



Cerebellar ataxia-dominant phenotype in patients with *ERCC4* mutations

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Abstract

Autosomal recessive cerebellar ataxias (ARCAs) are clinically and genetically heterogeneous neurological disorders. Through whole-exome sequencing of Japanese ARCA patients, we identified three index patients from unrelated families who had biallelic mutations in *ERCC4*. *ERCC4* mutations have been known to cause xeroderma pigmentosum complementation group F (XP-F), Cockayne syndrome, and Fanconi anemia phenotypes. All of the patients described here showed very slowly progressive cerebellar ataxia and cognitive decline with choreiform involuntary movement, with young adolescent or midlife onset. Brain MRI demonstrated atrophy that included the cerebellum and brainstem. Of note, cutaneous symptoms were very mild: there was normal to very mild pigmentation of exposed skin areas and/or an equivocal history of pathological sunburn. However, an unscheduled DNA synthesis assay of fibroblasts from the patient revealed impairment of nucleotide excision repair. A similar phenotype was very recently recognized through genetic analysis of Caucasian cerebellar ataxia patients. Our results confirm that biallelic *ERCC4* mutations cause a cerebellar ataxia-dominant phenotype with mild cutaneous symptoms, possibly accounting for a high proportion of the genetic causes of ARCA in Japan, where XP-F is prevalent.

Introduction

Cerebellar ataxia (CA) is a clinically and genetically heterogeneous neurological disorder. Recent advances in

whole-exome sequencing (WES) have contributed significantly not only to the discovery of novel mutated genes [1], but also to the identification of genetic causes of CA,

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even in unrecognized phenotypes [2], and to the comprehensive genetic diagnosis of CA patients [3, 4].

In this study, we identified three index Japanese patients from unrelated families who exhibited young adolescent- or midlife-onset CA with biallelic single nucleotide variants (SNVs) of *ERCC4*; two of these patients were found through WES analysis of 55 unrelated Japanese patients with autosomal recessive or sporadic CA and spastic paraplegia (SPG) of unknown cause. All four patients described in this study showed similar phenotypic features of CA, with choreiform involuntary movements and brain atrophy that included the cerebellum and brainstem. Although recessive loss-of-function mutations in *ERCC4* have been known to cause xeroderma pigmentosum complementation group F (XP-F) [5], Cockayne syndrome (XP-F/CS) [6], and Fanconi anemia (XP-F/CS/FA) phenotypes [6, 7], our patients lacked subjective skin symptoms as well as morphological, developmental, and hematological abnormalities. However, a careful history and examination revealed mild skin findings consistent with xeroderma pigmentosum (XP). Furthermore, we conducted an unscheduled DNA synthesis (UDS) assay of fibroblasts from the patient and revealed that removal of ultraviolet (UV)-induced DNA damage was impaired. While previous reports have described a low incidence of XP-F with neurological involvement [8], very recently a CA-dominant phenotype was described as a rare cause of autosomal recessive CA (ARCA) [9]. Our study also indicates that biallelic *ERCC4* mutations cause a CA-dominant phenotype with mild symptoms of XP, and may account for a high proportion of the genetic causes of ARCA in Japan.

Materials and methods

This study included 55 unrelated Japanese patients with autosomal recessive or sporadic CA ($n = 23$) or SPG ($n = 32$), with no mutations in genes known to cause either condition. Clinical information, radiological images, and blood samples were obtained from the patients and their family members after written informed consent was provided. Experimental protocols were approved by the Institutional Review Board of Yokohama City University Faculty of Medicine. All experiments were performed in accordance with institutional guidelines.

To identify pathogenic mutations, we performed WES on all 55 patients. Genomic DNA was processed using the SureSelect Human All Exon Kit v3~v5 (Agilent Technologies, Santa Clara, CA), and sequenced on an Illumina HiSeq 2000/2500 with 101-bp paired-end reads. Alignment, variant calling, and annotation were performed using Novoalign (<http://www.novocraft.com/>), Picard (<http://picard.sourceforge.net/>), Genome Analysis Toolkit ([\[www.broadinstitute.org/gatk/index.php\]\(http://www.broadinstitute.org/gatk/index.php\)\), and ANNOVAR \(<http://www.openbioinformatics.org/annovar/>\), as described previously \[10\]. For each patient, SNVs and insertion/deletions \(indels\) were extracted based on information regarding rare variants with a minor allele frequency <1% in dbSNP137, located in exons or splice sites \(within 10 bp of the boundaries\), and with a frequency <1% in exome data from 575 “in house” Japanese controls. Among these, we included frameshift, nonsense, or missense SNVs that were either homozygous or potentially compound heterozygous and were predicted to be disruptive to protein function when analyzed with Polyphen2 \[11\] and SIFT \[12\]. We carefully checked variants in common genes shared in two or more patients.](https://</p></div><div data-bbox=)

UDS assays were performed on cultured skin fibroblasts obtained from a normal control subject, patient 1, and a patient with XP-A, as described previously [13]. Briefly, cells were plated in multiwell plates (96-well) and UV-irradiated (20 J/m² of 254 nm UVC). Cells were incubated for 4 h in medium supplemented with 5 μM 5-ethynyl-2'-deoxyuridine (EdU) after UV irradiation. Incorporated EdU was conjugated with Alexa Fluor 488 azide and nuclei were stained with DAPI. Fluorescent (Alexa Fluor 488 azide and DAPI) images were obtained and data were analyzed using the ArrayScan VTI (Thermo Fisher Scientific, Waltham, MA).

The minimal erythema dose (MED) for UVB was measured using UV source Dermaray-100 (Toshiba Medical, Tokyo, Japan), with FL20S-E-30/DMR lump for patient 1, 2 and 3, or Dermaray-320 (Toshiba Medical) with FL32S-E-12/DMR lump for patient 4.

Results

ERCC4 mutations in CA patients

We found a homozygous missense *ERCC4* variant in one patient (patient 3) and potentially compound heterozygous variants of *ERCC4* in another patient (patient 1) with a similar CA phenotype, both from the Yokohama City University cohort ($n = 55$). Patient 2, who was the sister of patient 1 and whose primary symptom was secondary amenorrhea, had the same variants in *ERCC4*. We also found another homozygous missense *ERCC4* variant in one patient (patient 4) who was included in the additional CA cohort ($n = 11$). Although biallelic loss-of-function mutations in *ERCC4* have been known to cause XP-F, XP-F/CS, and XP-F/CS/FA phenotypes, the four patients described here did not demonstrate skin symptoms, developmental abnormalities, or anemia as critical clinical features. Sanger sequencing of the patients and the parents of patients 1 and 2 confirmed that the mutations were either homozygous or

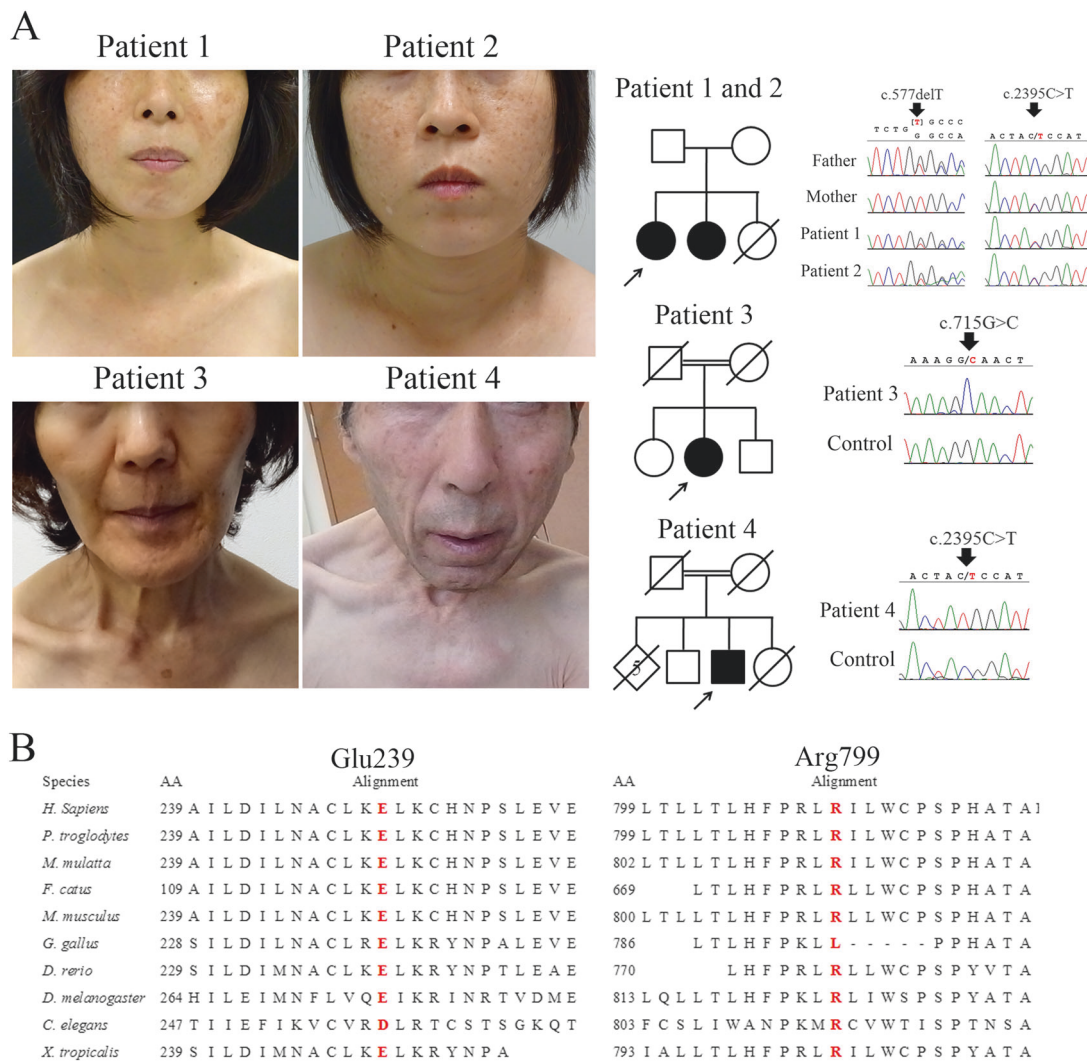


Fig. 1 Clinical manifestations of patients with *ERCC4* mutations. **a** Pictures of patients' exposed skin areas and their family pedigrees (Patients 1–4) are shown, as well as an electropherogram from Sanger sequencing. The arrow indicates the index patient in each family.

compound heterozygous, and were thus compatible with an autosomal recessive model (Fig. 1a). Patients 1, 3, and 4 were negative for the genetic alterations associated with spinocerebellar ataxia (SCA) 1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, SCA 31, and dentatorubral-pallidolusian atrophy. Among the detected variants, c.2395C>T [p.Arg799Trp] in *ERCC4*, which was found in patients 1, 2, and 4, has been reported as a pathogenic mutation in XP-F [14], while c.577del [p.Trp193Glyfs*6], found in patients 1 and 2, results in protein truncation, indicating that these variants were definitively pathological. In patient 3, c.715G>C [p.Glu239Gln] in *ERCC4* occurred in a highly conserved amino acid (Fig. 1b) and has been consistently predicted to be disruptive to protein function when analyzed with multiple tools, including Polyphen2, SIFT, and Mutation Taster [11, 12, 15]. The minor allele

Patients 3 and 4 were children born to first-cousin parents. The father of patients 1 and 2 has retinitis pigmentosa, their sister died from glioblastoma, and two sisters of patient 4 died from cancer. **b** Two missense mutations occurred at evolutionarily conserved amino acids

frequency (MAF) of the c.2395C>T mutation was 0.0052 (3/575) in our "in-house" Japanese control exome, and 0.00051 (62/121398) in the Exome Aggregation Consortium (ExAc) database (<http://exac.broadinstitute.org/>), respectively. The c.577del and c.715G>C mutations were not found in our "in-house" Japanese control exome or in the ExAc database. Patients 1, 3, and 4 did not have any homozygous or potentially compound heterozygous pathological mutations in genes related to other ARCAs or to autosomal recessive hereditary spastic paraplegias.

Clinical presentations

The patients' clinical presentations are summarized in Table 1. Early developmental milestones were normal in all cases. Age of disease onset ranged from 25 to 43 years.

Table 1 Clinical features of autosomal recessive cerebellar ataxia with *ERCC4* mutations

Patient (sex)	1 (female)	2 (female)	3 (female)	4 (male)
<i>ERCC4</i> mutation(s)	c.577del [p.Trp193Glyfs*6] /c.2395 C>T [p.Arg799Trp] compound heterozygous		c.715 G>C [p.Glu239Gln] homozygous	c.2395 C>T [p.Arg799Trp] homozygous
Consanguinity	–	–	+	+
Age at examination	38	35	66	66
Age of onset (years)	30	25	43	28
Mental retardation	–	–	–	–
Symptoms at onset	Dysarthria, involuntary movement	Dysarthria	Gait instability	Gait instability, involuntary movement
Cognitive deterioration	±MMSE 29/30	MMSE 30/30	+MMSE 26/30	+MMSE 17/30
Eye movement	Saccadic	Smooth	Saccadic	Saccadic
Dysarthria	+	+	+	+
Muscle tone	hypo	Normal	hypo	Normal, Gegenhalten +
Muscle weakness	–	–	–	–
Truncal ataxia	+	+	+	+
Limb ataxia	+	+	+	+
SARA	9/40	6.5/40	11.5/40	29/40
Extrapyramidal signs	–	–	–	–
Involuntary movements	Choreiform movements	Choreiform movements	Choreiform movements	Choreiform movements
Sensory involvements	–	–	Mild impairment of vibration and position sense	–
Hyperreflexia	+	+	+	+
Extensor plantar responses	–	–	–	–
Peripheral neuropathy (including nerve conduction study)	–	–	–	–
Bladder disturbance	–	–	–	–
Photosensitivity (severe sunburn)	In childhood	In childhood	In childhood	–
Solar lentiginos/Freckles	Normal~very mild	Mild	Normal~very mild	Mild
Skin atrophy	–	–	–	–
Skin cancer	–	–	–	–
MED for UVB (normal ≥ 132 mJ/cm ²)	132 mJ/cm ²	66 mJ/cm ²	66 mJ/cm ²	30 mJ/cm ²
Anemia	–	–	–	–
Audiological assessment (average of right and left at 500, 1000, 2000, and 4000 Hz)	15, 5, 5, and 15 dB	NA	35, 32.5, 40, and 47.5 dB	NA, 50, NA, and 40 dB
Others	secondary amenorrhea	secondary amenorrhea		Epilepsy +
Cerebellar atrophy	+	+	+	+
Cerebral atrophy	+	+	+	+
Brain-stem atrophy	+	+	+	+
EEG	6 Hz phantom +	Normal	NA	Bilateral spikes on photic stimulation

NA not available

Initial symptoms were gait unsteadiness, dysarthria, and/or involuntary movements. In addition, initially mild cognitive decline gradually worsened. Neurological symptoms were very slowly progressive. On neurological examination, all patients showed impairment of smooth eye pursuit,

dysarthria, and limb and truncal ataxia. Three patients showed choreiform involuntary movements of the neck, tongue, and upper and lower extremities. Patient 1 exhibited very mild choreiform movements in her upper extremities. All patients' tendon reflexes were slightly increased.

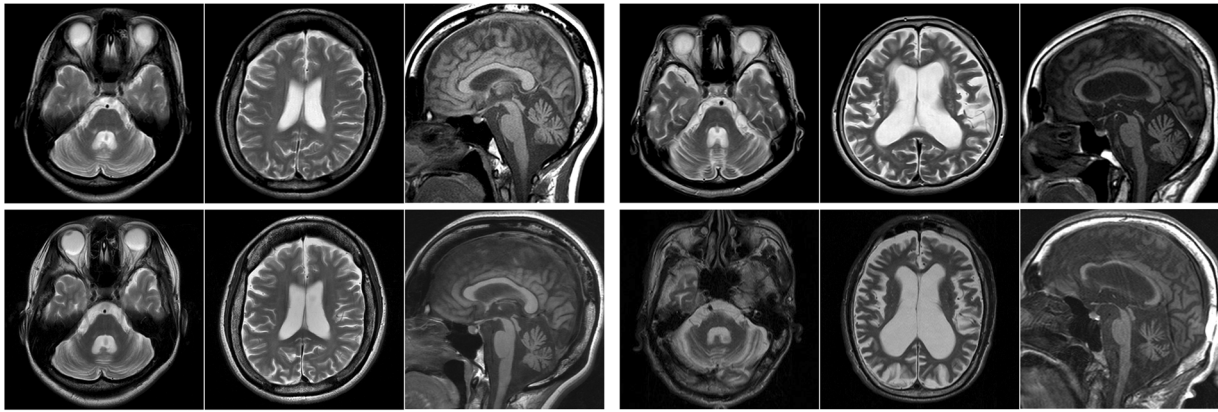


Fig. 2 Brain MRI of patients. Axial sections of T2-weighted images and sagittal sections of T1-weighted images are shown. Left panels show MRI results of Patient 1 at 31 years of age (upper) and at 37 years of age (lower). Right panels show MRI results of Patient 3 at

years of age (upper) and of Patient 4 at 66 years of age (lower). Age-dependent atrophy of the brain, including the cerebellum and brainstem, is observed

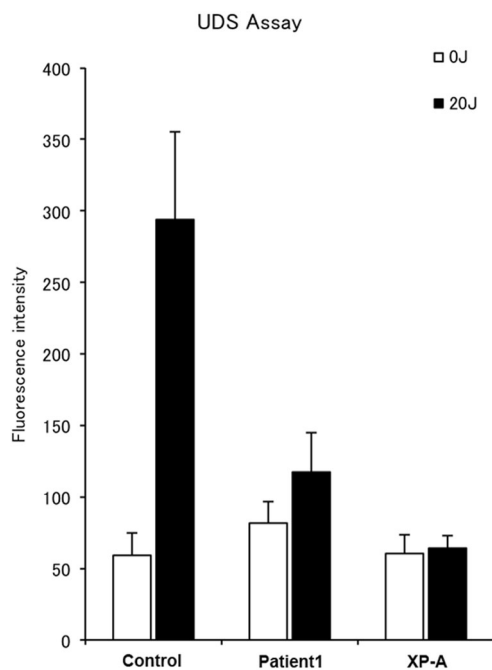


Fig. 3 UDS assay of fibroblasts. Fibroblasts from patient 1 show reduced UDS level compared to control cells (triplicate; filled bars, 20 J/m² UVC; open bars, no UVC)

Laboratory tests were essentially normal, including blood count. A nerve conduction study disclosed no abnormalities. Brain MRI in all patients showed brain atrophy, especially in the cerebellum and brainstem, along with dilation of the ventricles. The brain atrophy was progressive in an age-dependent manner (Fig. 2). The electroencephalogram (EEG) of patient 3, who had a seizure history, showed abnormal spikes on photic stimulation. None

of the patients experienced problems due to their cutaneous symptoms. During re-evaluation, a history of severe sunburn in childhood was confirmed in three patients, and normal to mildly abnormal solar lentigines and freckles on exposed skin areas were confirmed in all patients (Fig. 1a). The MED for UVB showed slight hypersensitivity to UVB in patients 2, 3, and 4 (Table 1). A UDS assay of fibroblasts from patient 1 revealed decreased level of UDS (Fig. 3), indicating impaired function of the nucleotide excision repair (NER) system.

Discussion

ERCC4 encodes XPF, a component of ERCC1/XPF endonuclease, which is mainly involved in incision of DNA damages in NER and interstrand crosslink (ICL) repair, and its mutations have been found in XP-F, XP-F/CS, and XP-F/CS/FA phenotypes [5–7]. A previous study demonstrated that impairment of global genome NER (GG-NER), transcription-coupled NER (TC-NER), and ICL repair were related to the XP, CS, and FA phenotypes, respectively [6]. Mutations of *ERCC4* variably affect these repair functions and cause diverse phenotypes [6, 7]. Neurological symptoms have been identified in several XP-F patients with *ERCC4* mutations [14, 16], but their incidence seems to be very low [8]. In accordance with the neurological presentation of XP-F patients described in previous reports [14, 16], patients in this study showed cognitive deterioration, CA, choreiform involuntary movements, and brain atrophy. Of note, none of the patients considered their skin manifestations to be symptoms of a disease, and indeed, the nature of these lesions remained unclear until genetic diagnosis. Such skin findings are likely to be missed unless the clinician has a low threshold for diagnostic evaluation.

To confirm that the skin symptoms were due to a pathological condition, we conducted a UDS assay to evaluate NER function in patient 1, and revealed that this CA-dominant XP-F patient showed decreased level of UDS. Furthermore, three of the four patients were hypersensitive to UVB, indicating that the patients were at potential risk of skin disorders such as skin cancer. Very recently, two individuals in a Caucasian cohort of CA patients were found to have *ERCC4* mutations with chorea, minimal skin symptoms, and cerebellar and cortical atrophy on MRI, and thus *ERCC4* mutations were reported as a rare cause of ARCA [9, 17]. Our cases similarly showed age-dependent, progressive atrophy of the cerebellum and brainstem, thus further characterizing the clinical phenotype and MRI findings in CA-dominant XP-F. As of this writing, eight cases, including the four in this study, have been reported as having XP-F with neurological involvement; [9, 14, 16] six out of eight have the c.2395C>T [p.Arg799Trp] mutation in at least in one allele. Considering the allele frequency of the c.2395C>T [p.Arg799Trp] mutation in Japanese control exome data (MAF = 0.0052), it is likely that the disease is underdiagnosed in CA cohorts, especially in Japan, because of the mild skin phenotype. Loss-of-function mutations of genes related to DNA repair pathways, for instance *TDP1*, *APTX*, and *ATM*, have been known to cause ARCA [18]. When complexed with ERCC1, XPF nuclease is known to incise damaged DNA strands 5' to the lesion in the process of NER, and is one of the major mechanisms for repairing DNA damage [18]. *ERCC4* mutations should be recognized as a potential cause of ARCA.

In conclusion, we found biallelic *ERCC4* mutations in three families with adult-onset CA. *ERCC4* mutations should be considered to be an important cause of adult-onset cerebellar ataxia and cognitive decline with choreiform movements as well as cerebral and brainstem atrophy. Elevated cancer risk in these patients may also be important in their clinical management.

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Author contributions HD wrote the main manuscript and prepared the figure and the table. HT, NM, and FT revised the manuscript and gave conceptual advice. HD, SK, SM, SN, MK, AK, RK, and KO collected the clinical data and samples. HD, SM, RF, SI, SK, KT, MT, KT, MN, YT, NMi, and HS conducted the analysis of the genetic data.

Compliance with ethical standards

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

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