients with late-onset ataxia [1]. Meanwhile, we found that three familial patients and one sporadic one had *RFC1* intronic pentanucleotide repeat expansions, and the frequency of *RFC1* intron expansions is 12% in familial patients and 8.5% in sporadic ones. Thus, the disease frequency in Japanese patients with CANVAS might be lower than that in European ones. In 91 Chinese patients with sporadic late-onset ataxia, no biallelic intronic AAGGG repeat expansion in *RFC1* was found [7]. Therefore, the frequency of CANVAS caused by biallelic *RFC1* AAGGG repeat expansions in Asians might be lower than that in Europeans, which is similar to the specific ethnic distribution of the Friedreich ataxia GAA expansion [8]. Since the sample size in the present study was limited, further elucidation of the carrier frequency of AAGGG repeat expansions in the Japanese population would contribute to elucidation of the prevalence of CANVAS in Japan.

We also found that biallelic ACAGG repeat expansion might have been responsible for CANVAS in one patient diagnosed clinically. The patient showed clinical features of cerebellar ataxia, vestibular failure, sensory neuropathy, chronic cough, which are characteristics of CANVAS. This novel repeat configuration has the same C/G content as the

**Table 1** Clinical features of the patients with familial or sporadic CANVAS

| | Family A-II-2 | Family B-II-2 | Family C-II-4 | Patient 1 |
|---|---|---|---|---|
| Sex | M | M | M | M |
| Age at onset | 48 | 77 | 64 | 55 |
| Age at examination | 60 | 89 | 70 | 72 |
| Inheritance | AR | AR | AR | S |
| Cerebellar ataxia | + | + | + | + |
| Neuropathy | + | NA | + | + |
| Vestibular failure | + | + | + | + |
| Chronic cough | + | NA | + | + |
| Upper limb reflexes | Normal | NA | Exaggerated | Normal |
| Lower limb reflexes | Normal | NA | Exaggerated | Decreased |
| Babinski signs | −/− | − | +/+ | −/− |
| Sleep apnea | + | − | − | − |
| MRI findings | Cerebellar atrophy | Cerebellar atrophy | Cerebellar atrophy | Cerebellar atrophy |
| Repeat expansion | AAGGG | AAGGG | AAGGG | ACAGG |

*M* male, *AR* autosomal recessive, *S* sporadic, *NA* not available

well-established disease-causing AAGGG repeat expansion and also showed a similar expansion size on Southern blot (Fig. 2). The short tandem repeat associated with CANVAS in the second intron of *RFC1* shows a lot of genetic heterogeneity. Expansions of AAAAG, AAAGG, AAGAG, and AGAGG have been reported but all were considered as being non-pathogenic [1, 3, 9]. Notably, AAAAG and AAAGG expansions tend to be smaller in size than that of pathogenic AAGGG repeats [1]. Further studies are required to elucidate the molecular mechanism by which ACAGG repeat expansion could cause CANVAS, and the identification of biallelic ACAGG expansion would provide clues as to the pathogenicity of these repeats.

Clinically, we found a male patient with sleep apnea syndrome (SAS), which has not been reported in CANVAS previously. He showed obstructive, central, and mixed SAS. The neuropathological findings of CANVAS indicated that there were no lesions in the brainstem or diencephalon, which are supposed to be the center of respiration and sleep [10]. Further neuropathological studies to reveal subtle microstructural changes in the brainstem of CANVAS patients might be required. The recognition of sleep disorders in CANVAS might be considered as well so as not to overlook a treatable symptom. In addition, Family C-II-4 presented bilateral Babinski signs, which had not been previously reported in CANVAS. This patient was not complicated by other neurological diseases, and the neuroimaging was unremarkable except for the finding of cerebellar atrophy. Similarly, brisk deep tendon reflexes in the upper and lower limbs were found in a quarter of cases reported in Europeans [9]. Therefore, we speculate that a pyramidal tract sign might be involved in the pathophysiology of CANVAS, which might need further investigation.

In summary, we demonstrated a relatively low frequency of CANVAS in Japanese patients with late-onset ataxia, possible responsibility of biallelic ACAGG repeat expansion for CANVAS, and SAS as a possible new symptom of CANVAS. Thus, this study might expand the clinical and genetic spectrum of CANVAS. *RFC1* expansions should be examined in patients with sensory ataxic neuropathy with or without cerebellar dysfunction and/or bilateral areflexia. In a patient with adult-onset ataxia, the presence of cough is also suggestive and should prompt testing.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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