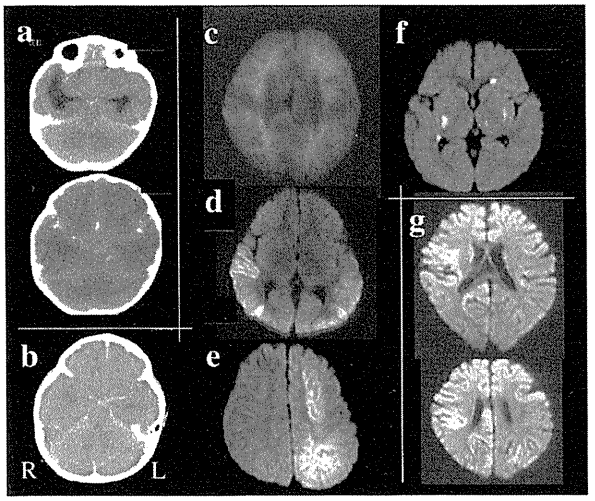

Fig. 1. (a) Brain CTs on 2nd and 21st day of hospitalization in Case 1 showing disappearance of cistern between brainstem and cerebellum (upper image) and severe brain edema (lower image). (b) Brain CT on admission showing severe brain edema in Case 2. (c–g) Brain MRIs showing abnormal high intensities in diffusion-weighted images in Cases 6,7,12,11 and 10, respectively.

3.4. Patient profile for the study of the blood ATP level

Twenty-five patients were included in this study. The diagnoses of the 25 patients were as follows: 10 patients with acute encephalopathy (mean age: 3 years and 11 months, age range: 7 months–10 years and 8 months, one IAE, one *Salmonella*-associated, one HHV-6-associated, three unknown virus-associated, one methylmalonic aciduria, one hepatic encephalopathy, one hemolytic uremic syndrome, and one septic encephalopathy (Case 2 in Table 1)), nine febrile seizure status (mean age: 1 year and 5 months, age range: 4 months–4 years 9 months), and six mitochondrial disease (mean age: 9 years and 8 months, age range: 2–25 years, two partial cytochrome c oxidase deficiency, three Leigh syndrome, and one chronic progressive external ophthalmoplegia). All 10 patients with acute encephalopathy were analyzed regarding the blood ATP levels in the acute phase (within 24 h of disease onset), and five of the 10 patients were also analyzed in the convalescent phase. Among the 15 patients who were analyzed for CPT II polymorphism, only Cases 2 and 12 were included in this study.

4. Methods

4.1. Analysis of CPT II polymorphism

Genomic DNA from whole blood was purified as previously described [15]. PCR of five exons of the CPT II gene was carried out with intron-based primers in genomic DNA. For haplotype analysis, the CPT II exon four region was cloned into the pCR® 2.1 vector (Invitrogen). The sequences of the PCR products and cloned CPT II gene were analyzed employing the ABI DyeDeoxy Terminator Cycle Sequencing Kit with an ABI-PRISM 3100 Genetic Analyzer (PE-Applied Biosystems). Each PCR product was sequenced at least twice independently.

4.2. Preparation of patients' lymphoblasts and culture

Blood samples (2 mL) were obtained from patients by venipuncture into a sterile EDTA blood collection tube. Lymphocytes were separated from peripheral blood, diluted (1:1, v/v) with sterile saline, by centrifugation ($800\times g$, 20 min) over 2 mL of Lymphoprep (Nycomed). The lymphocyte layer was recovered and washed twice with PBS by centrifugation at $250\times g$ for 10 min each, and then maintained in PRMI-1640 (GIBCO) supplemented with 12.5% FCS. Cells were incubated with 5% CO_2 at 37 °C for 7 days. Lymphoblastic cell lines were established by infecting peripheral blood lymphocytes with the Epstein Barr virus. Cells were grown in suspension in an SC flask (Greiner 658190) in an upright position, in 10 ml of PRMI-1640 medium that contained 12.5% FCS, maintained at 37 °C. Fluid was routinely changed every 2 days by removing the medium above the settled cells and replacing it with an equal volume of fresh medium.

4.3. Analysis of CPT II activity

CPT II activities of patients' lymphoblasts were analyzed as previously described [14]. To prepare whole cell extracts, cells were harvested and washed twice with PBS (−) at $250\times g$ for 10 min and then lysed with 0.5 mL of ice-cold lysis buffer (5 mM Tris–HCl buffer, pH 7.4, containing 1% Tween-20 and 0.5 M KCl), then centrifuged at $147{,}600\times g$ for 1 h at 4 °C. To analyze the heat stability of CPT II, cell lysates were pre-incubated at 30, 37 and 41 °C for 0–120 min. Protein concentrations in the cell lysates were measured using the BCATM Protein Assay Kit (Thermo SCIENTIFIC).

4.4. Measurement of blood ATP levels

ATP concentrations in whole blood lysate were measured by an ENLITEN® ATP assay system bioluminescence detection kit (Promega) according to the instructions provided by the manufacturer and the values were expressed as ATP levels in whole blood.

5. Results

5.1. CPT II polymorphism in the patients

As shown in Table 1, among the 15 patients studied, seven had a thermolabile F352C CPT II variant (1 F352C only and six [F352C + V368I]), four V368I only,

two [V368I + M647 V], and two no polymorphisms. In 12 patients with acute encephalopathy (Cases 1–12), six (Cases 1–3 and 5–7) had a thermolabile F352C CPT II variant (1 F352C only and five [F352C + V368I]), and five (Cases 8–12) had the V368I CPT II variant (4 V368I only and one [V368I + M647 V]) and one (Case 4) showed no CPT II variant. Two patients with acute encephalopathy who died (Cases 1 and 2) had a thermolabile F352C CPT II variant (1 F352C only and the other [F352C + V368I]). In three patients with febrile delirium associated with influenza infection (cases 13–15), only case 13 (brief febrile seizure and unusually long febrile delirium) had the [F352C + V368I] CPT II variant. No other reported CPT II mutations or polymorphisms were detected.

There was no significant difference in the age at onset (41.0 ± 23.3 vs. 24.3 ± 12.7 months of age, $p = 0.18$), duration of high fever (52.0 ± 35.3 vs. 63.0 ± 44.9 h, $p = 0.28$), and duration of seizures (40.5 ± 40.1 vs. 56.7 ± 23.4 h, $p = 0.12$) between the six patients with acute encephalopathy with a thermolabile F352C CPT II variant (Cases 1–3,5–7) and six patients with acute encephalopathy without this thermolabile variant (Cases 4,8–12) (Mann–Whitney U-test).

5.2. Lymphocyte CPT II activity in the patients

As shown in Fig. 2(b), CPT II activity using peripheral lymphocytes of a patient with a thermolabile F352C CPT II variant was significantly reduced to about 50% during incubation for 120 min at 41 °C as compared to those at 30 and 37 °C. All patients with a thermolabile F352C CPT II variant showed a significant reduction of CPT II activity at 41 °C.

Fig. 2(a) shows CPT II activity in a patient with the V368I CPT II variant without reduction even at 41 °C.

5.3. Blood ATP levels in patients with acute encephalopathy

As shown in Fig. 3, ATP levels in the extracts of whole blood in the acute phase of encephalopathy during high fever were significantly low (0.58 ± 0.16 mM, $n = 10$) compared with those in the convalescent phase (1.08 ± 0.27 mM, $n = 5$) and with those of patients with febrile seizure status (1.01 ± 0.36 mM, $n = 9$). The blood ATP levels in the acute phase of encephalopathy revealed no significant difference when compared to those of patients with mitochondrial disease exhibiting several symptoms (0.79 ± 0.39 mM, $n = 6$).

6. Discussion

Although the precise pathomechanisms of acute encephalopathy have yet to be clarified, it is postulated that some genetically-determined factors might be involved, because some types of acute encephalopathy are more frequent in Japanese than in Caucasians. Chen et al. [12] demonstrated that the thermolabile phenotype of CPT II variations such as the F352C CPT II variant or complex [F352C + V368I] CPT II variant might be a principal genetic background of IAE in Japanese. On the basis of the analysis of fatty acid oxidation and cellular ATP production in COS-7 cells transfected with wild-type and variant *CPT2* cDNAs at 37 and 41 °C, Yao et al. [14] suggested that the compound *CPT2* variants with thermolabile phenotypes are the main cause of multiple-organ failure, particularly in high ATP-consuming organs as well as endothelial cells and play a major role in the etiology of IAE.

In the 12 patients with acute encephalopathy studied, six patients (Cases 1–3 and 5–7) had thermolabile F352C CPT II variants (F352C CPT II variant alone in one case and complex [F352C + V368I] CPT II variants in five cases), which were reported to be frequently noted in severe IAE patients [12,14]. Of the six patients, two patients (Case 1, IAE and Case 2, *Hemophilus influenzae*-associated septic encephalopathy) died despite intensive care. Case 2, who died of fatal septic encephalopathy [16], showed a high acylcarnitine ratio ((C16 + C18:1)/C2:0.203) on admission. This value corresponded to the ratio (>0.09) of the high-risk group of patients with IAE showing a fatal outcome, thus reflecting the disorder of mitochondrial •-oxidation. [12]. The remaining six patients (Cases 4 and 8–12) with acute encephalopathy without a thermolabile F352C CPT II variant followed a relatively mild clinical course (Table 1). Out of the six patients, five had a V368I CPT II variant.

As shown in Fig. 2, the CPT II activities of lymphocyte in patients with the F352C CPT II variant showed thermal instability, that is, a marked activity reduction at 41 °C, while those in patients with the V368I CPT II variant did not. There was no significant difference in the age at onset, duration of high fever, and duration of seizures between the six patients with the F352C CPT II variant (Cases 1–3 and 5–7) and six patients without this variant (Cases 4 and 8–12). Therefore, taken together, it seems likely that a thermolabile F352C CPT II variant might be related to the severity of disease, that is, the rapidity of progression of brain edema. In Caucasians, two polymorphisms of CPT II, p.V368I and p.M647 V, occur with a frequency of 0.5 and 0.25, respectively, exhibiting a Hardy–Weinberg equilibrium. A third polymorphism, p.F352C, occurs with a frequency of 0.21 exclusively in the Japanese population [17]. Therefore, this thermolabile F352C CPT II variant might be one of the predisposing factors to trigger the pathomechanism of acute encephalopathy in Japanese.

The CPT system regulates the entry of long-chain fatty acids into the mitochondrial matrix for •-oxidation. Fatty acid oxidation is an important source of acetyl-CoA for maintaining the tricarboxylic acid cycle.

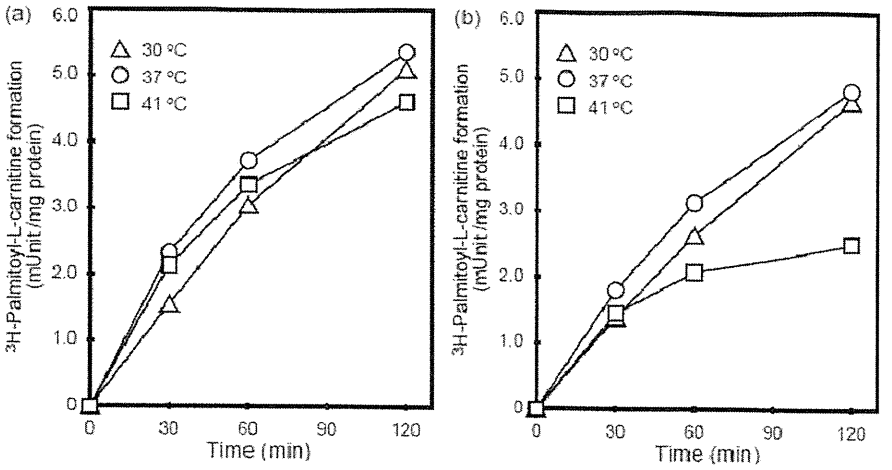

Fig. 2. (a) Lymphocyte CPT II activity in case 12 (influenza-associated encephalopathy) with V368I CPT II variant at 30, 37 and 41 °C. No definite reduction of CPT II activity was observed at 41 °C. (b) Lymphocyte CPT II activity in Case 1 (influenza-associated encephalopathy) with a thermolabile F352C CPT II variant at 30, 37 and 41 °C. At 41 °C, the CPT II activity decreased to about 50% of that at 37 °C after 2-h-incubation.

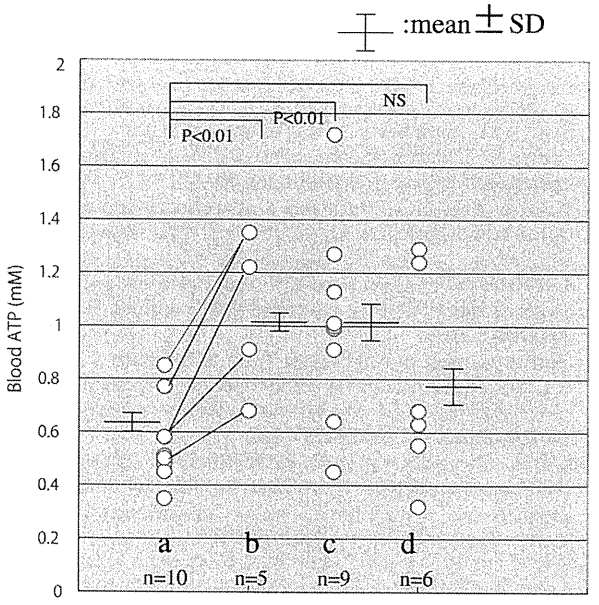

Fig. 3. ATP levels in whole blood in patients with acute encephalopathy (acute (a) and convalescent phase (b)), febrile seizure status (c) and mitochondrial disease (d). In five patients with acute encephalopathy, blood ATP level recovered at convalescent phase.

The CPT II is ubiquitously expressed in all tissues that require fatty acid oxidation as an energy-producing pathway [18]. CPT II deficiency is a disorder of long-chain fatty acid oxidation. It is classified into three clinical types based on the age at onset and disease severity: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. It is clear that our patients' clinical manifestations did not correspond to any of these three types. The thermolabile instability of the F352C CPT II variant in our cases explains the situation whereby impaired energy metabolism could occur during high fever due to a secondary CPT II deficiency in spite of the absence of symptomatic manifestations of CPT II disorder in daily life at a normal temperature [12,14].

Olpin et al. [19] reported based on mutation analysis that when CPT II activities are above 20% of controls, fatty acid oxidation in fibroblasts is usually within the normal range (>70% of controls). However, under heat stress, fasting, acidosis, and seizures, moderately lowered CPT II activity due to the thermolabile F352C CPT II variant may accelerate the disease process of acute encephalopathy.

Blood ATP levels in the acute phase of encephalopathy during high fever were significantly lower than those in the convalescent phase and also with those of patients with febrile seizure status. This suggests that mitochondrial energetic failure may be more severe in patients with acute encephalopathy, and the pathological process of acute encephalopathy should differ from the febrile seizure status. The low levels of ATP in the acute phase of encephalopathy were normalized in the convalescent phase in line with clinical recovery. Interestingly, blood ATP levels in the acute phase of encephalopathy corresponded to those of mitochondrial disease with several symptoms. Yao et al. [14] showed that COS-7 cells transfected with thermolabile [F352C + V368I] CPT II variants exhibited significantly decreased fatty acid oxidation and subsequent intracellular ATP reduction at 41 °C. The decreased ATP levels seemed to reflect systemic mitochondrial dysfunction including the blood brain barrier (BBB) at the acute phase of encephalopathy in our cases. The ATP demand per body weight is so high in infants that a thermolabile CPT II variant induced-ATP reduction might lead to a greater susceptibility to the pathophysiology of encephalopathy in children than in adults.

The brain capillary endothelium is characterized by a greater density of mitochondria than that of peripheral capillaries [20]. This greater mitochondrial density is required to maintain the significant active transport mechanisms, electrochemical gradients, autoregulatory adjustments, and regulation of tight junctional complexes. As such, the requirement of a constant ATP supply may make the BBB particularly susceptible to acute hypoxic insult [21]. From a similar perspective, BBB breakdown may occur at an initial stage of encephalopathy under the condition of ATP reduction, thus leading to subsequent brain edema due to complex cascade of hypercytokinemia, excitotoxicity, and oxidative stress. Although there is one hypothesis that cytokine storm due to virus–glial cell interaction might cause endothelial cell damage (BBB breakdown) leading to brain edema and neuronal injury [11], we consider that endothelial cell damage might induce in turn cytokine production resulting in neuronal damage in patients with thermolabile F352C CPT II variant irrespective of encephalopathy type .

In three patients with febrile delirium associated with influenza virus infection (Cases 13–15), Case 13 with a thermolabile F352C CPT II variant developed a short seizure and an intermittent confused state with visual hallucinations and agitation lasting 6 h. Cases 14 and 15 without F352C CPT II variant showed short-term consciousness alteration and abnormal behavior without seizures. All patients' brain MRIs were normal, and they fully recovered. Although more extensive study is needed, the grade of febrile delirium associated with influenza virus was more severe in a case with a thermolabile F352C CPT II variant when compared with that in cases without F352C CPT II variant.

Given that a thermolabile CPT II variant might be one of the predisposing factors for acute encephalopathy, we should revise the therapeutic strategy from the acute phase. Considering the rapid progression of encephalopathy and associated low CPT II activity during high fever, immediate hypothermia, sufficient glucose infusion, and L-carnitine supplementation should be adopted as treatment options. We speculate that the immediate hypothermia led to the recovery of the lowered CPT II activity and, thus, mitochondrial energy failure became minimal in many tissues including the brain capillary endothelium, leading to less severe damage to the central nervous system.

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Review article

Hereditary progressive dystonia with marked diurnal fluctuation

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Abstract

Hereditary progressive dystonia with marked diurnal fluctuation (HPD) is a dopa-responsive dystonia, now called autosomal dominant GTP cyclohydrolase 1 deficiency or Segawa disease, caused by mutation of the GCH-1 gene located on 14q22.1 to q22.2. Because of heterozygous mutation, partial deficiency of tetrahydrobiopterin affects tyrosine hydroxylase (TH) rather selectively and causes decrease of TH in the terminals of the nigrostriatal dopamine (NS DA) neurons, projecting to the D1 receptors on the striosome, the striatal direct pathways and the subthalamic nucleus (STN) and the D4 receptors of the tuberoinfundibular tract. The activities of TH in the terminal are high in early childhood decrease exponentially to the stational level around early twenties, and show circadian oscillatron. TH in HPD follows these variations with around 20% of normal levels and with development of the downstream structures show appears characteristic clinical symptoms age dependently.

In late fetus period to early infancy, through the striosome-substantia nigra pars compacta pathway failure in morphogenesis of the DA neurons in substantia nigra, in childhood around 6 years postural dystonia through the D1 direct pathways and the descending output of the basal ganglia. Diurnal fluctuation is apparent in childhood but decrease its grade with age.

TH deficiency at the terminal on the STN causes action dystonia from around 8 years and postural tremor from around 10 years, focal dystonia in adulthood.

Adult onset cases in the family with action dystonia start with writer's cramp, torticollis or generalized rigid hypertonus with tremor but do not show postural dystonia. TH deficiency on the D4 receptors causes stagnation of the body length in childhood. With or without action dystonia depends on the locus of mutation. Postural dystonia is inhibitory disorder, while action dystonia is excitatory disorder. The TH deficiency at the terminal does not cause morphological changes or degenerative process. Thus, levodopa shows favorable effects without any relation to the duration of illness.

Keywords: Autosomal dominant GCH-1 deficiency; Segawa disease; Postural type; Action type; Pteridine metabolism

1. Introduction

Hereditary progressive dystonia with marked diurnal fluctuation (HPD) is a dopa-responsive dystonia described by Segawa et al. in 1976 [1]. After discovery of the causative gene, the gene of GTP cyclohydrolase 1 (GCH-1) located on 14q22.1 to q22.2 [2], this is called as autosomal dominant GCH-1 deficiency or Segawa disease. Clinically there are two types, postural dystonia and action dystonia type [3,4]. It depends on the family or the loci of mutation. In postural type the clinical symptoms are similar inter- and intra-families, however, action type shows intra-familial variations.

In this article clinical characteristics, neurophysiological, biochemical, neuroimaging, and neuropathological findings are reviewed. Pathophysiologies of these two types are discussed. Lastly, the pathophysiology of the early onset cases, another phenotype are commented.

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2. Clinical characteristics

In most cases the symptoms at onset is dystonic posture, with rigidity in one lower extremity, pes equinovarus, around 6 years. Clinical course was clarified by an experience of 51 years female with postural type having clinical course of 43 years after onset at 8 years, that is postural dystonia expands to other limbs, and all limbs and trunk muscles are affected by late teens, the rigidity aggravates progressively until around 20 years of age, but the progression subsides in twenties and becomes stationary in the thirties. Postural tremor with high cycles of 8–10 Hz appears after 10 years in an upper extremity and expands to all limbs by thirties. However, locomotion is preserved throughout the course.

In action dystonia type besides postural dystonia dystonic movements of an upper extremity or action retrocollis appear around 8 years. The latter may associate oculogyric crises. From experiences of 38 years female patients with 30 years clinical course and of a childhood onset case with long term follow up, torticollis and writer's cramp appear in adulthood. In families with action dystonia type, there are adult onset cases who start with writer's cramp, torticollis or generalized rigid hypertonus with postural tremor, but do not show dystonic posture or apparent progression.

Symptoms show marked diurnal fluctuation in childhood. But it attenuates the grade with age and becomes unapparent with cessation of clinical progression. In adult onset cases it is not observed. Childhood onset cases show stagnation of body length with onset of dystonia. This is not observed in cases with onset after adolescence.

A few patients show migraineous headache, autistic features, depressive reaction or obsessive behavior. Patients with onset early in infancy start with delay in motor and psychomental development.

Childhood onset cases show marked female predominance. In our personal cases (41 cases from 20 families) F:M is 33:8, that is 4:1, and it is more marked in postural type 18:1 than action type 2:1. While adult onset cases show male predominance. Furukawa et al. [5] showed higher penetrance (87%) in females than males (38%).

Neurological examinations reveal rigid hypertonus [3,4]. But in contrast to Parkinson disease (PD) it is not plastic rigidity, and cases with tremor do not show cogwheel rigidity. These clinical signs, particularly rigidity and tremor, show asymmetry. Exaggeration of tendon reflexes, some with ankle clonus, are observed in child patients, however Babinski sign is negative. In advanced stage, pulsion is observed but freezing phenomenon is not detected because of preservation of locomotion. Cerebral or cerebellar sings or sensory abnormalities are not observed. Early onset patients show postural or truncal hypotonia, failure in locomotion in infancy, camptocormia in late childhood and parkinsonism in adulthood besides symptoms of HPD.

In child onset cases, a dose of 20 mg/kg/day of plain levodopa (levodopa without decarboxylase inhibitor) or 4–5 mg/kg/day with decarboxylase inhibitor show complete and sustained effects without side effects [3,4,6]. There are families in which plain levodopa is effective throughout the course. However, in other family, replacement to L-dopa with decarboxylase inhibitor is necessary from around 13 years, because of activation of the decarboxylation of dopa in the intestine [6]. In a few cases choreic movements develop by a rapid increase of dosage or by administration of a high dose of levodopa in initial stage of treatment [4]. In these patients by reduction of the dosage and slow titration to the optimal doses, favorable and sustained effect is obtained without unfavorable side-effects [6].

However, for action dystonia and related symptoms effects of levodopa may be incomplete, and action retrocollis and oculogyric crises may be aggravated by initial doses [3,4]. Furthermore, patients with this type may show levodopa induced dyskinesia.

The short stature caused by stagnation of the body length recovers completely, if levodopa is administered before adolescent.

Anticholinergics showed a marked and sustained effect for dystonia, but not for tremor [3]. Moderate effects of tetrahydrobiopterin (BH_4) with levodopa were reported, but no favorable effects with monotherapy [3].

Before the era of L-dopa stereotactic operations were performed on two patients, one patient with postural type dystonia [7] and the other with action type reported as juvenile parkinsonism [8] whose GCH-1 mutations were identified later [7,8]. Pallidotomy showed moderate effects on postural dystonia but not on tremor [7]. Ventrolateral thalamotomy was effective on tremor [7], rigidity and levodopa induced dyskinesia occurring in adult with action type [8], but no effect on postural dystonia [7].

The age related clinical course observed in a case with long clinical course is explained by following the age variation of the activities of tyrosine hydroxylase (TH) in the synaptic terminals of the caudate nucleus of the nigrostriatal (NS) DA neurons shown by McGeer and McGeer [9] with low levels of TH (Fig. 1). TH activities of the NS DA neurons do now show the state dependent variation in the SNc while show circadian oscillation in the terminals [9]. These evidences implicated that HPD is caused by non-progressive decrease of TH activities in the terminals of the NS DA neuron and develops clinical symptoms following the age and circadian variation of the TH activities of the terminals.

3. Studies for evaluation of the pathophysiology

Polysomnographies (PSGs) performed to clarify the sleep effects [3,4] revealed importance of REM sleep

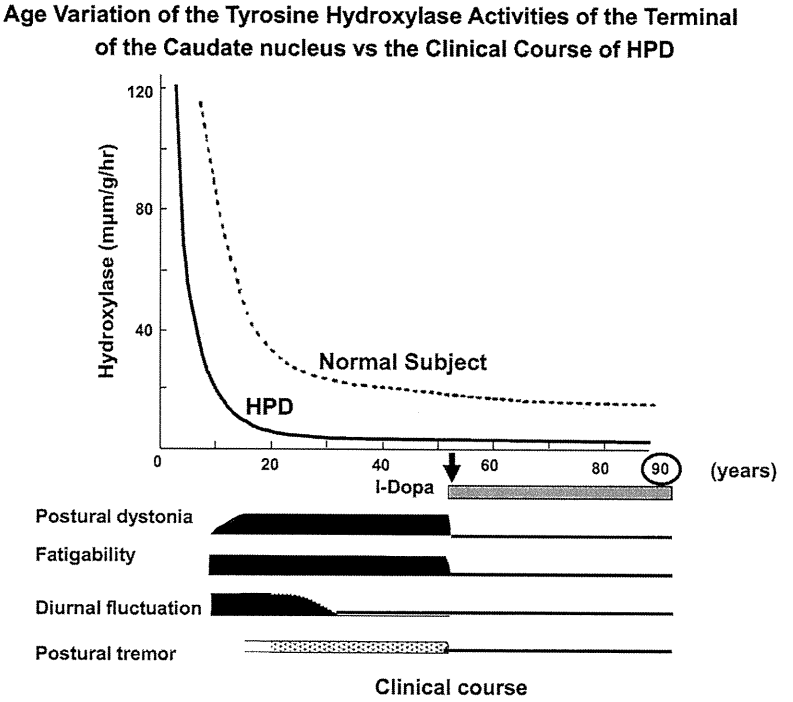

Fig. 1. Correlation of the 43 years clinical course of 51 years female postural type, with onset at 8 years and the age variation of the tyrosine hydroxylase activities of the terminals of the nigrostriatal dopamine neuron as the causative nucleus shown by McGeer and McGeer [9]. This case was completely recovered by L-dopa started at 51 years which continued without any side effects until 90 years.

(sREM) for the sleep effects by selective sleep stage deprivation studies [1]. Twitch movements (TMs) in sREM reflect the dopamine (DA) activities. In normal subjects TMs in sREM increase with sleep cycle and decrease with age. Degree of increment with sleep cycle reduces with age. The TMs of HPD patients followed these nocturnal and age variation with the lower levels of around 20% of normal values. In sREM of the 1st cycle it was markedly decreased less than 20% of the level in the last cycle of normal subjects, but in that of last cycle it exceeded more than 20%, which related morning recovery. Other parameters modulated by the brainstem aminergic neurons were preserved normally [3,4].

Fujita and Shintaku [10] revealed marked decrease less than 20% of the normal range of biopterin and neopterin in cerebrospinal fluid (CSF) of a case with HPD, by Furukawa et al. [11] confirmed the results and deficiency of GCH-1 was clarified as the cause of HPD [11]. In asymptomatic carriers these were 30–50% of normal levels. The activities of GCH-1 in the mononuclear blood cells decreased to less than 20% of normal levels in patients and 30–40% in asymptomatic carriers [2].

Ichinose found the gene of GCH-1 on 14q22.1 to q22.2 and examining seven HPD patients reveled this gene as the causative gene of HPD [2].

Neuroimaging, neurophysiological, neuropathological and neurohistological studies confirmed the pathophysiology speculated from clinical studies.

Magnetic resonance imaging (MRI) studies performed in various institutions revealed no abnormalities [3,4]. Brain positron emission tomography (PED) scans studies showed no definite abnormalities in [^{18}F] dopa uptake, [^{11}C] raclopride PET, [^{11}C] N-spiperone PET [3] and normal development of the D_2 receptors in HPD [3,4]. In our experience of two 38 years, female patients of action type with 30 years' clinical courses without treatments after the onset at 8 years, and one 59 years male with onset at 58 years showed normal [^{18}F] dopa uptake and [^{11}C] N-spiperone PET. These confirmed decrease of TH as the cause of DA deficiency.

Evaluations of voluntary saccades revealed abnormalities in both visually guided (VGS) and memory guided saccades (MGS), and implicated involvement of both the direct and the indirect pathways of the basal ganglia [12]. In adult onset patients abnormalities were observed only in MGS.

Paired pulse transcranial magnetic stimulation was studied in two institutes [13,14]. One study showed residual abnormalities in motor inhibition in levodopa-treated HPD patients even though clinically asymptomatic [13]. However, the other study revealed that dysfunction of GABAa inhibitory interneurons of the primary motor cortex does not contribute to the generation of postural dystonia of HPD [14]. Sensory evoked potential revealed normal gating in patients with postural type, while it was abnormal in patients with action type.

These neurophysiological studies revealed involvement of the striatal direct pathway and the descending output of the basal ganglia for the postural type and the indirect pathway and the ascending pathway for tremor and symptoms of action type dystonia.

Neuropathological and neurohistochemical studies were performed on a case with action dystonia reported as juvenile parkinsonism [8] and a 19 years old DRD female, postural type died by traffic accident [15], both of which were later confirmed GCH-1 gene mutation.

Neuropathological examinations revealed decrease in melanin, particularly in the ventral tier of the pars compacta of the substantia nigra (SNc) [8,15], and one with morphological immaturity of the neurons [8]. Histochemically, DA content was subnormal [16] or normal [8] in the SNc, while it showed marked decrement in the striatum [8,16]. The reduction was greater in the putamen than in the caudate nucleus, and subregionally, the decrement was more great in the rostral caudate and the caudal putamen similar to PD. However, in contrast to PD this case showed a greater DA loss in the ventral subdivision of the rostral caudate, the area rich in striosome, than its dorsal counterpart, though in the putamen, the dorsoventral DA gradient was similar to PD [16]. The activity and protein content of TH was decreased in the striatum, but within normal range in the SNc [16].

Furukawa et al. [17] showed marked reduction of total biopterin (84%) and neopterin (62%) in the putamen, despite normal concentration of aromatic acid decarboxylase, DA transporter and vesicular monoamine transporter. These authors [18] also demonstrated modest reduction of TH protein (52%) and DA (44%), despite marked reduction of striatal biopterin (82%) in an asymptomatic carrier, and implicated the levels of TH protein as a key for development of symptoms.

Up to now more than one hundred independent mutations have been identified in the coding region of GCH-1 which is identical in one family, but differs among families [2–4]. However, we found two occasions showing identical mutation in unrelated families.

It was shown molecular analysis remains unable to determine mutations in the coding region of the gene in approximately 40% of subjects with GCH-1 deficiency. In these cases, abnormalities in intron genomic deletion, a large gene deletion, an intragenic duplication or inversion of GCH-1 or mutation in as yet undefined regulatory gene modifying enzyme function may be present [3,4].

4. Pathophysiological consideration

As for the pathogenetic mechanisms for dominant inheritance with heterozygous mutation, classic dominant negative effects have been considered [3,4]. The rates of mutant GCH-1 messenger ribonucleic acid (mRNA) production against normal mRNA were 28% in a patient but 8.3% in an asymptomatic carrier [3,4]. However, the ratio varies depending on the locus of the mutation. Furthermore, the ratio differed among affected individuals in some families. These may cause inter- and intra-familial variation of the phenotype as well as the rate of penetrance. Furthermore, the loci of the mutation may also be involved in phenotype.

It is also necessary why a certain mutation relate particular clinical symptoms shown above.

As the enzyme for the synthesis of BH_4, GCH-1 deficiency may affect tryptophan hydroxylase (TPH) as well as TH. There is the difference of K_m value for TH and TPH. With heterozygous mutant gene, the BH_4 decreases partially in HPD. Thus TH with higher affinity to BH_4 is affected rather selectively [3,4]. However, in molecular conditions with marked decrease of BH_4, TPH is affected as well as TH and may produce symptoms induced by deficiencies of the 5HT neurons.

In basal ganglia disorders, contralateral or ipsilateral of the side of predominantly involved of the sternocleidomastoideus (SCM) and the extremities reflects the region of the causative lesion.

In postural type of AD GCH-1 deficiency, predominant side of rigid hypertonus is contralateral between the SCM and the extremities. This suggests the lesion in the afferent structure to the striatum with side predominance ipsilateral to the predominantly affected side of the SCM, that is, involvement of the NS DA neuron with predominance to the side predominantly affected SCM.

Postural tremor, torticollis and generalized rigid hypertonus develop later independently from postural dystonia. The side predominance of these symptoms is ipsilateral both in the extremities and in the SCM. This implicates a causative lesion located in the downstream of the striatum. As these symptoms are DA responsive, it is suggested that hypofunction of the DA neuron projecting to the D_1 receptor on the subthalamic nucleus (STN) is postulated to be involved [3,4].

Furthermore, results of the stereotactic surgeries and the paired pulse transcranial magnetic stimulation show involvement of the ascending output of the basal ganglia in tremor, focal and segmental dystonia and rigidity in adult onset cases. These processes are also involved in focal dystonia.

Whereas, dopa-responsive growth arrest seen in children with HPD postulates the involvement of D_4 receptor of the tuberoinfundibular tract. The D_4 receptor belongs to the D_2 receptor family, which matures early among D_2 families [3,4]. This implicates that the terminals of the NS-DA neuron in HPD connect to the receptors which develop early.

Pteridine metabolism develops in late fetal period with critical period in early infancy which extends to

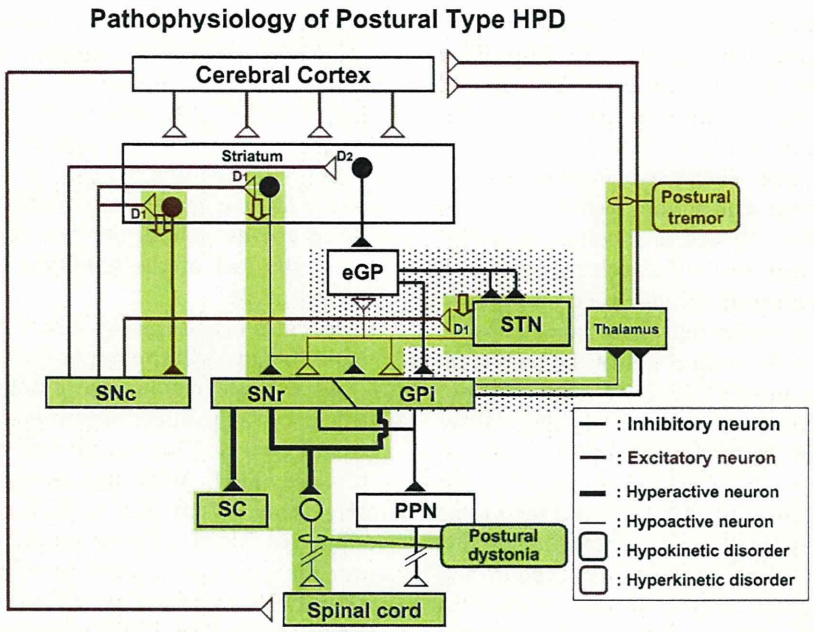

Fig. 2. Pathophysiologies of HPD; GPe: globus pallidus external segment; GPI: globus pallidus internal segment; STN: subthalamic nucleus; SNc: substantia nigra pars compacta; SN: substantia nigra pars reticula; SC: superior colliculus; PPN: pedunculopontine nucleus.

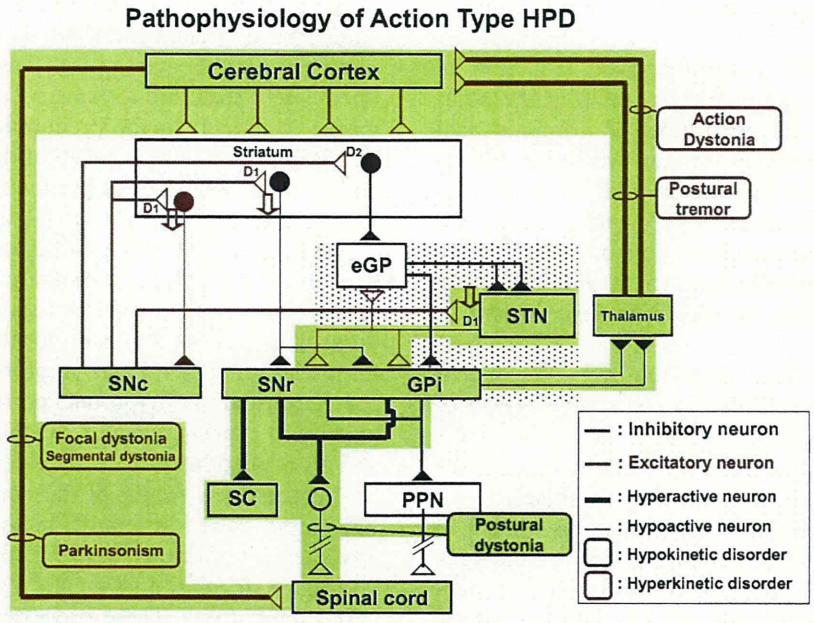

Fig. 3. Pathophysiologies of HPD; GPe: globus pallidus external segment; GPi: globus pallidus internal segment; STN: subthalamic nucleus; SNc: substantia nigra pars compacta; SN: substantia nigra pars reticula; SC: superior colliculus; PPN: pedunculopontine nucleus.

early childhood [19]. Study in stimulated mononuclear blood cells [20] also showed age-dependent decrement of the activities of GCH-1 in the first three decades of life. Thus pteridine metabolism may involve in the age related decrement of TH activity [10] particularly in its early phase.

Thus, the pathophysiologies of postural and action type dystonia of HPD are shown in **Figs. 2 and 3**.

GCH-1 deficiency caused by abnormalities of pteridine metabolism leads to the decrease of the TH protein or DA in the ventral area of the striatum or the striosome in early developmental course.

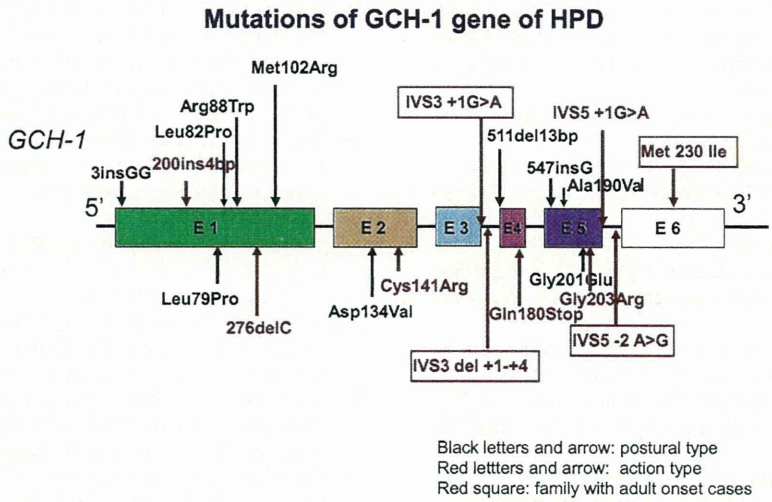

Fig. 4. Locus of the mutation of the GCH-1 gene in HPD. The mutations shown with black letter are those of postural type, these shown with red letters are mutations of action type. Those surrounded by red square are mutations of the families with adult onset cases.

The D_1 DA receptors develop early among DA receptors. Thus, in HPD deficiency of TH or DA at the terminal of the NS DA neuron projecting to the D_1 receptors provides characteristic symptoms along with development of the downstream structures.

Thus disfacilitation of the D_1 receptors on the striosome causes failure in morphogenesis of the DA neurons in the SNc by early infancy with suppressing the striosome–SNc pathway, which matures earliest among the striatal outputs, with GABAergic neuron, excitatory at the age.

The striatal direct pathway and the descending output of the basal ganglia mature earlier than the indirect pathway and the ascending outputs [3,4]. Thus hypofunction of the D_1 receptor on the striatal direct pathway causes postural dystonia in childhood by disinhibition of the descending pathway of the basal ganglia to the brainstem reticular formation. Hypofunction of DA terminals on the STN provides tremor and symptoms of action dystonia type by disfacilitating the late maturing ascending pathways of the basal ganglia to the specific nucleus of the thalamus in late childhood, and focal dystonia and generalized rigidity with tremor in adulthood [4].

Postural dystonia is a hypokinetic disorder, while the action dystonia and other symptoms related to the STN are hyperkinetic disorders.

However, it is not clarified why these pathophysiologies develop under normal DA activities in the SNc.

Postural type and action type are differed by the mutation (**Fig. 4**). But there observed no particular coding region for these types.

Failure in development and modulation of postural tone and locomotion and psychobehavioral function from early infancy observed in compound heterozygotes and early onset phenotypes are considered to be caused by deficiency of 5HT activities. The deficiency of the 5HT activities further causes insufficiency in restriction of atonia into sREM and induces hypofunction of the pedunculopontine nucleus (PPN), which induce hypofunction of the DA neurons in the SNc and the ventro tegmental area, and cause parkinsonian and psychological symptoms. Preservation of interlimb coordination or locomotion in classic HPD suggests minimum or no involvement of the 5HT neuron [4].

Female predominance might depend on a genetically determined gender difference of the DA neuron. However, correlation of levels of the striatal TH protein for development of symptoms and gender difference in penetrance, suggest involvement of gender difference in modulation of striatal TH protein for female predominance.

Diagnosis of HPD is usually not difficult by characteristic clinical symptoms. Gene analysis for the mutation of GCH-1 gene is most definitive diagnosis. However, there are cases whose mutation is not detected. In these cases estimation of neopterin and biopterin in CSF, and GCH-1 activity in peripheral mononuclear cells are reliable.

A part of this study was presented at the 10th Asian and Oceanian Congress of Child Neurology which was held in Daegu, Korea, June 10–13, 2009.

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Dandy–Walker Malformation Associated With Heterozygous *ZIC1* and *ZIC4* Deletion: Report of a New Patient

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We report on a female patient with Dandy–Walker malformation possibly caused by heterozygous loss of *ZIC1* and *ZIC4*. The patient presented with mental retardation, epilepsy, and multiple congenital malformations including spina bifida, mild dysmorphic facial features including, thick eyebrows, broad nose, full lips, macroglossia, and hypoplasia of the cerebellar vermis with enlargement of the fourth ventricle on brain magnetic resonance imaging, which is consistent with Dandy–Walker malformation. A chromosome analysis showed interstitial deletion of chromosome 3q23-q25.1. Fluorescence in situ hybridization (FISH) and microarray-based genomic analysis revealed the heterozygous deletion of *ZIC1* and *ZIC4* loci on 3q24. Her facial features were not consistent with those observed in blepharophimosis–ptosis–epicanthus inversus syndrome (BPES) involving *FOXL2* abnormality. Other deleted genes at 3q23-25.1 might contribute to the dysmorphic facial appearance. A milder phenotype as the Dandy–Walker malformation in our patient supports the idea that modifying loci/genes can influence the development of cerebellar malformation.

Key words: Dandy–Walker malformation; interstitial deletion 3q; *ZIC1*; *ZIC4*

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INTRODUCTION

Dandy–Walker malformation (DWM) is an abnormality in the development of the central nervous system that is defined by hypoplasia and upward rotation of the cerebellar vermis and cystic dilatation of the fourth ventricle [Hart et al., 1972; Parisi and Dobyns, 2003]. DWM is etiologically heterogeneous in association with a wide variety of chromosomal anomalies, various Mendelian disorders, multifactorial disorders, and environmental factors [Murray et al., 1985; Chitayat et al., 1994].

Grinberg et al. [2004] described seven nonrelated patients of DWM with de novo interstitial deletions of chromosome 3q. Cytogenetic investigation of these patients showed the first critical region involved in DWM, which encompasses genes *ZIC1* and *ZIC4*. There are five *Zic* genes encoding zinc finger proteins in humans and mice [Grinberg and Millen, 2005]. *ZIC1* and *ZIC4* are tightly linked on human chromosome 3 and mouse chromosome 9 [Grinberg et al., 2004; Grinberg and Millen, 2005]. A heterozygous deletion of these two linked genes in mice resulted in a phenotype that closely resembles DWM [Grinberg et al., 2004], strongly suggesting that heterozygous deletion of both *ZIC1* and *ZIC4* is the cause of DWM in humans. This is the second report on a new patient of DWM with heterozygous *ZIC1* and *ZIC4* deletion.

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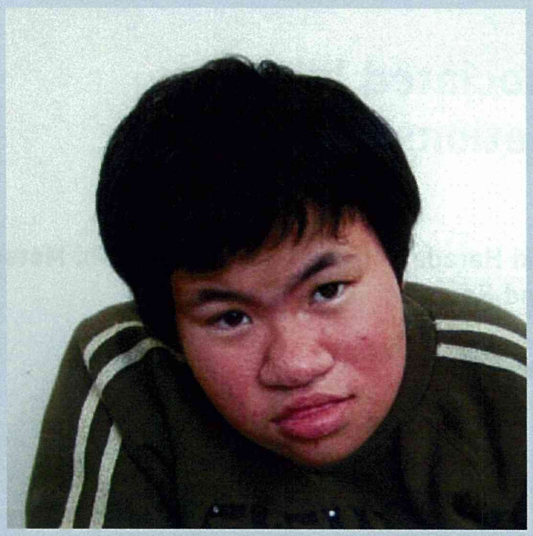

FIG. 1. Photograph of our patient at 21 years of age. Thick eyebrows, broad nose, and full lips are notable.

CLINICAL REPORT

A girl, the third child of healthy nonconsanguineous parents, was born at 41 weeks gestation by normal vaginal delivery following an uneventful pregnancy. Her elder brother has had epilepsy since age 10, however, his mental development was normal. Her birth weight was 2,880 g, length was 48 cm, and occipitofrontal circumference was 33 cm. A sacral dimple associated with occult spine bifida was found at birth. Computed tomography (CT) of the brain showed no sign of hydrocephalus. Follow-up CT at the age of 3 years showed mild dilatation of lateral ventricles. No treatment including surgical intervention was required at that time. Dislocation of both hip joints was also noted. After delivery, the patient received tube feeding for 3 months because of feeding difficulties and poor body weight gain. Her development was severely delayed: she could crawl by herself at age 1 year, and walk alone at 8 years. Hip joint dislocation was surgically repaired at 3 years. At 11 years, she developed generalized seizures, which were uncontrollable by various anti-epileptic drugs. On admission at 11 years, she showed dysmorphic features including thick eyebrows, a broad nose, full lips, and macroglossia, but no blepharophimosis/ptosis (Fig. 1). Mild scoliosis was also noted. Neurological examination revealed she had left hemiparesis with contracture of the lower and upper limbs. Both lower limbs were atrophic. Myoclonic movements on the left limbs were observed. Signs of cranial nerve impairment or cerebellar ataxia were evident. Electroencephalography identified a right-sided delta activity in the frontal lobe. Radiogram of facial bone showed no abnormal findings. Magnetic resonance imaging (MRI) of the brain showed hypoplasia and upward rotation of the cerebellar vermis and enlargement of the fourth ventricle, indicating DWM (Fig. 2A,B). The lateral ventricles were also enlarged. At 19 years, she could not speak. She could not walk alone because of deteriorating left hemiparesis with frequent seizures. She showed regular menstruation after menarche at 15 years.

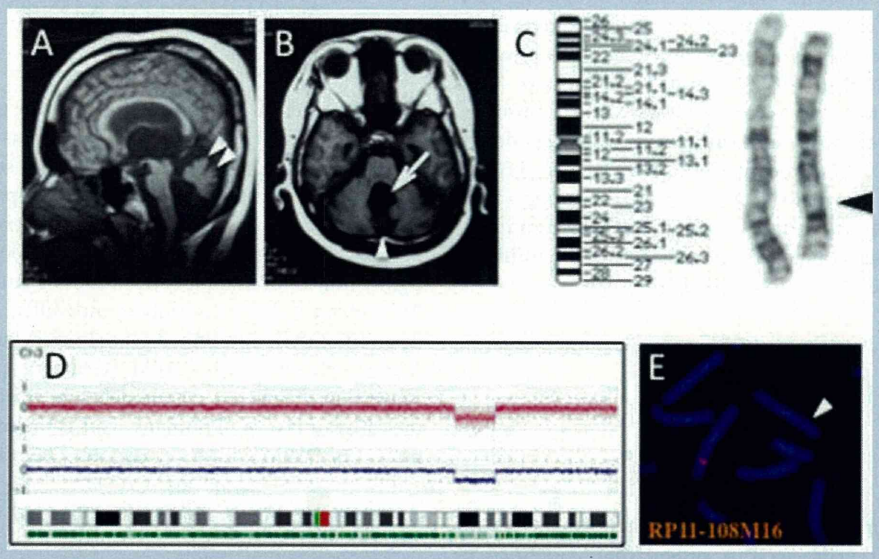

FIG. 2. A: Brain magnetic resonance imaging of the patient. Midline sagittal T1-weighted image shows hypoplasia and upward rotation of the cerebellar vermis (white arrowheads). B: Brain magnetic resonance imaging of the patient. Transverse T1-weighted image shows hypoplasia of the cerebellar hemisphere and vermis, and enlarged the fourth ventricle (white arrow). The fourth ventricle communicates with the posterior fossa fluid space (white arrowhead). C: Partial karyotype of the patient: del(3)(q23q25.1). The arrowhead indicates deletion. D: SNP chip analysis. A 14-Mb deletion is clearly demonstrated using CNAG 2.0 software. E: FISH analysis. RP11-108M16 covering *ZIC1* and *ZIC4* shows a heterozygous deletion (white arrowhead).

Cytogenetic and Genomic Analysis

Chromosomal analysis of her peripheral blood lymphocytes revealed that her karyotype was 46,XX,del(3)(q23q25.1) (Fig. 2C). Normal karyotype was confirmed in her parents. Her elder brother was not examined because abnormal features were not observed. To define chromosome 3q deletion, we performed genomic copy number analysis by GeneChip 250K Nsp Array (Affymetrix, Santa Clara, CA) and CNAG 2.0 software [Nannya et al., 2005] using the DNA of the patient's peripheral blood leukocytes. Copy number analysis clearly demonstrated the 14-Mb interstitial deletion: arr 3q23q25.31 (142,479,100–156,504,521) × 1 (Fig. 2D). According to the UCSC Genome Browser Human February 2009 Assembly, the deletion contains 67 RefSeq genes including following OMIM genes, *ATR*, *PLOD2*, *ZIC4*, *ZIC1*, *AGTR1*, *HPS3*, *CP*, *CLRN1*, *P2RY12*, and *MME*. Fluorescence in situ hybridization (FISH) using three BAC clones, RP11-108M16 (covering *ZIC4* and *ZIC1*), RP11-1001A3, and RP11-71N10 at 3q24, confirmed the deletion (Fig. 2E).

DISCUSSION

This patient presented with multiple congenital malformations, including occult spina bifida, dysmorphic facial features, and hypoplasia, and upward rotation of the cerebellar vermis with enlargement of the fourth ventricle on brain MRI, which were consistent with DWM. A cytogenetic analysis showed interstitial deletion of chromosome 3q23 to 3q25.1, and her FISH study confirmed the heterozygous deletion of *ZIC1* and *ZIC4* on 3q24. Recently, Grinberg et al. [2004] reported that locus 3q24 is the first critical region involved in DWM, encompassing genes *ZIC1* and *ZIC4*. The human ZIC gene family encoding zinc-finger transcription factors is comprised of five members [Grinberg and Millen, 2005]. The ZIC gene family is expressed in central nervous systems, including the cerebellum, and *Zic* genes are found to be expressed in the adult mouse cerebellum in a highly restricted manner [Aruga et al., 1994, 1996, 2002]. *Zic1* plays essential roles in cerebellar development, and the *Zic1* gene deletion could cause the extracerebellar phenotype as those reported in the *Zic1* knockout mice [Aruga et al., 1998; Ogura et al., 2001]. Human *ZIC1* and *ZIC4* are both mapped to 3p24. A mouse model for only $Zic1^{+/-}$ or $Zic4^{+/-}$ showed a slightly hypoplastic cerebellum, however, 15% of these double heterozygotes have severe cerebellar hypoplasia [Grinberg et al., 2004]. The authors conclude that heterozygous loss of *ZIC1* and *ZIC4* is the cause of DWM in individuals with deletion of 3q2. In our patient, we also demonstrated the heterozygous deletion of both *ZIC1* and *ZIC4* by FISH and SNP array. After the original report of seven cases, this is the second report dealing with the eighth case of DWM with heterozygous *ZIC1* and *ZIC4* deletion.

The original seven patients showed various phenotypes such as DWM. A wide variety of deletion in size was noted. The cerebellar hypoplasia in our patient is moderately severe as compared with those in the original seven cases. Chromosomal deletion in our patient ranges from 3q23 to 3q25.1. Grinberg et al. [2004] stated that the severity of DWM does not correlate with the size of chromosomal deletion. In the $Zic1^{+/-}$ $Zic4^{+/-}$ mice, 85% had a mild cerebellar phenotype whereas 15% were severely affected. The authors speculated that the variable expressivity observed in humans and mice might support the idea of modifying loci influencing the development of cerebellar malformation.

Three of the seven individuals in the report of Grinberg et al. [2004] have facial changes observed in the blepharophimosis–ptosis–epicanthus inversus syndrome (BPES), representing a recognizable contiguous gene syndrome [Smith et al., 1989; Ishikiriyama and Goto, 1993]. BPES is also an autosomal dominant inheritance and maps to 3q23 [Amati et al., 1995]. Crisponi et al. [2001] identified the forkhead transcription factor gene 2 (*FOXL2*) gene as responsible for BPES, which is located 3q22.3-q23. The facial dysmorphology of our patient is not consistent with BPES. To define her deletion, we performed a SNP array-based genomic copy number analysis and found that her interstitial deletion did not involve *FOXL2*. Only one of the five patients with 3q23-q25 deletion in the earlier reports showed no clinical features resembling BPES [Franceschini et al., 1983; Alvarado et al., 1987]. Dysmorphic facial features of this patient included synophrys of the eyebrows, broad nose, and full lips [Franceschini et al., 1983], partially resembling those of our case. Other deleted genes may affect the dysmorphic facial features in our patient.

DWM is a relatively common malformation of the central nervous system, but this condition has etiologic heterogeneity. After finding the first critical region of DWM, DeScipio et al. [2005] identified six children with subtelomeric deletions of 6p25, which is the second locus of DWM, and Aldinger et al. [2009] reported that the alteration of *FOXC1* at 6p25.3 contributed to DWM. In addition, Jalali et al. [2008] reported on a male patient with a heterozygous deletion of distal 2q, and identified a candidate locus for DWM with occipital cephalocele at 2q36.1 by linkage analysis. Their family showed an autosomal dominant mode of inheritance.

The recurrence risk for DWM is also heterogeneous, depending on the etiology, which is not yet fully explained despite intensive cytogenetic investigations of many cases. Empiric recurrence risk for DWM in the absence of a known disorder is relatively low [Murray et al., 1985]. High-resolution chromosomal analysis of patients may provide critical information necessary for genetic counseling related to DWM.

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Inflammatory changes in infantile-onset *LMNA*-associated myopathy

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Abstract

Mutations in *LMNA* cause wide variety of disorders including Emery–Dreifuss muscular dystrophy, limb girdle muscular dystrophy, and congenital muscular dystrophy. We recently found a *LMNA* mutation in a patient who was previously diagnosed as infantile onset inflammatory myopathy. In this study, we screened for *LMNA* mutations in 20 patients suspected to have inflammatory myopathy with onset at 2 years or younger. The diagnosis of inflammatory myopathy was based on muscle pathology with presence of perivascular cuffing and/or endomysial/perimysial lymphocyte infiltration. We identified heterozygous *LMNA* mutations in 11 patients (55%), who eventually developed joint contractures and/or cardiac involvement after the infantile period. Our findings suggest that *LMNA* mutation should be considered in myopathy patients with inflammatory changes during infancy, and that this may help avoid life-threatening events associated with laminopathy.

Keywords: Inflammatory myopathy; Laminopathy; Emery–Dreifuss muscular dystrophy; Limb girdle muscular dystrophy; Congenital muscular dystrophy; *LMNA*; Infantile; Pathology; Steroid therapy; Muscle image

1. Introduction

Laminopathy is a group of disorders caused by mutations in the *LMNA* gene encoding A-type lamins that includes autosomal forms of Emery–Dreifuss muscular dystrophy (AD- and AR-EDMD) and limb girdle muscular dystrophy type 1B (LGMD1B). EDMD is characterized by the triad of: (1) early contractures of the elbows, Achilles tendons, and posterior cervical muscles; (2) slowly progressive muscle weakness and atrophy that begins in a humeroperoneal distribution; and (3) cardiomyopathy with conduction defects which culminates in complete heart block and atrial paralysis [1]. LGMD1B patients show progressive proximal dominant muscle involvement and

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cardiomyopathy with conduction defects, but joint contracture is not prominent. The onset of these diseases is usually 2 years or later. Recently, *LMNA*-related congenital muscular dystrophy (L-CMD) was reported as a novel and severe form of laminopathy [2]. L-CMD has variable severity and can be divided in two main groups: a severe group with absent motor development and patients with dropped-head syndrome.

We recently came across an infantile-onset laminopathy patient with marked mononuclear cell infiltrations in his muscle mimicking inflammatory myopathy (Patient 1 in Table 1, Fig. 1A). This patient showed hypotonia and delayed motor milestones with elevation of serum CK levels from 3 months of age. Although, he became ambulant at 15 months of age, he presented proximal dominant muscle weakness and atrophy with no dropped-head at 2 years of age. Corticosteroid therapy was started based on the muscle pathological findings that had beneficial effects on his motor development. *LMNA* gene analysis was done at 6 years of age when his ankle and elbow joint contractures appeared and a heterozygous p.Glu358Lys mutation was identified.

From this result, we screened *LMNA* mutation in the 20 patients with the onset at 2 years or younger who were pathologically suspected as inflammatory myopathy.

2. Patients and methods

2.1. Patients

All clinical materials used in this study were obtained for diagnostic purposes and written informed consent was obtained from guardians of all patients. This work was approved by the Ethical Committee of National Center of Neurology and Psychiatry (NCNP). We retrospectively recruited patients with onset at 2 years or younger who were pathologically suspected to have inflammatory myopathy from a total of 10,874 muscle biopsies stored in the

Table 1
Clinical, radiological, and genetic findings of patients with *LMNA* mutations and inflammatory changes.

Patient #/gender/ *LMNA* mutations	Age at onset /age at biopsy/ age at last consultation	Initial signs/ CK at biopsy	Muscle pathology	Steroid treatment: responsiveness/ age at start of administration/ duration of administration	Age at acquired ambulation/ maximum motor ability	Cardiac involvement	Joint contracture	Respiratory dysfunction	CT/MRI (age)/imaging at thigh	CT/MRI (age)/imaging at calf
1/M/E358K*	3 m/2 y/11 y	Motor delay/900	IC: marked, diffuse; NR: moderate; Fib: mild	Effective/2 y/9 y	15 m/Ambulant	No	6 y: Ankles, elbows, 8 y: rigid spine	No	MRI (8 y)/ selective involvement of VL, VI, VM	MRI (8 y)/ selective involvement of SO, mGC
2/M/R249W*	10 m/10 m/12 y (Died by respiratory failure)	Motor delay/1000	IC: marked, pathy; NR: mild; Fib: mild	Effective/10 m/11 y	Unknown/ambulant	9 y: Heart failure	4 y: Ankles, knees	9 y: Nocturnal NPPV	ND	ND
3/M/N39D	11 m/1 y/16 y	Motor delay/1100	IC: marked, pathy; NR: marked; Fib: mild	Effective/1 y/15 y	18 m/Ambulant	13 y: 200B0 A–V block, 15 y 3° A–V block, pacemaker implantation	1 y: Ankles, knees, hips, Rigid spine from childhood	No	CT (13 y)/DI with relative sparing of RF, GR, SA	CT (13 y)/DI
4/F/R249Q*	2 y/2 y/15 y	High CK/2000	IC: moderate, focal; NR: moderate; Fib: moderate	Effective/3 y/6 m	14 m/Ambulant	12 y: 1° A–V block	3 y: Ankles, 8 y: elbows	No	CT (6 y)/DI with relative sparing of RF, GR	CT (6 y)/ selective involvement of SO, mGC
5/M/R28Q	5 m/1 y/11 y	Motor delay/800	IC: marked, pathy; NR: moderate; Fib: moderate	Ineffective/1 y/2 y	18 m/9 y: Inability to walk	Atrial fibrillation, A–V block, PAC, PVC	No	No	CT (11 y)/DI with relative sparing of RF, GR, SA	ND
6/M/R41S	9 m/1 y/13 y	Motor delay/900	IC: moderate, diffuse, NR: moderate Fib: moderate	Ineffective/1 y/8 y	16 m/9 y: Inability to walk	11 y: PSVT attack	6 y: Ankles, elbows	11 y: Nocturnal NPPV	MRI (10 y)/ DI/DI	MRI (10 y)/ DI/DI
7/F/K32del*	1 y/2 y/6 y	Unsteady gait/800	IC: mild, focal; NR: mild; Fib: mild	Ineffective/2 y/8 m	15 m/5 y: Inability to walk	No	2 y: Ankles	No	CT (4 y)/DI with relative sparing of RF, GR/Selective involvement of SO, mGC	CT (4 y)/DI with relative sparing of RF, GR/Selective involvement of SO, mGC
8/M/R249W*	11 m/1 y/24 y (Died by arrhythmia)	Motor delay/600	IC: marked, pathy; NR: mild; Fib: moderate	Ineffective/1 y/unknown	2 y/12 y: Inability to walk	17 y: 2° A–V block, 23 y complete A–V block	17 y: Ankles, knees	No	ND	ND
9/F/L292P	1 y/8 y/10 y	Motor delay/300	IC: mild, focal; NR: moderate; Fib: marked	Unadministered	16 m/4 y: Inability to walk	6 y: LV dysfunction, 8 y: PAC, PVC	No	No	MRI (8 y)/DI with relative sparing of RF, GR, SA	MRI (8 y)/DI
10/F/R377C*	2 y/4 y/7 y (Died by heart failure)	Unsteady gait/1000	IC: moderate, focal; NR: moderate; Fib: moderate	Unadministered	10 m/ambulant	7 y: DCM (EF:32%)	5 y: Ankles	No	ND	ND
11/F/N456H	2 y/5 y/10 y	Unsteady gait/3000	IC: moderate, focal; NR: moderate; Fib: marked	Unadministered	12 m/ambulant	No	6 y: Ankle, knee, neck, 8 y: rigid spine	No	MRI (10 y)/ DI with relative sparing of RF, GR, SA	MRI (10 y)/ DI

A–V block = atrioventricular conduction block, CK = creatine kinase, CT = computed tomography, DI = diffuse involvement, EF = ejection fraction, Fib = endomysial fibrosis, GR = gracilis, IC = inflammatory cellular infiltration, LV = left ventricle, mGC = medial head of gastrocnemius, MRI = magnetic resonance imaging, NPPV = noninvasive positive-pressure ventilation, NR = necrotic and regenerating process, PAC = premature atrial contraction, PSVT = paroxysmal supraventricular tachycardia, PVC = premature ventricular contraction, RF = rectus femoris, SA = Sartorius, SO = soleus, VI = vastus intermedius, VL = vastus lateralis, VM = vastus medialis.

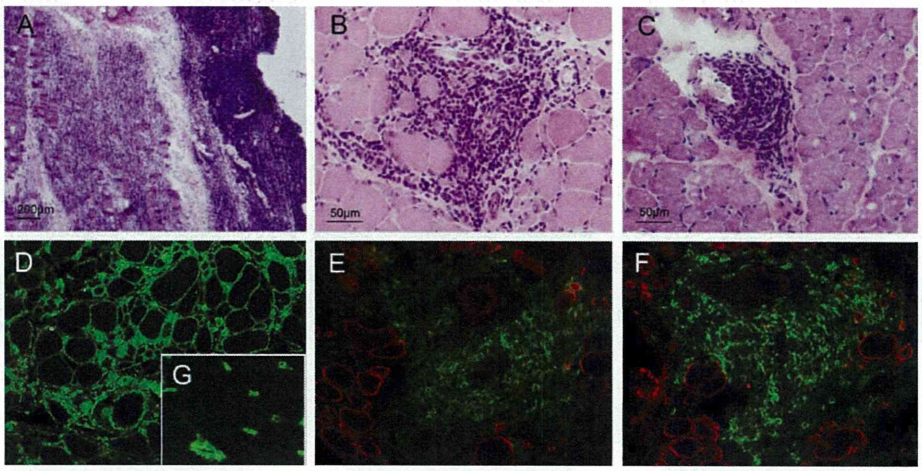

Fig. 1. Inflammatory cellular infiltration observed in the patients with *LMNA* mutations on hematoxilin and eosin staining (A: Patient 1, B: Patient 3, C: Patient 9). Serial frozen sections of muscle from Patient 5 were immunostained with HLA-ABC (D), double immunostained with CD4 (green) and dystrophin (red) (E), and CD20 (green) and dysrophin (red) (F). HLA-ABC stain in control muscle is shown in (G).

National Center of Neurology and Psychiatry. The diagnosis of inflammatory myopathy was based upon the mononuclear cell infiltrations at perimysial, endomysial, and perivascular sites [3]. Patients suspected to have dermatomyositis with skin rash and/or perifascicular atrophy on muscle pathology were excluded in this study. Then we gathered a total of 20 patients including one patient (Patient 2) who had previously been reported as infantile polymyositis [4].

2.2. Histopathological studies

All biopsied samples were taken from biceps brachii. Muscle specimens were frozen in isopentane chilled in liquid nitrogen. Serial frozen sections were stained with hematoxylin and eosin, modified Gomori trichrome, and a battery of histochemical methods. Immunohistochemical analysis was performed as described previously [5]. Antibodies used in this study are: dystrophin (DMDP-II [6], DYS1, DYS2, and DYS3 from Novocastra, Newcastle upon Tyne, UK); sarcoglycans (SGCA, SGCB, SGCG, and SGCD: Novocastra); laminin-α2 chain (ALEXIS, Farmingdale, NY); α-dystroglycan (Upstate Biotech, Lake Placid, NY); caveolin-3 (BD Transduction Laboratories, Franklin Lakes, NJ); dysferlin (Novocastra); emerin (Novocastra); collagen VI (Novocastra); CD4 and CD8 (Nichirei, Tokyo, Japan); CD20, and HLA-ABC (DAKO, Glostrup, Denmark).

2.3. Mutational analysis of LMNA

Genomic DNA was extracted from either frozen muscles or peripheral lymphocytes using standard protocols [7]. All exons and their flanking intronic regions of *LMNA* were amplified by PCR and directly sequenced using automated 3130 sequencer (PE Applied Biosystem, Foster City, CA). Primer sequences are available upon request.

2.4. Clinical information

Clinical characteristics collected from attending physicians were demographic data, age of onset, initial signs, motor functions, presence of cardiac involvement, presence of joint contractures, respiratory function, effectiveness of steroid, and pertinent laboratory examinations including serum creatine kinase (CK), electrocardiogram, Holter electrocardiogram, and echocardiogram.

2.5. Muscle imaging

Muscle computed tomography (CT) or magnetic resonance imaging (MRI) was done with some modifications depending on the facilities in each hospital. Scans were performed at thigh (the largest diameter of thigh) and calf (the largest diameter of lower leg) levels. Involvement of each muscle was evaluated at both scan levels.

3. Results

Ten types of heterozygous single nucleotide substitutions in *LMNA* were identified in 11 of 20 patients. Four (p.Arg249Gln, p.Leu292Pro, p.Asn456His and p.Arg377 Cys) mutations were previously reported in patients with AD-EDMD or LGMD1B, one (p.Arg249Trp) was found only in L-CMD patients, and two (p.Lys32del and p.Glu358Lys) were identified in AD-EDMD, LGMD1B, or L-CMD patients [2,8–10]. Another three (p.Arg28Gln, p.Asn39Asp, p.Arg41Ser,) were novel mutations and not detected in 300 control chromosomes. All 11 patients had neither consanguinity nor family history of myopathy or

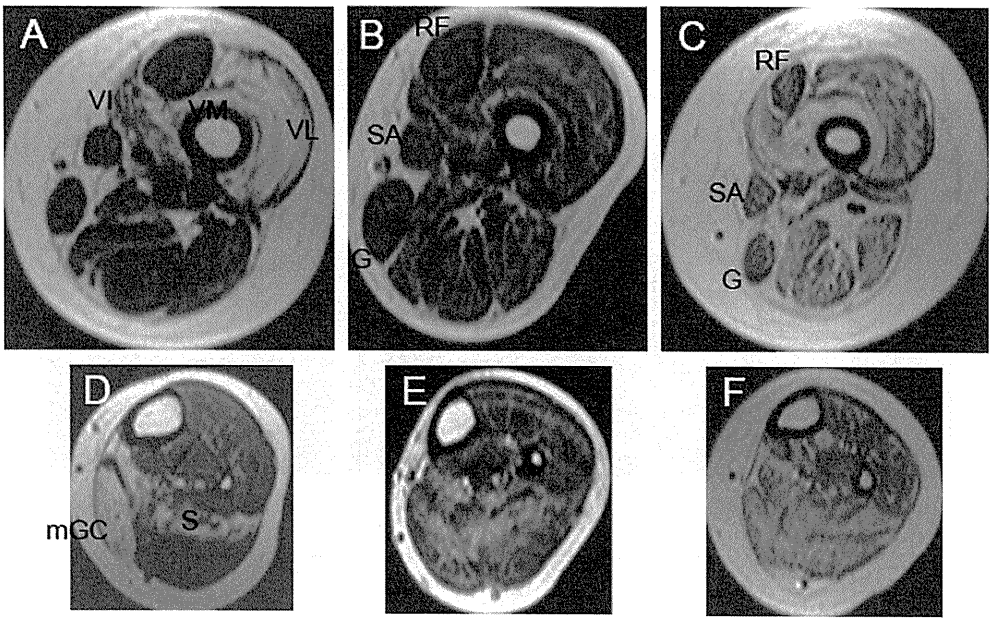

Fig. 2. Selective muscle involvement of thigh and calf muscles. Transverse sections of T1 weighted magnetic resonance imaging of thigh (A–C) and calf (D–F) in patients with *LMNA* mutations. Selective involvements of vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), soleus (S), and medial head of gastrocnemius (mGC, A, D: Patient 1), relatively mild and diffuse involvements with relative sparing of rectus femoris (RF), gracilis (G), sartorius (SA, B, E: patient 11), and diffuse and severe involvement with relative sparing of rectus femoris, gracilis, sartorius (C, F: patient 9) are observed.

cardiomyopathy. DNA samples from the parents of 11 patients were not available.

Table 1 shows clinical summary of the 11 patients with *LMNA* mutations. Initial clinical signs were motor developmental delay or progressive muscle weakness. Head drop was not observed in any patient. Serum CK levels were mildly to moderately elevated in all patients. Joint contractures, spinal rigidity, and cardiac involvement were not observed at the time of the biopsy but became prominent in some patients in later age. Importantly, Patient 6 had an episodic paroxysmal supraventricular tachycardia during general anesthesia at age 11 years, and Patient 3 received pacemaker implantation due to complete atrioventricular conduction block at age 15 years. Patient 8 succumbed to sudden death due to arrhythmia at age 24 years and Patient 10 died by cardiac failure at age 7 years. Two patients developed chronic respiratory failure requiring non-invasive positive-pressure ventilation. Patient 2 died by respiratory failure at age 12 years. Steroid was used in eight patients but beneficial effects such as improvement of muscle power and reduction of serum CK levels were seen only in four.

On muscle biopsy, the most striking inflammatory change was observed in Patient 1 showing numerous inflammatory cells predominantly located in the perimysial connective tissue (Fig. 1A). This finding was diffusely seen in the whole muscle specimen. The other 10 patients also showed variable degrees of mononuclear cellular infiltration with active necrosis and regenerating process (Fig. 1B, C, Table 1). Fiber size variation and endomysial fibrosis were also seen. Fiber type grouping, groups of atrophic fibers, and abnormal oxidative stains were not observed. Immunohistochemically, sarcolemmal HLA staining was increased in many fibers in all patients examined (Fig. 1D). Infiltrated mononuclear cells were positive for lymphocyte markers of CD4 (Fig. 1E), CD8 (data not shown), or CD20 (Fig. 1F). No abnormal immunostaining was seen for the antibodies associated with muscular dystrophy (data not shown).

Muscle imaging was performed in relatively later stages of the disease in eight out of 11 patients with *LMNA* mutations (Fig. 2). At the level of thigh, Patient 1 showed selective involvement of vastus lateralis, vastus intermedius and vastus medialis. Patient 6 showed diffuse involvement of all thigh muscles. The remaining six patients showed diffuse involvement of thigh muscles with relative sparing of sartorius, gracilis and rectus femoris. At lower leg levels, three patients (Patients 1, 4, and 7) showed selective involvement of soleus and medial head of gastrocnemius. The remaining four patients showed diffuse involvement of calf muscles.

4. Discussion

In our series, surprisingly, more than half of the infantile patients showing inflammatory changes are due to *LMNA* mutations. Prominent mononuclear cell infiltrations can sometimes be evident in biopsies from muscular dystrophy patients including CMD, LGMD, and facioscapulohumeral muscular dystrophy, leading to misdiagnosis of inflammatory myopathy [11–16]. Apparently, however, frequency of inflammatory changes is much higher in infantile striated muscle laminopathy patients, suggesting a possibil-