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SHORT COMMUNICATION

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Adult onset limb-girdle type mitochondrial myopathy with a mitochondrial DNA np8291 A-to-G substitution

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Abstract We analyzed mitochondrial DNA (mtDNA) from 7 patients in four families with adult onset limb-girdle type mitochondrial myopathy to clarify their genetic background. The patients, 2 men and 5 women, showed common clinical features, characterized by isolated skeletal myopathy, high serum creatine kinase level, ragged-red fibers and cytochrome c oxidase-defective fibers. Analysis of muscle biopsy specimens indicated that cytochrome c oxidase activity was decreased relative to that of citrate synthase in 5 of the 7 patients. Southern blotting and direct sequence analyses showed an A-to-G homoplasmic transition at np8291 and intergenic COII/tRNA(Lys) 9bp deletion in all patients. This substitution was detected in only 2 of 600 control individuals including healthy subjects and patients with other neuromuscular disorders; these 2 individuals had diabetes mellitus and myotonic dystrophy, respectively. Consequently, the mtDNA transition at np8291 was a rare polymorphism. However, the 7 patients we studied had identical clinical, pathological, biochemical, and genetic features. Therefore, limb-girdle type mitochondrial myopathy with this rare polymorphism may form a subgroup of adult onset mitochondrial myopathy.

Key words Adult onset · Limb-girdle type · Mitochondrial myopathy · Familial · Mitochondrial DNA · np8291 A-to-G substitution

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Introduction

Mitochondrial diseases involve many organs and their clinical features vary from systemic disorders to single organ involvement (DiMauro et al. 1998). Many mitochondrial DNA (mtDNA) mutations have been reported in mitochondrial myopathy/encephalomyopathy (DiMauro and Bonilla 1997). However, mitochondrial myopathy with isolated skeletal muscle involvement and pathogenic mtDNA mutation is relatively rare (Goto et al. 1992; Bindoff et al. 1993; Moraes et al. 1993; Manfredi et al. 1995; Weber et al. 1997). There may be many patients with pure myopathy but unknown genetic defects in the mtDNA. We studied seven patients in four families with adult onset limb-girdle (L-G) type mitochondrial myopathy, and found a rare mtDNA substitution in all patients.

Subjects and methods

Patient evaluation

Neurological examinations and blood sampling in the seven patients and four healthy members of four families were performed by the authors. An ergometer exercise test was performed by the method reported previously (Nakagawa et al. 1995). Muscle biopsy specimens were obtained from the biceps brachii of all patients and examined histochemically and biochemically.

Oxidative phosphorylation complex activity assay

Spectrophotometric assays were used to measure NADH-cytochrome c reductase (complex I+III), succinate-cytochrome c reductase (complex II+III), cytochrome c oxidase (complex IV), and citrate synthase activities in the biopsied muscle homogenate (Bresolin et al. 1985).

Table 1 Clinical and laboratory findings in seven patients in four families

Families	1			2		3	4
Patients	1	2	3	4	5	6	7
Relationship		Brother of patient 1	Son of patient 1		Sister of patint 4		
Age (years) /sex	70/F	64/M	38/M	71/F	52/F	58/F	42/F
Age of onset	50	63	35	69	45	44	39
Muscle weakness and atrophy	PD	PD	PD	PD	PD	PD	PD
CK level[a]	4-10 ×	4 ×	2-3 ×	n-2 ×	2-10 ×	8-10 ×	2-7 ×
Ergometer exercise test	L/P↑	L/P↑	L/P↑	L/P↑	Not done	Normal	Not done
Ragged-red fibers (%)	5.0	6.0	5.5	4.0	3.5	6.5	1.0
CCO defective fibers (%)	5.0	5.0	7.0	4.5	5.0	7.4	7.0
Brain MRI/CT	Multiple infraction; cerebral hemorrhage	Not done	Small high intensity spots in white matter; systematic lipomatosis	Normal	Not done	Laterality of lateral ventricle	Normal
Complications	Hypertension; hyperlipidomia	AMI		Hypothyroidism; hyperlipidemia			
Mithochondrial enzyme activities (μmol/min per g of tissue)							
Complex I + III [7.80±1.94, 5.41-12.01]	4.24 (0.81)	8.54 (0.77)	4.86 (0.70)	6.21 (1.07)	10.47 (0.69)	11.69 (0.85)	8.11 (0.89)
Complex II + III [2.07±0.62, 1.30-2.92]	1.05 (0.45)	2.36 (0.21)	1.42 (0.21)	1.32 (0.23)	2.36 (0.16)	2.09 (0.15)	1.89 (0.21)
Complex IV [7.55±2.47, 4.50-11.70]	2.35 (0.45)	5.39 (0.49)	2.97 (0.43)	5.46 (0.94)	12.09 (0.80)	6.35 (0.46)	4.86 (0.53)
Citrate synthase [9.03±1.64, 6.69-12.50]	5.25	11.10	6.90	5.81	15.07	13.79	9.10

[a] Ratio to normal upper limit of creatine kinase;
CCO, Cytochrome c oxidase; PD, proximal dominant; AMI, acute myocardinal infraction; L/P, lactate/ pyruvate ratio; MRI, magnetic resonance imaging; CT, computed tomography; n, normal

Mitochondrial DNA analysis

Southern blotting analysis was performed, using DNA extracted from the biopsied muscles (Nakagawa et al. 1995). For the sensitive detection of mtDNA deletion, we employed the primer sets reported by Johnston et al. (1995). The whole mtDNA sequence was analyzed using an ABI PRISM 377TM automated sequencer (Perkin-Elmer, Norwalk, CT, USA), using 30 primer sets covering the entire mtDNA. mtDNA polymorphism frequency was examined by polymerase chain reaction-restriction fragment length polymorohism (PCR-RFLP) in 600 individuals; 241 healthy controls, 187 patients with cerebrovascular disease, 20 with mitochondrial encephalomyopathy, 43 with diabetes mellitus, 30 with myotonic dystrophy, and 79 with other neuromuscular diseases.

All studies were performed after informed consent had been obtained from all participants.

Results

Pedigree analysis

The seven patients in the four families had common clinical and pathological findings characterized by adult onset L-G type muscle weakness and atrophy, high serum creatine kinase (CK) level, ragged-red fibers (RRFs), and cytochrome c oxidase (CCO)-defective fibers in biopsied muscle. They had no central nervous system involvement, except for one patient, who died of cerebral hemorrhage (Table 1). One family originated from a different prefecture, while the other three were from different cities in Kagoshima prefecture. Results of genealogical analysis suggested maternal inheritance in family 1 (Table 1).

Case reports

Patient 1 (family 1-1). A 70-year-old woman, whose parents were second cousins, had begun to experience difficulty in climbing stairs at the age of 50. She had proximal muscle weakness and atrophy in the extremities but showed neither mental retardation nor eye movement disorders. Electromyography showed a myogenic pattern. Ergometer exercise test showed an abnormal increase in the lactate/pyruvate (L/P) ratio. Her muscle weakness progressed gradually and she had mild dyspnea on light work. She had cerebellar infarction at the age of 69 and died of cerebral hemorrhage at the age of 70.

Patient 3 (family 1-3). A 38-year-old man, the third son of patient 1, had no muscle weakness and normal CK level at the age of 27. He noticed muscle weakness in the extremities at the age of 37. He had symmetrical subcutaneous lipomas that were confirmed by biopsy. His elder sister had the same clinical features.

Patient 4 (family 2-1). A 71-year-old woman exhibited general weakness, dysarthria, dysphagia, and hypertension after a common cold infection at the age of 70. On examination, she had proximal dominant muscle weakness and atrophy in the extremities. Hypothyroidism was detected after admission and she was diagnosed as having mitochondrial myopathy with hypothyroidism.

Oxidative phosphorylation complex activity assay

The activities of complex I +III, II+III, and IV in biopsied muscle were decreased in patient 1, and the activities of complex I +III and IV were decreased in patient 3. The activity of complex IV relative to that of citrate synthase was reduced to about 50 % of that in normal controls in five of the seven patients (Table 1).

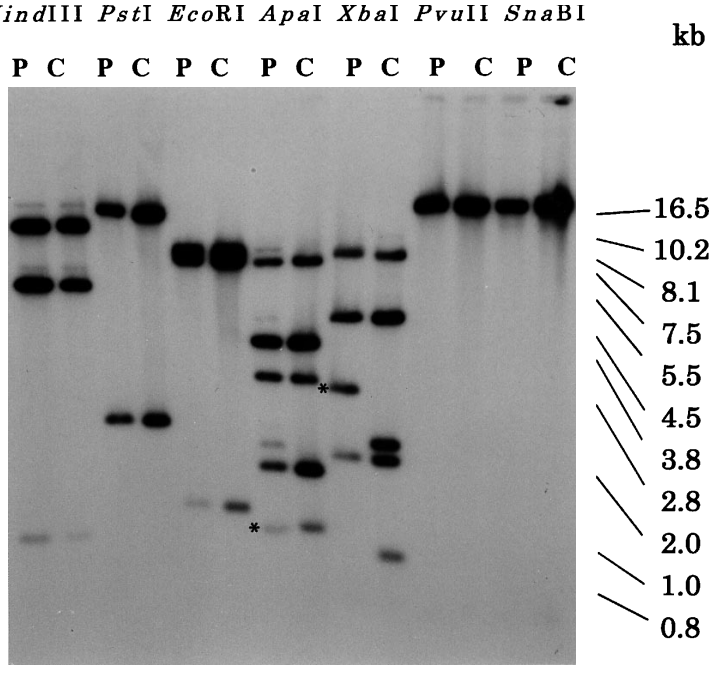

Fig.1. Southern blotting analysis of mtDNA in patient 3. No large mtDNA deletion was detected after *Pvu*II digestion, but a band shorter than the normal 1kb band was detected after *Apa*I digestion and loss of an *Xba*I recognition site was detected at np8286. *Asterisks* indicate these mutant bands. *P*, patient; *C*, control

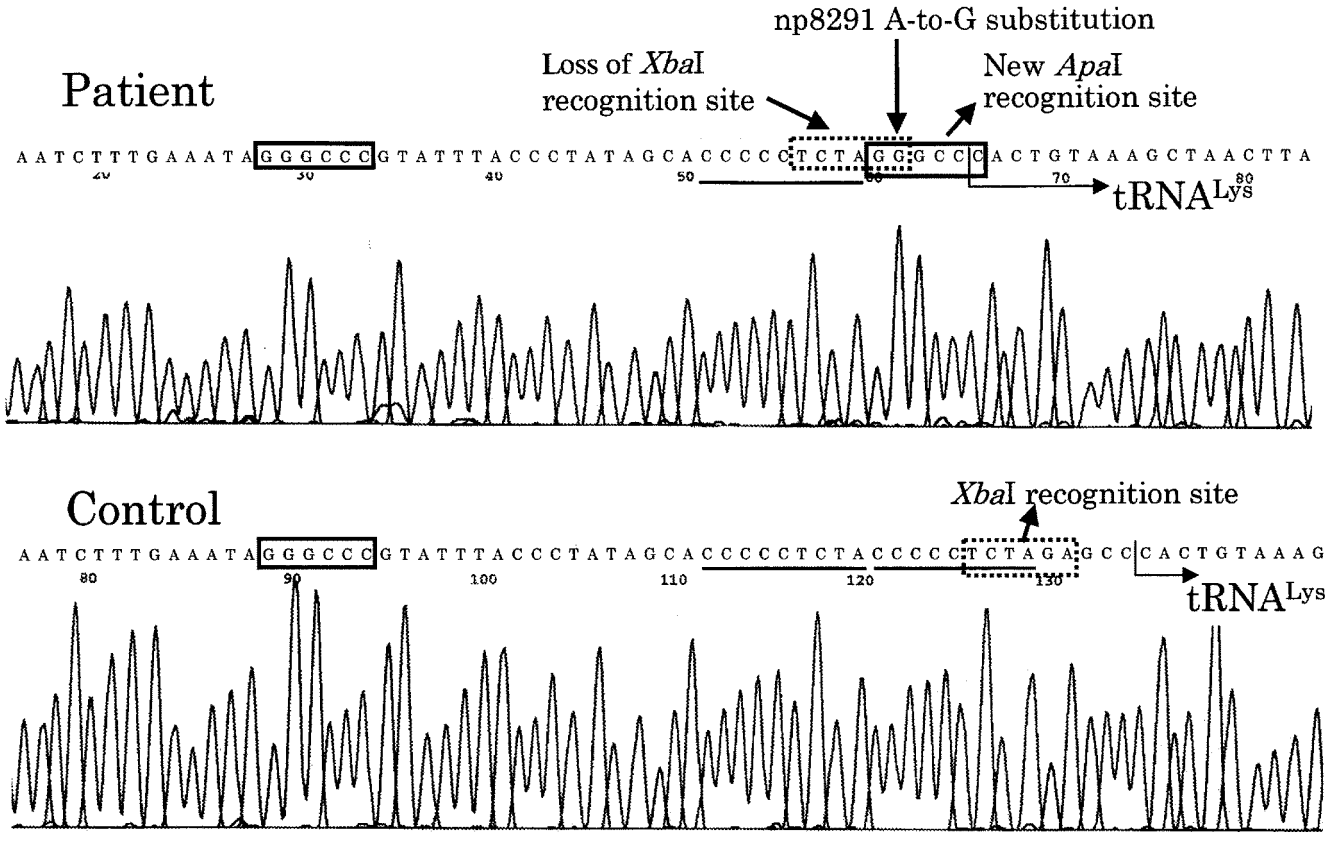

Fig. 2. Mitochondrial DNA sequences in np8236 and 8312 regions in patient 3. All patients had an A-to-G homoplasmic transition at np8291. This substitution generated a new *Apa*I recognition site and loss of an *Xba*I recognition site in this region. Moreover, a 9bp deletion between np8272-8280 was detected in all patients. *Underlines* indicate the 9-bp repeat sequence, and *Apa*I and *Xba*I recognition sites are indicated by *boxes with solid and broken lines*, respectively

Mitochondrial DNA analysis

Southern blotting analysis showed a band shorter than the normal 1 kb band after *Apa*I digestion and loss of the *Xba*I recognition site at np8286 (Fig. 1). Southern blotting and PCR analysis showed no large mtDNA deletions. We determined the whole mtDNA sequence of patient 3 and compared the sequence with the reference sequence of Anderson et al. (1981). The following 28 homoplasmic substitutions and one small deletion were detected in the mtDNA from patient 3; C511T, A512G, C4612T, C7232T, C7256T, G7316A, T7705C, C7810T, C7868T, C7891T, G7912A, A8021G, G8065A, C8140T, G8152A, A8291G, a 9bp deletion between np8272-8280, A10403G, T11335C, C11447G, G11719A, G11969A, G13702C, G14199T, G14272C, G14365C, G14368C, A15326G, and T15670C. We checked the mtDNA from the other six patients for these substitutions and the deletion except for a synonymous one, and for those substitutious reported as a polymorphism (Howell et al. 1995; Ikebe et al. 1995). All seven patients showed a common A-to-G homoplasmic transition at np8291. This substitution generated a new *Apa*I recognition site and loss of an *Xba*I recognition site in this region. Moreover, a 9bp deletion between np8272-8280 was detected in all patients (Fig. 2). Siblings without muscle weakness also had this homoplasmic substitution and deletion.

Polymorphism frequency

The np8291 A-to-G transition was detected as a homoplasmic substitution in only 2 of the 600 control individuals. These 2 individuals, 1 with insulin-dependent diabetes mellitus (IDDM) and 1 with myotonic dystrophy, also had the 9 bp deletion. In addition, 5% of healthy control subjects in Kagoshima had the 9bp deletion without the np8291 A-to-G transition.

Discussion

The clinical and pathological features in our seven patients were characterized by adult onset L-G type muscle weakness and atrophy, high CK level, RRFs, CCO-defective fibers, mildly decreased CCO activities in biopsied muscle, an A-to-G transition at np8291 and the 9 bp deletion. These patients except for one patient with cerebrovascular disorder, showed no central nervous involvement.

Other healthy members of the patients' families had the

same homoplasmic transition and 9bp deletion, without the muscle weakness. There are two possible explanations of the significance of this transition and deletion. This transition may be a rare polymorphism and not a disease-causative mutation, and other genetic factors, i.e. abnormality of nuclear DNA coded mitochondrial proteins exclusively expressed in skeletal muscle, may be present in these patients. Alternatively, as the nucleotide at np8291, four bases prior to the 5′ end of tRNA(Lys), is conserved in mammals, an A-to-G transition at this position may influence tRNA(Lys) translation. The frequency of the 9 bp deletion is relatively low in the Southern part of Japan (Horai et al. 1996). The clinically normal family members may have subclinical myopathy, because muscle biopsy was not performed in these subjects. Therefore, this transition with the 9 bp deletion may be a disease-causative mutation which results in a decrease in CCO activity in skeletal muscle, and some environmental factor(s) may influence the onset of the disease. However, it is difficult to explain the presence of the same substitution and deletion in the two control patients one with IDDM and one with myotonic dystrophy.

Johnston et al. (1995) reported late-onset mitochondrial myopathy with multiple mtDNA deletions. The clinical features of their study population were similar to those of our patients. However, our patients showed no large mtDNA deletion, earlier disease onset, and higher incidences of RRFs and CCO-defective fibers. Therefore, the mitochondrial abnormalities detected in our patients must be different from those in the patients reported by Johnston et al. and do not seem to represent an exaggerated form of the normal aging process (Brierley et al. 1998).

Interestingly, one patient had symmetrical subcutaneous lipomas. mtDNA mutations have been reported in multiple symmetric lipomatosis (MSL) with mitochondrial myopathy (Campos et al. 1996; Klopstock et al. 1997). The suspected mitochondrial dysfunction in our patients may have been related to the development of symmetrical subcutaneous lipomas.

In conclusion, we reported seven patients in four families with adult onset L-G type mitochondrial myopathy with a rare mtDNA polymorphism. L-G type mitochondrial myopathy with this rare polymorphism may form a subgroup of adult onset mitochondrial myopathy. Further genetic studies are needed to clarify the pathological mechanism of this disease.

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