The PCR products were separated in a 2% agarose gel by electrophoresis and stained with ethidium bromide.

Results

Phenotypic features The mode of inheritance was autosomal dominant transmission because male-to-male transmission was observed in the pedigree (Fig. 1). The clinical findings in the ten afflicted patients are summarized in Table 1. Most cases showed hyperreflexia in all four extremities and spasticity of the lower limbs. Patellar contracture and ankle contracture were seen in one and three patients, respectively. In seven patients (70%), gait disturbance was noticed before 2 years of age. Four cases (40%) had urinary dysfunctions and eight cases (80%) demonstrated impaired vibration sense in the lower limbs. Some patients occasionally suffered from paroxysmal low backache or abdominal pain. All four female patients experienced miscarriages. According to clinical information on the patients III-7 and III-8, miscarriages could be due to infertility of unknown etiology.

Neuroimaging studies (data not shown) Cranial MRI showed no abnormalities in the three patients examined (III-8, IV-1, and IV-2). In patient III-8, spinal MRI revealed lumbar spondylosis and spondylotic canal stenosis at L4/5 to L5/S1, while SPECT using ^{99m}Tc-ECD showed hypoperfusion in the cerebellum and brainstem. In patient IV-1, spinal MRI revealed a narrow spinal canal at the middle cervical level and bulging of the nucleus pulposus in L4/L5 and L5/S1, while SPECT using ^{99m}Tc-ECD revealed slight hypoperfusion of the bilateral frontal and right temporal regions. In patient IV-2, spinal MRI revealed syringomyelia at the C6 and C7 levels, while SPECT using ^{99m}Tc-ECD showed no abnormalities.

Electrophysiological studies Electroencephalograms and visual-evoked potentials were normal in all three patients examined. On examination of the auditory brainstem responses, the bilateral I-III durations were prolonged (left, 2.67 ms; right, 2.47 ms; normal values, 2.0–2.4 ms) in patient IV-2. The short latency somatosensory potentials evoked by stimulation of the bilateral median nerves were normal, while those evoked by stimulation of the bilateral tibial nerves were below the detectable level in all three patients. On nerve conduction studies, the motor conduction velocities were normal. The compound muscle action potentials (CMAPs) of the median nerve were moderately reduced in two patients. Patient IV-1 showed a decrease in the proximal CMAP/distal CMAP ratio (1.55:8.67) in the median nerve. The distal motor latencies were within the

Table 1 Clinical findings for the ten patients in the present pedigree

Patient	Age/sex	Age at onset	Spasticity (UE/LE)	Hyperreflexia (UE/LE)	Subjective urinary dysfunction	Ankle clonus	Babinski reflex	Pes cavus	Vibration sense decrease	Miscarriage	Transfer
II-3	79/M	<2	−/+	+/contracture	−	Contracture	+	−	Severe		Wheelchair
II-5	74/F	<2	−/+	+/+	−	Contracture	+	−	Mild	+	Wheelchair
II-7	65/M	<2	−/+	−/+	+	Contracture	+	−	Severe		Wheelchair
III-6	52/F	40	−/+	+/+	+	+	+	−	Mild	+	Independent
III-7	50/F	47	−/+	+/+	+	+	+	−	Mild	+	Independent
III-8	45/F	<2	−/+	+/+	+	+	+	−	Mild	+	Wheelchair
III-9	37/M	–[a]	−/+	+/+	−	−	−	−	Moderate		Independent
III-10	35/M	<2	−/+	+/+	−	+	−	−	−		Independent
IV-1	25/M	<2	−/+	+/+	−	+	−	−	–		Independent
IV-2	18/M	<2	−/+	+/+	−	+	+	+	Mild		Cane

M male, *F* female, *UE* upper extremities, *LE* lower extremities

[a] No subjective symptoms

normal range or mildly prolonged. The sensory conduction velocities and the amplitudes of sensory nerve action potentials (SNAPs) were within the normal ranges or mildly reduced in the upper limbs. Sural nerve SNAPs were not detectable in two of the three patients examined. Needle electromyography showed reduced interference, which was greater in the lower limbs (all patients), and giant potentials that were thought to indicate a neurogenic pattern in the rectus femoris (patient IV-2).

CSF examination Lumbar punctures showed normal cell counts and normal protein and glucose levels. Mildly increased total tau levels were observed in the CSF of two of the three patients examined (patient III-8, 195 pg/ml; patient IV-1, 249 pg/ml; patient IV-2, 258 pg/ml; normal values, <200 pg/ml).

Neurophysiological studies The Hasegawa Dementia Scale—Revised scores for patients III-8, IV-1, and IV-2 were 26 out of 30, 28 out of 30, and 24 out of 30, respectively. Using the Wechsler Adult Intelligence Scale—Third Edition, the verbal, performance and full scale IQ values were determined to be 71, 84, and 75 in patient III-8; 77, 84, and 78 in patient IV-1; and 70, 65, and 65 in patient IV-2, respectively.

Genetic analyses Linkage analyses provided the highest multipoint LOD score of 2.64 at 2p23-21 (D2S165-D2S367-D2S2259; Fig. 2) where the *SPAST* gene is located. Although we sequenced the entire coding regions of *SPAST* by a direct sequencing method, we detected no substitutional mutations and no small insertions or deletions. We conducted real-time quantitative PCR analyses to evaluate the copy number variants of *SPAST* and its neighboring gene, *DPY30*. The results are shown schematically in Fig. 3a. The patients had

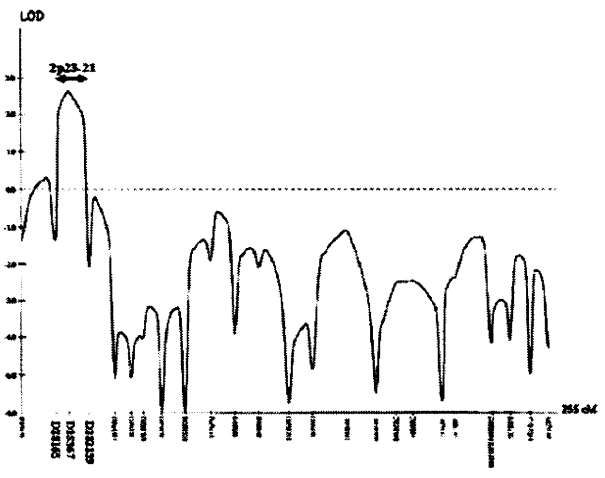

Fig. 2 Multipoint LOD scores at chromosome 2. The highest multipoint LOD score of 2.64 is observed at D2S367

single copies of exons 1 to 4 of *SPAST* and exons 1 to 3 of *DPY30*, but had two copies of exons 5 to 17 of *SPAST* and exons 4 and 5 of *DPY30*. *SPAST* and *DPY30* are adjacent genes in a head-to-head manner and their intergenic region is about 24 kb in length (Fig. 3a). On the other hand, the control members had two copies of all exons and introns examined. These data suggest that the patients had a large heterozygous deletion involving not only exons 1 to 4 of *SPAST* but also exons 1 to 3 of *DPY30*. To examine the deletion in detail, we performed PCR analyses using the specific primer pair DPY30in3.13F and SPASTin4.33R, resulting in a PCR product of 900 bp, in the patients examined by real-time PCR. Sequencing of the PCR product revealed the precise locations of the breakpoints of the recombination event and the size of the deletion (69,821 bp; Fig. 3b). The results of the deletion-specific PCR amplification are shown in Fig. 3c. A deletion-specific PCR fragment of 900 bp was observed in all the affected individuals (II-3, II-5, II-7, III-6, III-7, III-8, III-9, III-10, IV-1, and IV-2), but not in the unaffected individuals (II-1 and III-1). The normal allele was also amplified as a 555-bp fragment in all the affected and unaffected individuals, indicating a partial heterozygous deletion of *SPAST* and *DPY30* in the affected individuals.

Discussion

The cardinal clinical features of the present family were as follows: (1) slowly progressive spastic paraplegia, (2) decreased vibration sense at the ankles, (3) urinary disturbance, (4) early childhood onset, (5) mild cognitive impairment, (6) peripheral neuropathy, and (7) miscarriages in the female patients. The genetic features of the family were partial heterozygous deletion of not only *SPAST* but also *DPY30*, which is located upstream of *SPAST* and arranged in a head-to-head manner.

The clinical features (1) to (3) described above are common symptoms of SPG4. Regarding early childhood onset and the cognitive impairment in our cases, we do not consider these symptoms to be particularly rare in patients with SPG4 because there are many previous reports of such cases and it seems that these symptoms are not determined by the type of mutation [7–24, 28]. In our cases, lumbar punctures showed normal cell counts and normal protein and glucose levels, which were also observed in other previous cases [25, 26]. Although CSF tau in SPG4 patients has not been mentioned in previous studies, the elevated tau protein levels in the CSF of our cases are thought to be intriguing. tau is a microtubule-associated protein that is primarily localized in neurons [29, 30]. Increased CSF total tau levels have been reported in patients with mild cognitive impairment or Alzheimer disease [31]. In the

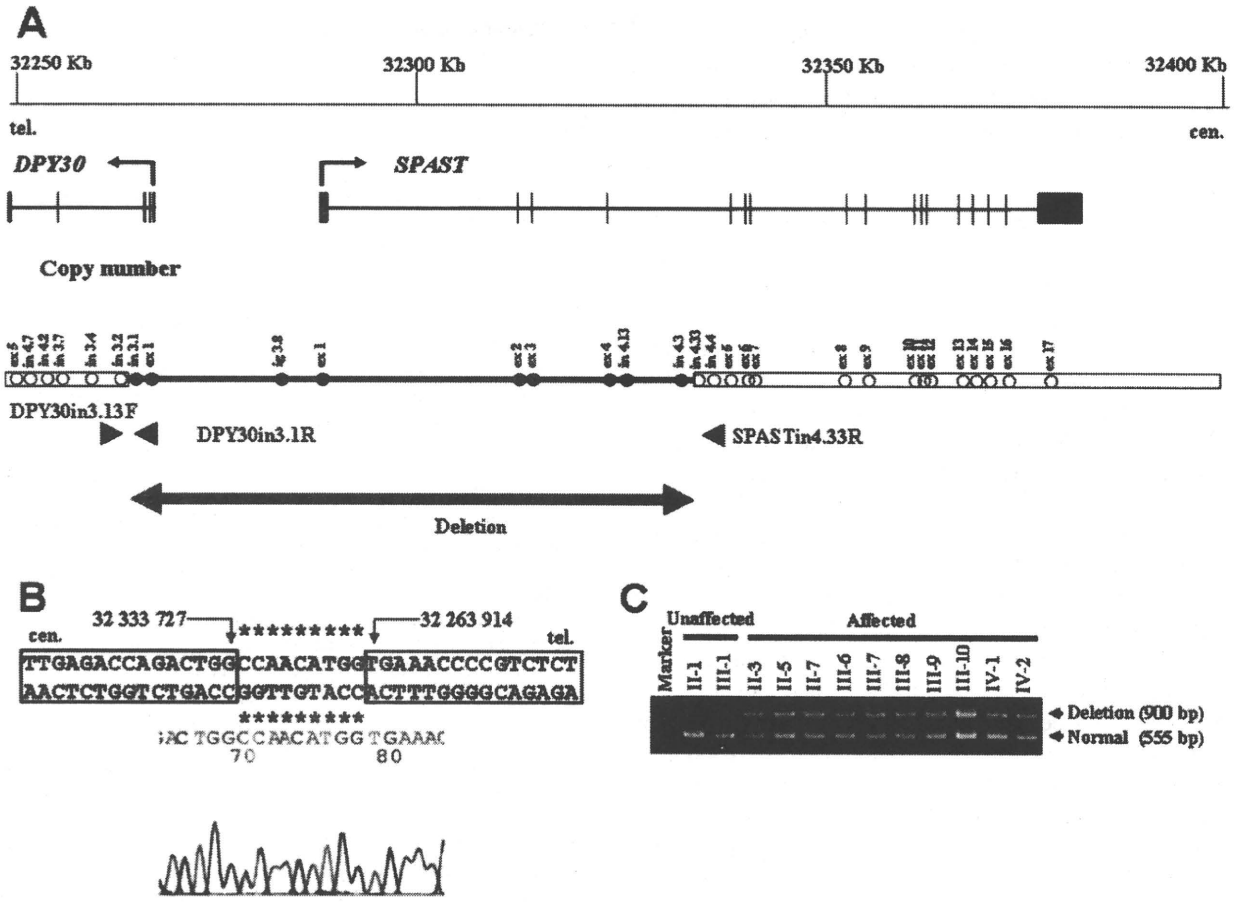

Fig. 3 Identification and analysis of the deletion in the present family. **a** Analysis of the copy numbers of *SPAST* and *DPY30* by real-time quantitative PCR. The structures of *SPAST* and *DPY30* are shown at the *top*. Exons are shown as *black lines* or *boxes*. *Arrows* indicate the transcription directions of each gene. *SPAST* and *DPY30* are composed of 17 and 5 exons, respectively, and arranged in a head-to-head manner. The regions where the copy numbers were determined by real-time quantitative PCR are shown schematically in the *middle* as exons (*ex*), introns (*in*), and intergenic regions (*ig*). *White circles* represent positions with two copies, and *black circles* represent positions with a single copy in the patients. The *single and double lines* show the deleted and non-deleted regions, respectively. The *arrowheads* indicate the PCR primers used to obtain the data shown in **b** and **c**. The genomic region deleted in this pedigree is shown at the *bottom*. The telomeric breakpoint is located between in3.1 and in3.2 of *DPY30*, and the centromeric breakpoint is located between in4.3 and in4.33 of *SPAST*. **b** DNA sequence of the recombinant junction fragment. A DNA fragment encompassing the recombinant junction was amplified using the primer pair DPY30in3.13F and SPASTin4.33R. The sequence of the resulting fragment was compared with the published human genome sequence (Build 37.1), and the breakpoints were estimated. The centromeric and telomeric sequences are *boxed*, and common nucleotides are marked by *asterisks*. **c** Deletion-specific PCR. The examined individuals in the family are indicated. The primer pairs DPY30in3.13F and SPASTin4.33R, and DPY30in3.13F and DPY30in3.1R, shown in **a**, were used for detection of the mutant and normal alleles, respectively. A deletion-specific PCR product of 900 bp is observed in each affected family member (II-3, II-5, II-7, III-6, III-7, III-8, III-9, III-10, IV-1, and IV-2), while a product for the normal allele of 555 bp is detected in all the family members examined

present study, the two SPG4 patients showing elevated CSF tau levels manifested mild cognitive impairment. The presence of tauopathy in SPG4 has been controversial. There is one report of an autopsy that did not reveal tau-related changes in an SPG4 patient who had not only an SPG4 mutation but also an SPG6 mutation [21]. On the other hand, there are several neuropathological reports showing evidence of tau pathology in SPG4 patients with or without cognitive impairment [13, 28]. Therefore, tau-related changes in the central nervous system could be associated with our cases.

The main pathological lesions of SPG4 are in the corticospinal tracts and dorsal column pathway [13, 14, 21, 28]. The issue of whether neuropathy is associated with SPG4 remains controversial, but there are increasing lines of evidence for dysfunctions in sensory motor peripheral neurons, which were mainly associated with axonal impairment in SPG4 patients by neurophysiological exami-

nations [17, 18, 23, 32]. There is also a report of cytopathological changes in the lower motor neurons in SPG4 patients [28]. Similarly, in our cases, peripheral axonal neuropathy was revealed by nerve conduction studies and needle electromyography. Thus, it is important to examine the peripheral nervous system of SPG4 patients.

In the present family, heterozygous SPG4 with a 69,821-bp deletion including both exons 1 to 3 of *DPY30* and exons 1 to 4 of *SPAST* was confirmed. There are homologous sequences of nine nucleotides between the regions around the telomeric and centromeric breakpoints, suggesting that the recombination occurred by non-allelic homologous recombination [33]. The frequencies of exonic deletions of *SPAST* are 18–20% in patients with mutation-negative autosomal dominant hereditary SPG and 3–11% in patients with autosomal dominant hereditary SPG [8, 9, 24]. To date, there have been some reports of SPG4 patients with deletions including exon 1 of *SPAST* [7–11]. However, there is only one report in which the 5′ extent of the deletion was investigated, showing a heterozygous 2,307-bp deletion ranging from the 5′-UTR after a transcriptional initiation site to intron 1 of *SPAST* [7]. Therefore, this is the first report of a deletion including the entire region of exon 1 of *SPAST*. Moreover, this is also the first report of a partial deletion of *DPY30*. This partial deletion includes the entire region of exon 1 of *DPY30* and may lead to a loss of DPY30. DPY30 is an essential component of the *Caenorhabditis elegans* dosage compensation complex and also an important subunit of the histone methylation complex [34, 35]. Null mutations in *dpy-30* cause XX-specific lethality and *dpy-30* is required for wild-type development of XO males in nematodes [35]. Although the pathological role of the partial deletion including the entire region of exon 1 of *DPY30* in the present pedigree remains unclear and there is no description of a disease caused by mutations in *DPY30* (MIM 612032), from a speculative point of view, it is possible that the deletion may be associated with the miscarriages in the female patients. It will be interesting to see whether some other *SPAST* exon 1 deletions involve *DPY30* and what the associated phenotypes are.

In conclusion, this is the first report of SPG4 associated with partial deletions of both *SPAST* and *DPY30*, and female patients experienced miscarriages. Therefore, detailed analysis of deletions in *SPAST* and *DPY30* may be important in SPG patients, especially for patients with a past history of miscarriages. The prompting extended investigation of other *SPAST* exon 1 deletion cases is expected, and it is worthy to examine the mutation analysis of *DPY30* in the women who had cryptogenic miscarriages.

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Conflicts of interest The authors have no conflicts of interest.

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