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Case report

Analysis of the hypothalamus in a case of X-linked lissencephaly with abnormal genitalia (XLAG)

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Abstract

X-linked lissencephaly with abnormal genitalia (XLAG) is characterized by lissencephaly, absent corpus callosum and ambiguous genitalia. We examined hypothalamic dysfunctions in a XLAG case with a novel mutation of the ARX gene, and performed immunohistochemical evaluation of the diencephalons in autopsy brain. A 1-year-old boy showed intractable epilepsy, persistent diarrhea and disturbed temperature regulation. This case had abnormalities in circadian rhythms and pituitary hormone reserve test. He died of pneumonia. The globus pallidus and subthalamic nucleus was not identified, and the putamen and thalamus were dysplasic. The suprachiasmatic nucleus was absent. A few neurons immunoreactive for vasopressin seemed to form the ectopic supraoptic-like nucleus. The diencephalons were disturbed differently in each sub-region, and the changes may be related to various hypothalamic dysfunctions.

Keywords: Lissencephaly; XLAG; ARX gene; Hypothalamus; Diencephalon

1. Introduction

X-linked lissencephaly with abnormal genitalia (XLAG) is characterized by lissencephaly, absent corpus callosum and ambiguous genitalia [1]. Kitamura et al. identified loss-of-function mutations in the ARX gene in individuals affected with XLAG [2]. XLAG boys show intractable epilepsy of neonatal onset, severe diarrhea and early death [3]. Altered hypothalamic functions are speculated, but the detailed endocrine analysis has rarely been done in XLAG patients [4]. We reported that immunohistochemistry can characterize the intermingled diencephalon in cases of alobar holoprosencephaly [5]. We attempted to correlate the immunohistochemical findings in the diencephalon with data in endocrine tests in an autopsy case of XLAG with a novel mutation of ARX gene.

2. Case report

A boy was the first child born to non-consanguineous healthy parents. He was born at 40 weeks after uneventful pregnancy. Apgar scores were 4 and 7 at 1 and 5 min, respectively. Birth weight was 2552 g, length 54.5 cm, and occipito-frontal circumference 33.5 cm. He showed respiratory distress at birth, and clonic convulsion in the upper limbs with myoclonus at eyelids. Micropenis, hypospadias and cryptorchism were observed. He

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showed systemic muscle hypotonia. Blood test for TORCH complex and various metabolic tests in the urine, blood and cerebrospinal fluid were unremarkable. Magnetic resonance imaging (MRI) of the brain revealed total absence of the corpus callosum and lissencephaly with a posterior-to-anterior gradient of severity, consisting of frontal pachygyria and posterior agyria (Fig. 1a). The cerebral cortex was thick, and the basal ganglia was hypoplasic. The posterior lobe of pituitary gland was detected (Fig. 1b). Chromosome test showed 46, XY in G-band and ish Yp11.3 (SRYx1) in SRY FISH. He demonstrated a deletion of 4 bases (980–983delAACA) in exon 2 of the ARX gene, a novel mutation [6], but his parents had no abnormalities in the ARX gene. His convulsions were refractory. His body temperature fluctuated and often showed an abnormal dip. He repetitively suffered from bacterial infections, and developed intractable diarrhea. At 16 months, he died of pneumonia.

2.1. Endocrine examination

At neonate, concentration of hypocretin-1 was normal (230 pg/ml) (220–360 pg/ml in controls) in the cerebrospinal fluid. Serum level of testosterone was low (68 ng/dl) (100–200 ng/dl in controls), whereas that of free triiodothyronine (3.0 pg/ml) and free thyroxin (fT4, 1.35 ng/dl) was unremarkable. Subcutaneous administration of adrenocorticotropic hormone (ACTH) increased serum level of cortisol from 2.8 to 48.8 μg/dl, and intravenous administration of corticotrophin-releasing hormone (CRH) could induce an elevation of serum levels of ACTH (38.3–48.1 pg/ml) and cortisol (1.4–3.3 μg/dl). At 1 month, pituitary hormone reserve was tested with simultaneous intravenous administration of CRH (250 $\mu g/m^2$, body surface), thyrotropin-releasing hormone (10 μg/kg) and luteinizing hormone-releasing hormone (3 μg/kg). Thyroid stimulating hormone (TSH) showed prolonged reaction. Gonadotropins demonstrated high value before stimulation and showed prolonged reaction. Serum levels of prolactin and ACTH were high before provocation, and the former was increased after stimulation, whereas the latter seems to be unchanged. Since insufficient secretion of CRH in the hypothalamus was speculated, replacement therapy with hydrocortisone started at 2 months. Serum level of fT4 decreased without increased serum level of TSH, and became less than 0.9 ng/dl at 3 months (0.97–1.79 ng/dl in controls), and replacement therapy with levothyroxine sodium was initiated. Serial recording of rectal temperature was performed for 1 month to assess circadian rhythm of core body temperature at 3 months. The core body temperature ranged from 34 to 38 °C, lacking definite circadian rhythm (Fig. 2a). At 4 months, wakefulness–sleep circadian rhythm was monitored with an actogram by means of Actiwatch (Mini Mitter) for 7 consecutive days. Actogram identified the wake and sleep states in a day, indicating the presence of ultradian rhythm, whereas there seemed to be no circadian rhythm (Fig. 2b).

2.2. Neuropathological findings

The brain weighed 270 g at autopsy, and the brain was very small. The surface of cerebrum was smooth predominantly in the occipital lobe. The olfactory bulbs and tracts were absent, the optic chiasm and pituitary infundibulum were found. The corpus callosum, anterior and posterior commissurae and Probst bundle were absent. Formalin-fixed brain was cut coronally and embedded in paraffin. Each brain section was stained with hematoxylin–eosin, Klüver–Barrera (KB), Bodian and Holzer methods. In serial sections of the diencephalon (Fig. 3a), we performed immunohistochemistry using

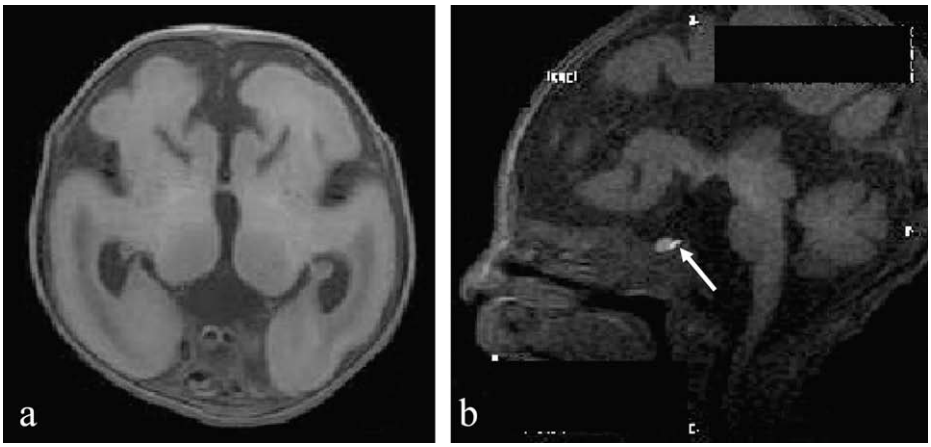

Fig. 1. Brain MRI findings at the age of 1 day. Axial view of T1-weighted image demonstrated total absence of the corpus callosum and lissencephaly, consisting of frontal pachygyria and posterior agyria (a). Sagittal view of T1-weighted image demonstrated normal high signal in the posterior lobe of the pituitary grand (arrow) (b).