Fig. 2. Body temperature and wakefulness–sleep rhythms. Date and time are shown in the vertical and horizontal axes, respectively. (a) Core body temperature at the age of 3 months ranged from 34 to 38 °C, lacking a definite circadian rhythm. (b) Actogram at the age of 4 months identified the wake and sleep states in a day, and vertical lines correspond to the frequency of body movements. No definite circadian rhythm was noticed.

mouse monoclonal antibodies to calretinin, parvalbumin, calbindin D28K (1:100, Novocastra), tyrosine hydroxylase (TH) (1:400, Affinity Bioreagents), tryptophan hydroxylase (1:100, Calbiochem) and rabbit polyclonal antibodies to vasopressin (VP) (1:100, Biomeda) and hypocretin-1 (1:100, Oncogene). The cerebral cortex consisted of a superficial cell-sparse layer, a thin neuron layer and a thick layer of randomly scattered neurons. Myelination was delayed in the white matter. Adjacent to the third ventricle, medium sized neurons immunoreactive for both VP and TH formed a group, mimicking the paraventricular nucleus. The optic tract-like myelinated fibers were transferred in the lateral diencephalon, whereas a few neurons immunoreactive for both VP and TH were found in the vicinity of myelinated fibers, mimicking the supraoptic nucleus (Fig. 3b and c). A few neurons immunoreactive for hypocretin-1 were scattered in the ventromedial diencephalons, but neither the suprachiasmatic nucleus nor the arcuate nucleus was identified. The basal ganglia composed of TH-immunoreactive neuropil and small bundles of myelinated fibers. Therein a few large and small neurons were found with fibers immunoreactive for TH (Fig. 3d and e). Neither the globus pallidus nor the subthalamic nucleus could be identified. The expressions of calcium-binding proteins were reduced in the basal ganglia. The

thalamus-like structure in the caudal part of diencephalon consisted of mixture of neurons and irregular myelinated fibers. Immunohistochemistry for TH identified the compact zone of the substantia nigra, periaqueductal gray matter, locus ceruleus and dorsal vagal nucleus, while that for tryptophan hydroxylase stained the raphe nuclei throughout the brainstem. The pyramidal tract was hypoplastic, including the cerebral peduncle, longitudinal fibers in the pontine base and medullary pyramis in the brainstem.

3. Discussion

This case was suspected as having XLAG because of typical clinical and MRI findings, and deletion in the ARX gene confirmed the diagnosis. Neuropathological findings such as the apparent three layers in the cerebral cortex correspond to those in the previous report. This is the first detailed analysis on the hypothalamic function and the neuropathological features of the diencephalons with basal ganglia in genetically confirmed XLAG cases [4,7]. We attempted to correlate the hypothalamic dysfunctions with the immunohistochemical abnormalities in the diencephalons similarly like the previous paper [8]. Absent circadian variations in rhythms of sleep–wakefulness and core body
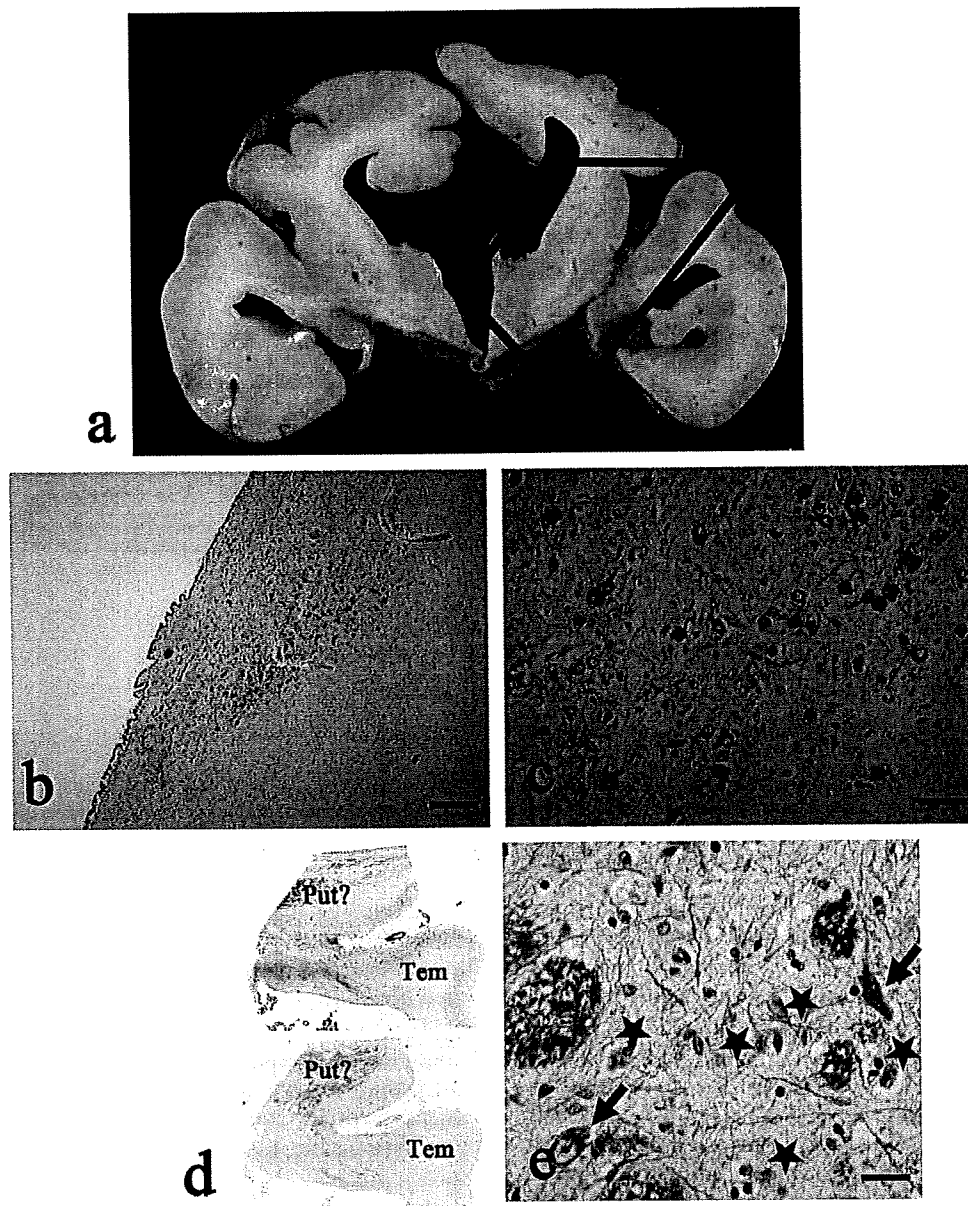
Fig. 3. Neuropathology of the diencephalons. The cross section of cerebrum at the supposed median eminence showed the non-demarcated diencephalons (a), and trapezoid lines trimmed the area, being examined immunohistologically (d). A group of neurons immunoreactive for vasopressin (b, bar = 250 μm) and tyrosine hydroxylase (TH) (c, bar = 50 μm) was found adjacent to the third ventricle. In the left diencephalon (Tem, the temporal cortex), the putamen-like structure (d, Put?) was composed of bundles of myelinated fibers (upper photograph, KB staining) and TH-immunoreactive neuropil (lower photograph). The putamen-like structure was composed of large neurons (arrows) and small neurons (stars) (e, KB staining, bar = 25 μm).

temperature may be related to malfunction of brain clock in the suprachiasmatic nucleus, which was not identified in the autopsy brain. In contrast, the monoaminergic neurons immunoreactive for TH and tryptophan hydroxylase seemed to be comparatively well preserved in the brainstem. Prolonged reactions of gonadotropins on pituitary hormone provocation test suggest altered control in the ventromedial hypothala-mus, which was in accordance with the reduction of neurons immunoreactive for hypocretin-1 in the ventromedial diencephalons. High basal level and prolonged increase after provocation of prolactin indicated the abnormal regulation of dopaminergic neuron systems. Although the dopaminergic neurons immunoreactive for both VP and TH were recognized in the apparent paraventricular and supraoptic nuclei,

they showed ectopic location and reduced occurrence of neurons. Persistent TSH reaction on provocation with hypothyroidism reflects the disturbed control of thyrotropin-releasing hormone secretion in the arcuate nucleus, which was deficient in the autopsy brain. Accordingly, it is likely that the diencephalons showed different changes in each sub-region, which may correspond to various hypothalamic dysfunctions. Taking these findings with the previous study in holoprosencephaly [5], we conclude that the detailed endocrine tests and immunohistochemical analysis in the autopsy brain can be useful for improving the hypothalamic disorders in patients with severe brain anomalies.

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Case report

Abnormal glucose metabolism in aromatic L-amino acid decarboxylase deficiency

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Abstract

We report sibling cases of aromatic L-amino acid decarboxylase (AADC) deficiency, which is a very rare congenital metabolic disorder. These patients were born to healthy and non-consanguineous parents, and presented oculogyric crises, paroxysmal dystonic attacks, and severe psychomotor retardation since early infancy. In cerebrospinal fluid the levels of homovanilic acid and 5-hydroxyindoleacetic acid were very low and the level of L-dopa was very high. The diagnosis was confirmed by the lack of AADC activity in plasma, and a point mutation in the *AADC* gene. MRI revealed a slightly small volume of the prefrontal areas and normal myelination in both patients. Positron emission tomography using 2-deoxy-2[^{18}F] fluoro-D-glucose was performed in one patient, which revealed hypometabolism in the prefrontal cortex and bilateral basal ganglia with a little laterality. These findings suggested that the severe dystonic features were caused by abnormal function of bilateral basal ganglia and severe psychomotor retardation could be due to abnormalities in prefrontal cortical activity.
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Keywords: AADC deficiency; MRI; PET; Prefrontal cortex; Caudate nucleus

1. Introduction

Aromatic L-amino acid decarboxylase (AADC or dopa decarboxylase; DDC) deficiency (OMIM #608643) is an extremely rare congenital metabolic disorder and one of the infantile movement disorders, which is very intractable to treat [1–4]. Although less than 100 cases have been reported worldwide [1–8], a relatively high occurrence rate was reported in Taiwan [7]. AADC converts L-dopa to dopamine and 5-hydroxy tryptophan to serotonin, and its deficiency results in the depletion of

both dopamine and serotonin in the brain. As a consequence, several characteristic symptoms are caused.

We experienced sibling cases of AADC deficiency, confirmed by enzymatic and genetic analysis. We report magnetic resonance imaging (MRI) findings in both cases, and positron emission tomography (PET) using 2-deoxy-2[^{18}F] fluoro-D-glucose (FDG) between dystonic attacks was performed in patient 1.

2. Case reports

2.1. Patient 1

This 3-year-old boy was born to healthy and unrelated parents with mild asphyxia at full term. He cried

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Table 1
The concentration of catecholamine of the CSF.

	L-Dopa	HVA	MHPG	5-HIAA
Patient 1	13.6	5.7	<1.0	<1.0
Patient 2	27.4	12.2	<1.0	<1.0
Normal range	<2.0(ng/ml)	28–200(ng/ml)	6.5–51(ng/ml)	17–116(ng/ml)

HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxy-phenylglycol; 5HIAA, 5-hydroxyindoleacetic acid.

weakly, was motion-less since birth, and needed tube feeding for 1 week. He first showed oculogyric crisis at 3 months of age, and had similar attacks several times a week. Oculogyric crisis usually lasted about 30 min. He also suffered from generalized dystonic attacks for 30–120 min several times a week. Opisthotonus or bicycle-riding movements were observed during these attacks. He showed visual pursuit at 6 months of age, but had not yet obtained head control or rolling over.

He had a severe intellectual and motor developmental delay. He was always nasally congested and his face was frequently running with sweat during wakefulness.

A neurological examination between dystonic attacks revealed general hypotonia, paucity of movement, slightly exaggerated deep tendon reflexes and pathological reflexes. Eye movement was normal. Ordinary blood analyses were normal. An electroencephalogram (EEG) showed no paroxysmal discharges during either dystonic attacks or inter-
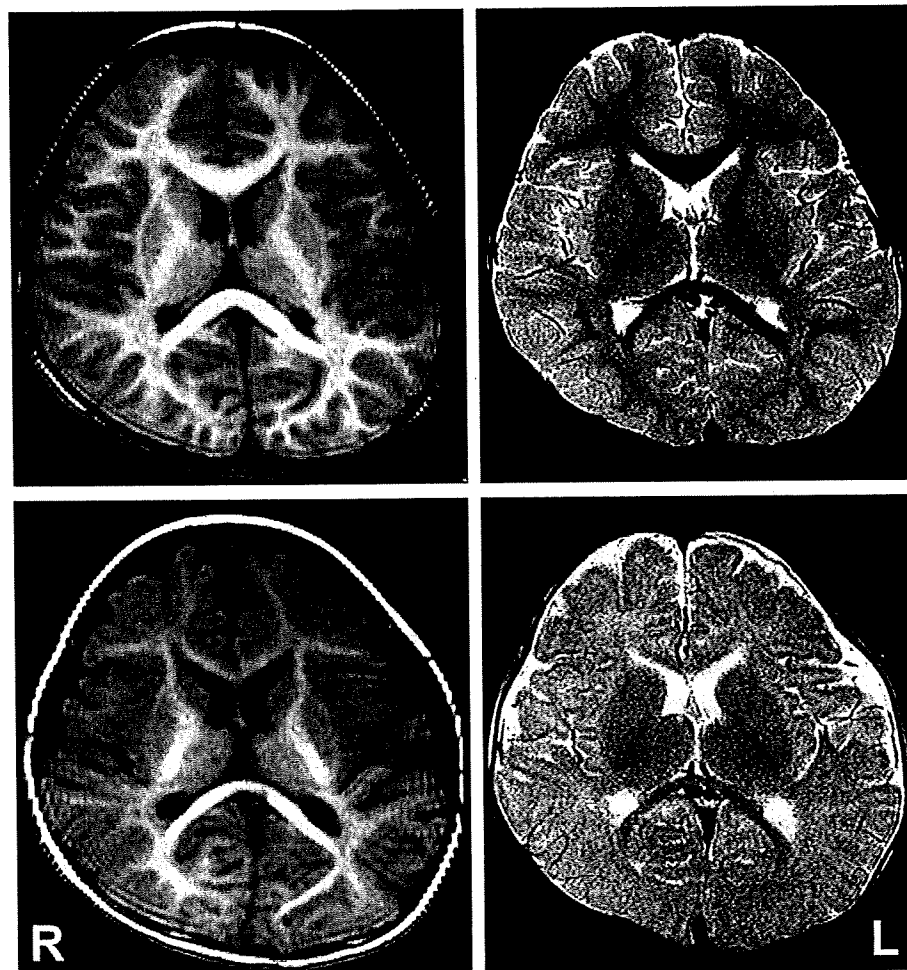

Fig. 1. Axial T1-weighted (left) and T2-weighted (right) MRI at the level of the putamen. Upper row is patient 1 and lower row is patient 2. MRI shows a slightly small volume of the prefrontal areas in both patients. The volumes of basal ganglia and brain cortex are normal, and myelination is also normal. No abnormal intensity areas are seen.

mittent states. A catecholamine analysis of the cerebrospinal fluid (CSF) revealed a very high concentration of L-dopa and a very low concentration of homovanilic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) (Table 1). These results strongly suggested AADC deficiency.

2.2. Patient 2

This 6-month-old girl was the younger sister of patient 1. She was born healthy with no adverse events. She also showed oculogyric crisis since 1 month of age, and paroxysmal general hypertonia lasting for a few hours since 3 months of age but she was alert during the attack. She had not yet obtained head control or rolling over. She also disclosed general hypotonia and paucity of movement between hypertonic attacks. Her CSF revealed a high concentration of L-dopa and a very low concentration of HVA and 5-HIAA (Table 1).

2.3. AADC activity

AADC activity was measured in the serum to confirm the diagnosis using previously reported methods [9].

Serum AADC activity was very low in both patients (AADC activity: 0.5 pmol/min/ml in patient 1, 0.4 pmol/min/ml in patient 2; normal; 50–100).

2.4. Gene analysis

The *AADC* gene mutation was analyzed after obtaining informed consent from the parents of the patients. Genomic DNA from peripheral blood of the patients was extracted according to standard procedures. Each exon of the *AADC* gene was amplified by PCR using primers designed to amplify the coding and flanking non-coding *AADC* regions. Bidirectional cycle sequencing reactions were performed with the ABI Big Dye Terminator Sequencing Kit (Applied Biosystems: Foster city, CA, USA), and the purified products were subject to an automated capillary array sequencer (ABI 3100, Applied Biosystems). Sequencing results revealed a heterozygous point mutation (g.329C > A). The other mutation was not detected. We confirmed that this point mutation was not present in 50 normal Japanese controls.
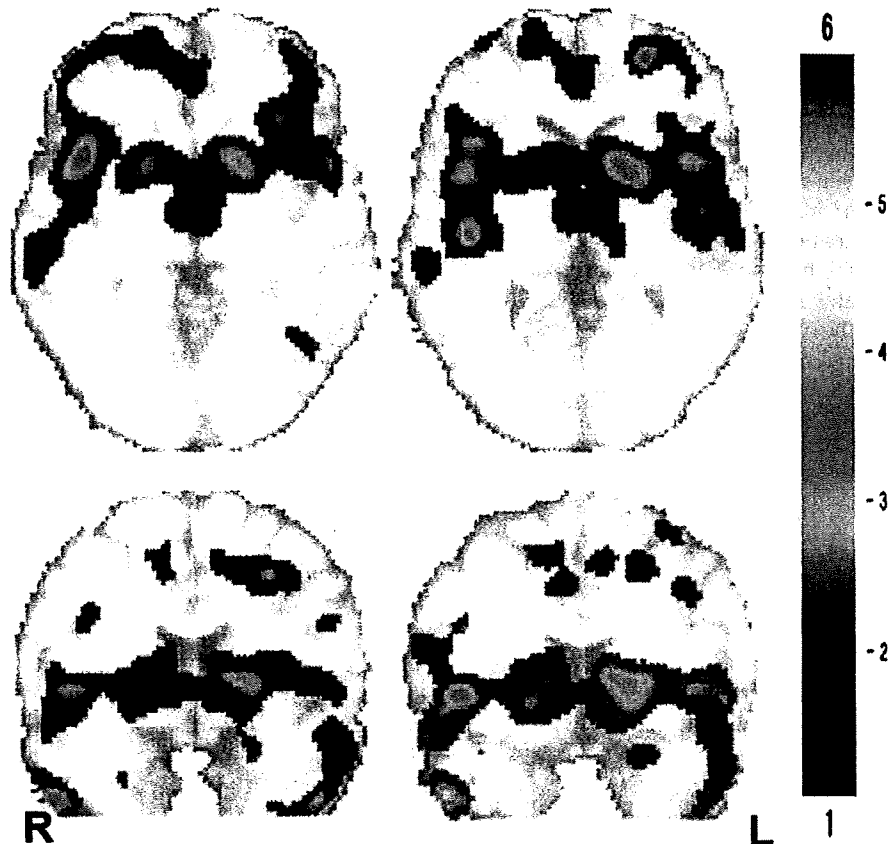

Fig. 2. Easy Z-score imaging (eZIS) analysis of FDG-PET in patient 1. Hypometabolim is observed in bilalateral caudate nuculei to putamina (lower in the left side) and insular cortex with some laterality. Upper; axial section, lower; coronal section.

3. Neuroradiological studies

MRI: Brain MRI in both patients revealed a slightly small volume of the prefrontal areas (Fig. 1) and normal myelination. No abnormal findings in the basal ganglia were observed.

PET: Glucose metabolism was evaluated by FDG-PET in patient 1. We evaluated the results by using an easy Z-score imaging system (eZIS) [10], eZIS revealed hypometabolism in both caudate nuclei and putamina with some laterality (lower in the left side) (Fig. 2) and prefrontal cortex (Fig. 3). The area in which the level of the area was lower than −2SD compared with the standard is colored with purple or blue and the area lower than −3SD is colored with green.

4. Discussion

Patient 1 was at first assumed to have cerebral palsy (CP) because he was born with mild asphyxia. He had been diagnosed with a dystonic type of CP before patient 2 was born. Patient 2, who was born healthy, showed oculogyric crises and dystonic attacks. Since these symptoms were the same as those in patient 1, it was presumed that they both had a basic disorder. Repeated attacks of dystonia reminded us of childhood movement disorders, especially neurotransmitter diseases, and the catecholamine in the CSF indicated an abnormality in the level of neurotransmitters. The low activity of AADC confirmed the diagnosis of AADC deficiency. The gene analysis of the *AADC* showed heterozygous mutation. Since we examined all exons and intron–exon junctions, there must be other mutation in other area. After the diagnosis was established, both patients were treated with a monoamine oxidase (MAO) inhibitor and a dopamine agonist, but showed no favorable response.

In MRI studies, the volume of the prefrontal area was reduced in both cases by visual inspection, although we did not performed volumetric study. The volume of the basal ganglia was normal.

We performed FDG-PET in patient 1 to investigate the brain glucose metabolism. The eZIS analysis revealed hypometabolism in both basal ganglia and prefrontal cortex. To our knowledge, these findings have not yet been reported in other patients with AADC deficiency [3].

In AADC deficiency, both dopamine and serotonin depletion must have occurred in the brain. Dopamine is mostly involved in substantia nigra and basal ganglia circuits. Hypometabolism in caudate nuclei shown in this FDG-PET study probably could be the cause the symptoms of dystonia and muscle tone abnormality.

The mechanism for the slightly small size and hypometabolism in the prefrontal cortex was not identified. Mesencephalic dopaminergic neurons are known to project to the prefrontal cortex and striatum [11]. The dopamine depletion probably causes dysfunction in dopaminergic innervation, and depleted dopaminergic pathways in the prefrontal cortex probably cause the occurrence of prefrontal cortical dysfunction. Similar dysfunction could occur in the serotonergic pathways. Most patients with AADC deficiency have both severe motor developmental and severe intellectual disability, which might be explained by the prefrontal cortical dysfunction.

Both dopamine and serotonin depletion could produce not only basal ganglia dysfunction but also prefrontal cortical dysfunction, especially in the developing brain.
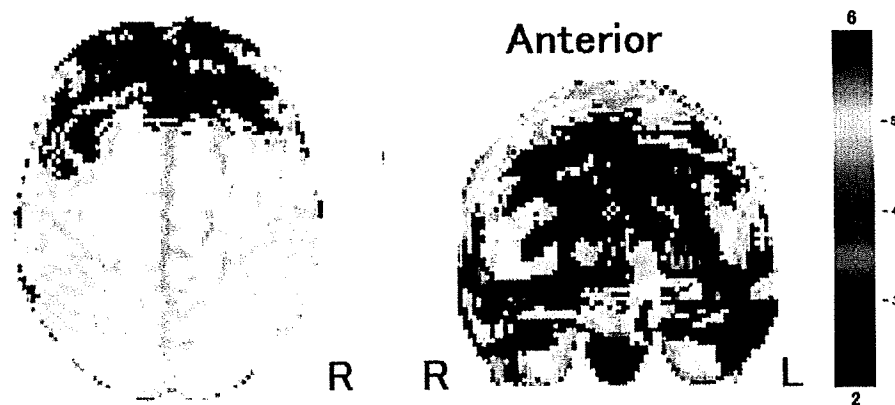
Acknowledgments

Fig. 3. eZIS analysis of FDG-PET in the projected view show hypometabolism in the prefrontal cortex. Left: from the upper side. Right: from the anterior side.

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Epilepsia

Frameshift mutations of the *ARX* gene in familial Ohtahara syndrome.

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Frameshift mutations of the *ARX* gene in familial Ohtahara syndrome.

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Supporting Information

Figure e-1.   EEG of Patient 2.

Abstract

Purpose: Ohtahara syndrome is one of the most severe and earliest forms of epilepsy

and is frequently associated with brain malformations, such as hemimegalencephaly.

Recently, longer expansion of the first polyalanine tract of *ARX* was found to

be causative for Ohtahara syndrome without brain malformation, while premature

termination mutations of *ARX* were found to cause severe brain malformations,

such as lissencephaly or hydranencephaly.   Both are designated as

ARX-related interneuronopathies.   Methods: We investigated the molecular

basis of Ohtahara syndrome in two families consisting of 6 male patients in two

generations demonstrating X-linked inheritance.   Results: Novel frameshift mutations

in the terminal exon of the *ARX* gene (Ala524fsX534 and E536fsX672) were

identified in two patients (2 years and 13 years, each) from both families.   Two

patients developed West syndrome, and one of these later developed Lennox-Gastaut

syndrome.   Brain MRI of all patients showed no brain malformations in contrast to the

patients with a premature termination mutation in other exons of *ARX*.

Discussion: The etiology of Ohtahara syndrome is heterogeneous; however, the

molecular analysis of *ARX* should be considered in sporadic or familial male

patients with Ohtahara syndrome.

Introduction

Ohtahara syndrome, or early-infantile epileptic encephalopathy with suppression-burst,

is one of the most severe and earliest forms of epilepsy.   The onset of the initial seizure

usually occurs during the neonatal period, and most patients with Ohtahara syndrome

show severe developmental delay (Yamatogi and Ohtahara 2002).   Tonic spasms are

the main seizure type and frequently occur everyday.   This is shown on an

electroencephalogram (EEG) as an initial suppression-burst pattern, which shifts to

hypsarrhythmia, leading to West syndrome several months after onset.   Ohtahara

syndrome is classified as a symptomatic epileptic syndrome and was believed to be

non-inherited in most cases because of its frequent association with

hemimegalencephaly or other brain malformations (Epilepsy 1989).

Recently, two genes, *ARX* and *STXBP1*, have been found to be responsible for Ohtahara

syndrome (Kato et al., 2007; Saitsu et al., 2008).   *ARX* (*aristaless*-related homeobox)

consists of five exons encoding a 562 amino acid protein (Kitamura et al., 2002).   The

ARX protein has four polyalanine tracts, in which 7-16 alanine residues are sequentially

repeated.   It is located on human chromosome Xp22.13 and is expressed in

GABAergic interneurons in the fetal brain.   Mutations of *ARX* cause a wide variety of

X-linked phenotypes, such as non-syndromic mental retardation, Partington syndrome,

West syndrome, Proud syndrome, and X-linked lissencephaly with abnormal genitalia

(Gecz et al., 2006; Kato et al., 2004).   There is a strong correlation between genotype

and phenotype (Kato et al. 2004).   Premature termination or null mutations cause brain

malformations, such as lissencephaly, hydranencephaly, and agenesis of the corpus

callosum, while expansion of polyalanine tracts gives rise to non-malformation groups,

such as mental retardation, dystonia, and epilepsy including West syndrome and

Ohtahara syndrome.   The expansion length of Ohtahara syndrome (33-bp) is longer

than that of patients with non-syndromic mental retardation (6 or 9-bp) or West

syndrome with dystonia (21-bp) (Guerrini et al., 2007), and the length of the first

polyalanine tract is correlated with the severity and onset of diseases (Kato et al. 2007).

Patients with Ohtahara syndrome caused by polyalanine expansion of *ARX* show similar

findings and clinical courses, such as hypoplastic external genitalia, progressive atrophy

of the cerebrum, in addition to the OS phenotype (Kato et al. 2007).   Interestingly,

deletion of exon 5 was reported in a small family with severe West syndrome without

brain malformation (Stromme et al., 2002), and the size of truncated mRNA was found

to be unrelated with the severity of brain malformations if the mutations are in exons 1

to 4 (Kato et al. 2004), suggesting the involvement of nonsense-mediated mRNA decay

in the pathogenesis of *ARX*-related diseases.

In this report, we show that frameshift mutations in exon 5 of *ARX* also causative for

two pedigrees involving Ohtahara syndrome and other epileptic encephalopathies

without brain malformations.

Clinical findings

In Family 1 (Figure 1A), Patient 1 (III-2) was the second child of unrelated healthy

Japanese parents.   He had two sisters by two different fathers, and his elder sister had a

developmental speech delay and growth hormone deficient short statue, 97.9 cm

(−2. 6SD) at 5 years and 6 months.   His mother had a healthy younger sister and two

affected elder brothers (II-1 and 2) with similar histories and conditions, such as

convulsions, dystonic posture, profound psychomotor delay in addition to being

bed-ridden, and died in infancy.   He was born at 42 weeks gestation by spontaneous

vaginal delivery after an uneventful pregnancy.   His birth weight was 2,798 g (-1.0

SD), his height was 48.0 cm (-0.9 SD), and his head circumference was 30.5 cm (-2.1

SD).   At the age of 1 month, he was diagnosed with Ohtahara syndrome after he

presented with tonic spasms, which sometimes occurred in clusters associated with a

suppression-burst pattern on EEG (Figure 2A).   The EEG finding evolved to

hypsarrhythmia (Figure 2B) from 3 months of age, which was compatible with West

syndrome.   He was frequently treated with mechanical ventilation due to recurrent

aspiration pneumonia until a tracheal separation procedure was performed as part of a

tracheostomy at the age of 29 months.   He unexpectedly showed dystonic posture from

27 months.   At the age of 28 months, his height was 84.8 cm (-0.9 SD), his weight was

8,070 g (-3.1 SD), and his head circumference was 44.1 cm (-3.1 SD).   He had severe

scoliosis, mild undescended testes with a normal penis, severe spastic quadriplegia,

profound mental retardation, and generalized tonic seizures with upward eye gazing

lasting several seconds frequently each day.   Treatments with pyridoxal phosphate,

valproic acid, benzodiazepines including clonazepam, barbiturates including

phenobarbital, and zonisamide were ineffective.   Topiramate showed partial

effectiveness against his seizures from 34 months of age, but his EEG still showed a

suppression-burst pattern during sleep state (Figures 2C and 2D).   Blood tests showed

elevation of liver transaminase (AST: 147 IU/L, ALT: 101 IU/L, LDH: 414 IU/L),

hyponatremia (Na: 129 mEq/L), and myoglobinemia (myoglobin: 215 ng/ml, CK: 647

IU/L) associated with severe pneumonia.   His chromosome arrangement was 46,XY.

His brain MRI was normal in early infancy, but then showed slightly progressive

ventricular dilatation and cortical atrophy.   His basal ganglia demonstrated a slightly

high spotted signal at 33 months of age.   His scoliosis began improving at 35 months.

In Family 2 (Figure 1B), Patient 2 (III-2) was the second son of a healthy mother.   The

pregnancy was uneventful, and delivery occurred at term without asphyxia.   His birth

weight was 2,968 g, and his head circumference was 32 cm.   Tonic spasms involving

flexion of the neck and extremities started from 22 days of age and occurred in clusters.

An interictal EEG at 43 days showed a suppression-burst pattern (figures e-1A and

e-1B).   An ictal video-EEG demonstrated two different patterns, a high-amplitude slow

wave combined with tonic spasms and a diffuse irregular polyspike-and-wave (figure

e-1C) with generalized myoclonic seizures or upward eye gazing.   His brain MRI at 47

days of age was normal.   High doses of pyridoxine and TRH injection were ineffective,

and valproic acid could not be administered because of vomiting.   At the age of two

months, his seizures temporarily disappeared after 3 days of ACTH injection

(0.01mg/kg/day).   At the age of three months, he started to have hypokinetic seizures

with oral automatism and cyanosis lasting one minute, which were similar to complex

partial seizures, and secondary generalized tonic-clonic seizures lasting one to three

minutes that occurred several times a month and was treated with carbamazepine (figure

e-1D).   Myoclonus and spasms appeared from the age of 5 months, which were

controlled by clonazepam after 8 months.   No epileptic discharges were shown on

waking EEG from the age of 6 months.   He remained seizure free from the age of 29 to

44 months with an increased dose of carbamazepine and diazepam for muscular

hypertonus.   Tonic seizures with cyanosis lasting 30 to 60 seconds arose at 45 months

of age.   At the age of 13 years, he showed marked growth failure (height: 108 cm,

weight: 11.6 kg); microcephaly (47 cm, −5.1 SD); severe spastic quadriplegia with no

voluntary movements; myoclonic seizures or tonic seizures, which occurred as a single

seizure or as clusters of seizures 10 to 20 times a day; no social contact; and no reaction

to optic or acoustic stimuli.   His external genitalia showed a relatively small penis (40

mm in length) and testes (1 ml in volume) because of delayed puberty.   An EEG at the

age of two years showed infrequent spikes during sleep state.   A brain MRI at the age

of 46 months demonstrated mild dilatation of the lateral ventricles without worsening

compared to that at the age of 13 years.   All tests for metabolic disorders and prenatal

infections were normal except for high blood and cerebrospinal fluid levels of lactate

(26.9 and 24.6 mg/dl, respectively) and pyruvate (1.08 and 1.70 mg/dl, respectively) in

early infancy.   Chromosomal analysis revealed a pericentric inversion of chromosome

9, 46,XY,inv(9)(p11q13), which was supposed to be a normal variant based on the

public databases (Starke et al., 2002).

Patient 3 (Figure 1B, III-1) was the elder half-brother of Patient 2.   He was

spontaneously born at term without asphyxia.   He started to have tonic spasms in

flexion associated with irregular diffuse polyspikes and wave bursts with multifocal

spikes on interictal sleep EEG from 3 weeks of age.   The spasms were increasingly

grouped in clusters.   His sleep EEG demonstrated a suppression-burst pattern

compatible with Ohtahara syndrome from 46 days to 3 months and then changed to

show hypsarrhythmia compatible with West syndrome from 4 months.   Treatment with

valproic acid and clonazepam reduced the seizure frequency until 8 months, and then

the clusters of tonic spasms worsened.   At the age of 11 months, administration of

ACTH (0.015 mg/kg/day) stopped his seizures after 10 days, but hypsarrhythmia was

still observed on EEG, and solitary spasms reappeared in spite of a second course of

ACTH therapy (0.02 mg/kg/day) at 13 months.   At the age of 27 months, he

demonstrated several types of generalized seizures, such as myoclonic seizures and

tonic seizures when awake, brief tonic seizures in clusters during sleep, and clonic

seizures provoked by high fever.   His EEG showed frequent multifocal or diffuse

paroxysmal discharges.   A brain MRI at 35 months demonstrated microcephaly.   At

the age of 6 years and 2 months, he suffered from tonic-clonic convulsions, sometimes

followed by brief tonic seizures that occurred in clusters and were associated with bursts

of diffuse slow spike-waves during both sleep and wakefulness, which were compatible

with Lennox-Gastaut syndrome.   He showed microcephaly (46 cm, -3.8 SD), profound

mental retardation with no social contact, severe motor disturbance with no head control

at the time of his death of gastroenteritis-related dehydration at the age of 6 years and 7

months.   Blood and urine tests including metabolic disorders and prenatal infections