Detection of the ACAGG Repeat Motif in *RFC1* in Two Dutch Ataxia Families

Cerebellar ataxia, neuropathy and vestibular areflexia Syndrome (CANVAS) is a genetic disorder caused by biallelic repeat expansions in intron 2 of *RFC1*. An AAGGG repeat expansion motif was discovered to be the cause of CANVAS and was identified in patients with the full or incomplete CANVAS phenotype across multiple populations.[1]

Recently, a novel pathogenic ACAGG repeat expansion motif was found exclusively in the Asian population.[2-6] In addition to detecting this novel motif, an expansion of the phenotype with motor neuropathy (fasciculations) was reported.

Here, we report three Dutch CANVAS patients carrying this novel ACAGG repeat expansion motif. The first patient is unrelated to the other 2 patients and was found to be compound heterozygous for the two pathogenic repeat expansions: ACAGG and AAGGG. The second patient was homozygous for the ACAGG repeat expansion. In both, the ACAGG repeat was a complex repeat: $(ACAGG)_{1400}(ACAGA)_{108}(AAAAG)_{14}$ for the first patient and $(ACAGG)_{1100–1400}(ACAGA)_{115}(AAAAG)_{16}$ for the second patient. The third patient was a deceased sibling of the second patient; DNA from this sibling was no longer available, but previous testing showed a singleshare region of homozygosity containing *RFC1*, indicating that she was also homozygous for the ACAGG repeat.

The original AAGGG expansion allele and the "Asian" ACAGG expansion allele share a core haplotype of four single-nucleotide polymorphisms (SNPs) in *RFC1*, suggesting a single origin of these alleles.[2] The Dutch patients that we report here had no Asian roots by history. For the 2 siblings, Dutch ancestry was confirmed back to AD 1800. Haplotype analysis in patients 1 and 2 surprisingly showed that two of the four SNPs in the core haplotype around ACAGG were different from the reported haplotype (-RS2066790 > G and RS17584703 > T; core haplotype contains A and C, respectively). Additionally, an extended 1.2-Mb haplotype around the ACAGG repeat was shared between the Dutch patients. This suggests that the complex ACAGG repeat expansion in *RFC1* in the Dutch patients may have a different origin from that in Asians.

A clinical evaluation of 2 patients and examination of medical records from the deceased third patient were performed, showing the core CANVAS phenotype and some additional features. An overview of the phenotype of the 3 patients is presented in Table 1.

Although motor neuropathy with fasciculations, muscle atrophy, and weakness were suggested to be specific for patients carrying ACAGG expansions, we detected only fasciculations and elevated creatine kinase in 1 Dutch patient. Moreover, these features were recently found in CANVAS patients with AAGGG expansions,[6,7] indicating that motor neuropathy and fasciculations can be part of the *RFC1*-associated syndrome independent of the repeat motif.

In conclusion, we report 3 additional non-Asian CANVAS patients with the novel ACAGG repeat expansion motif in *RFC1*. Haplotype analysis showed that the ACAGG allele in these Dutch patients very likely has originated independently from the Asian allele. Our clinical observations do not confirm a specific phenotypic signature of this repeat composition, but more patients need to be identified to robustly study genotype–phenotype correlations.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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TABLE 1 *Genetic and clinical features, demographics and results of auxiliary investigations*

Genetic and clinical features	Patient 1	Patient 2	Patient 3*	Totals
Repeat expansion	AAGGG/ACAGG	ACAGG/ACAGG	ACAGG/ACAGG	
Number of repeats	~770/~1500	~1570/~1270	~1570/~1270[a]	
Age at onset	71	50	~55	
Age at evaluation	75	66	62	
Disease duration (y)	4	16	7	
Sex	Female	Male	Female	
Family history or consanguinity	No	Yes[b]	Yes[b]	
First symptom/sign	Walking instability	Difficulty dosing strength, myoclonus hands	Sensory neuropathy	
Cerebellar ataxia				
Oculomotor	+	+	+	3/3
Speech	−	+	+	2/3
Gait	+	+	+	3/3
Limb	+/−	+	+	3/3
SARA score	13	22	NA	
Sensory disturbances	+	+	+	3/3
Vestibular dysfunction	+	+	NA	2/2
Autonomic dysfunction	+	−	−	1/3
Chronic cough	+	+	NA	2/2
Muscle weakness	−	+	+	2/3
Muscle atrophy	−	+	+	2/3
Fasciculation	−	+	−	1/3
Tendon reflexes	Absent	Absent	Absent	
Pyramidal sign	−	−	−	0/3
Parkinsonism	−	−	−	0/3
Sleep disorders	−	+ (apnea, RLS)	NA	1/2
Cognitive impairment	+	−	−	1/3
Creatine kinase	NA	Slightly elevated (261 μmol/L)	NA	
EMG	Sensory neuronopathy	Sensorimotor axonal polyneuropathy	Sensorimotor axonal polyneuropathy	
MRI brain	Cerebellar atrophy	Cerebellar atrophy	No cerebellar atrophy	

*Patient deceased.
[a]Assumed repeat expansion based on shared ROH with patient 2.
[b]Patients 2 and 3 are siblings with consanguineous parents (third cousins).
Abbreviations: SARA, Scale for the Assessment and Rating of Ataxia; NA, not available; RLS, restless legs syndrome; EMG; electromyography; MRI, magnetic resonance imaging; ROH, region of homozygosity.

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