Aberrant Neuronal Migration in the Brainstem of Fukuyama-Type Congenital Muscular Dystrophy

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Abstract. We examined the brainstem of 10 patients with Fukuyama-type congenital muscular dystrophy (FCMD). In the midbrain we noted leptomeningeal glioneuronal heterotopia (LGH) (n = 9) and intramural “micropolygyria” (n = 1) in the tectum, as well as tyrosine hydroxylase-positive ectopic neurons/fibers ventral to the cerebral peduncle (n = 3). In the pontomedullary region, glial fibrillary acidic protein-positive subpial tissue intermingled with neurons and myelinated fibers was present in the ventrolateral pontine surface in all cases and extended over the lateral surface of the upper medulla oblongata. This subpial gliotic band was often contiguous with the extra-pial LGH tissues. The gliotic band protruded from the ventrolateral pontine surface in 3 cases and appeared to include ectopic neurons of the pontine nucleus. Disarrangement of the arcuate nuclei (n = 3) was also noted in the medulla oblongata. We hypothesize that both the radial and tangential neuronal migration systems are disrupted in the FCMD brainstem in addition to altered neuronal migration in the cerebral and cerebellar cortex. Fukutin protein may play a part in the morphogenesis of certain neuronal structures in the brainstem and the dysplastic structure termed “aberrant pyramidal tract” in previous reports may essentially result from an ectopic migration of pontine nucleus neurons.

Key Words: Fukutin; Pontine migratory stream; Precerebellar neuroepithelium; Subpial migration pathway; Substantia nigra.

INTRODUCTION

Fukuyama-type congenital muscular dystrophy (FCMD) is characterized by micropolygyria of the cerebral and cerebellar cortex (1–3). Previous studies have shown breaches of basal lamina/glia-limitans in fetal FCMD cases (4, 5), through which cortical neurons had overmigrated, suggesting that a fragile glia-limitans is the primary cause of micropolygyria. However, after the fukutin gene was identified as the causative agent for FCMD (6), immunohistochemical (7) and in situ hybridization (8) studies have revealed expression of fukutin protein in the migrating neurons and at lower levels in the glial cells (9) that form the glia-limitans. The reduced expression of fukutin protein in the developing FCMD cortex (7) suggests that the fukutin protein may be involved primarily in the settling process of migrating neurons. However it remains unclear whether or not the fukutin protein is involved in the stabilization of glia-limitans and/or neuro-glial interaction during corticogenesis.

Neuropathological studies of the FCMD brainstem have identified leptomeningeal glioneuronal heterotopia (LGH) and an “aberrant pyramidal tract” (1, 3). LGH is defined as microscopic pial buds or nests of disorganized glioneuronal tissue within the subarachnoid space (10), and is often associated with congenital anomalies or perinatal destructive lesions of the brain (11, 12). It is unclear whether the presence of LGH in the FCMD brainstem is related to a process of breakdown of the fragile glia-limitans, as is hypothesized for the cerebral cortex. The pathology underlying the aberrant pyramidal tract also remains obscure.

In this study, we demonstrate that certain neuronal structures are selectively vulnerable in FCMD brainstem. Based on these observations, we hypothesize that the migration anomalies may result not only from the physical fragility of the glia limitans, but also from disrupted neuro-glial interactions in specific populations of migrating neurons.

MATERIALS AND METHODS

Brains from 10 FCMD patients (aged 2 to 27 years; 5 males, 5 females) and 12 control subjects (aged 1 to 17 years) were examined. The former patients were diagnosed with FCMD based on clinical features and pathological findings on muscle
biopsy. All patients had motor and intellectual disabilities (Table 1). Sudden unexpected death occurred in 5 cases. Analysis of the fukutin gene, carried out in 6 cases, revealed a heterogeneous mutation consisting of a 3-kb insertion in 5 cases (patients 2, 3, 5, 8, and 10). The clinical and neuropathological features of 3 patients (patients 2, 4, and 5) have been previously described (13).

Brain tissues were obtained at autopsy within 24 hours after death. After macroscopic inspection, the formalin-fixed brains were cut coronally and several portions of the cerebrum, cerebellum, midbrain, pons, and medulla oblongata were embedded in paraffin. In both the FCMD and control cases, we examined microscopically at least 5 sections of each brainstem, and selected identical areas of upper midbrain (through the superior colliculus and red nucleus), lower midbrain (through the inferior colliculus and decussation of the superior cerebellar peduncle), upper pons (through the locus ceruleus), lower pons (through the facial nucleus), and upper medulla oblongata (through the inferior olive). Anatomical structures without apparent dysplasia were identified according to the atlas of Paxinos (14) and the text of Olszewski and Baxter (15). Whenever necessary, the sections were examined blind to whether the tissues were taken from FCMD or control patients. Four-μm-thick sections were stained with hematoxylin and eosin (H&E), Luxol-fast-blue/periodic acid Schiff or by Klu¨ver-Barrera and Holzer methods, as well as immunohistochemically using a polyclonal antibody against glial fibrillary acidic protein (GFAP) (DAKO Corporation, Santa Barbara, CA). Since we found ectopic neurons ventral to the cerebral peduncle in FCMD brains, we used a monoclonal antibody against tyrosine hydroxylase (TH) (Chemicon International, Temecula, CA) to characterize such ectopic neurons. The brainstem sections were immersed in 3% hydrogen peroxide for 5 min to abolish endogenous peroxidase activity. Microwave treatment for 14 min at 95°C was performed to retrieve the TH antigen. The sections were incubated with the primary antibody (diluted at 1:1,000 for GFAP and 1:100 for TH) overnight at 4°C (GFAP) or for 2 hours at 37°C. 

Fig. 1. Neuropathological findings of the midbrain in Fukuyama-type congenital muscular dystrophy (FCMD). A–C: Dysplastic glioneuronal tissue noted in the lower tectum of patient 1. B, C: Higher magnification of the tissue within the inset in panel A. D: The leptomeningeal glioneuronal heterotopia (LGH) over the tectum in patient 8. E–G: Ventral surface of the cerebral peduncle in a 2-year-old control subject (E) and patient 1 (F, G). Dotted lines indicate the border between the cerebral peduncle and the glial tissue. F: A probable neuron within the gliotic tissue is shown in the inset. G: Tyrosine hydroxylase (TH)-immunoreactive neurons and fibers (inset) located in the area ventral to the cerebral peduncle. cp: cerebral peduncle. A, B: Luxol fast blue-Periodic acid Schiff staining; C–F: GFAP immunostaining; G: TH immunostaining. Scale bar = 200 μm.

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<th>Patient</th>
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<th>Consanguinity/familial history</th>
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<th>Intelligence</th>
<th>Neurophysiological assessment</th>
<th>Cardiac involvement</th>
<th>Cause of death</th>
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<td>Sentences</td>
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<tr>
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<td>Sentences</td>
<td>Febrile convulsion ABR delayed V wave; VEP; SSEP normal</td>
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<td>Single words</td>
<td>EEG frontal spikes</td>
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<td>Heart failure</td>
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<td>17 yr</td>
<td>F</td>
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<td>Keep sitting</td>
<td>Sentences</td>
<td>EEG dysrhythmia</td>
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<td>10</td>
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<td>M</td>
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<td>Walk</td>
<td>Sentences</td>
<td>Normal EEG</td>
<td>DCM</td>
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TABLE 1
Clinical Features of the FCMD Cases

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<th>Patient Age Sex</th>
<th>Consanguinity/familial history</th>
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<td>Sudden death</td>
</tr>
<tr>
<td>4 14 yr F</td>
<td>(−)</td>
<td>Keep sitting</td>
<td>Sentences</td>
<td>Normal EEG</td>
<td>NE</td>
<td>Sudden death</td>
</tr>
<tr>
<td>5 15 yr F</td>
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<td>Sentences</td>
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ABERRANT NEURONAL MIGRATION IN FCMD BRAINSTEM
room temperature (TH). In negative control experiments, the primary antibodies were omitted and replaced with normal rabbit or mouse antisera. The avidin-biotin complex conjugated with peroxidase was used to visualize antibody binding. Between each step, the sections were thoroughly washed with phosphate-buffered saline 3 times. The immuno-products were Tween each step, the sections were thoroughly washed with hydrogen peroxide and sections were counterstained with hematoxylin.

RESULTS

General Examinations (Table 2)

Micropolygyria of the cerebral cortex of varying severity was observed both macroscopically and microscopically in all cases. Patient 1, who had a heterozygous fukutin gene mutation, had the most widespread micropolygyria as well as prominent agyria/pachygyria in the temporal lobes. Cerebellar micropolygyria was identified in all cases. The middle cerebellar peduncle and basal pons appeared hypoplastic in 3 cases. The ventral medulla was flat and the protrusion of pyramids was indistinct in 8 cases.

Midbrain Neuropathology of FCMD

In the lower tectum of patient 1 there was an anomalous structure (Fig. 1A, B) resembling the cerebral cortical micropolygyria, in which GFAP-positive glial cells and fibers (Fig. 1C), neuronal somata, and blood vessels (Fig. 1B) were intermingled. The other 9 patients had LGH with multiple pial-glial bridges in the tectum, the extent of which varied among patients (Fig. 1D). The LGH was less conspicuous in the lateral and ventral border of the midbrain surrounding the cerebral peduncle. However, the subpial GFAP-positive layer was often thicker (Fig. 1F) than the glia-limitants of control subjects (Fig. 1E), and occasionally contained neuronal somata (Fig. 1F, inset). Some of these neurons and their axons were immunopositive for TH in 3 FCMD cases (Fig. 1G).

Ponstine Neuropathology of FCMD

The longitudinal fascicles in the basal pons were disorganized (Fig. 2A, B) to varying degrees and were partly shifted to the ventral-most area at the lower pontine level (Fig. 2C, D) in 3 cases. The transverse fibers were also disorganized and the circumscribing fascicles of pontocerebellar fibers that course into the middle cerebellar peduncle (Fig. 2B) were often hypoplastic or fragmentary (Fig. 2A).

The ponstine nuclei exhibited some neuronal clusters that protruded into the subarachnoid space at the ventrolateral ponstine surface, in association with blood vessels and myelinated fibers (Fig. 2E, F). This parenchymal protrusion extended ventromedially, tangential to the ponstine surface, which was consistent with the migratory trajectory of the ponstine nucleus neurons during development. In addition, 2 patients had an LGH-like portion of neuronal leakage through multiple bridges into the glial tissue that was lying over the surface of the ponstine base.
Fig. 2. Morphology of the pons in FCMD. Upper (A: patient 1; B: 2-year-old control subject) and lower (C: patient 4; D: 3-year-old control subject) pontine levels are shown. C: Longitudinal fibers are shifted to the ventral-most area (arrows). E, F: Protruding tissue in the ventrolateral region of the basal pons (E: the area shown as the inset 1 in panel (A), F: patient 9). A, E: Luxol fast blue-Periodic acid Schiff staining; B–D, F: Klüver-Barrera staining. Scale bar = 200 μm.
(Fig. 3A). These protruding and LGH-like lesions were generally immunopositive for GFAP (Fig. 3B), but contained only a small number of astrocyte cell somata. In the basal pontine regions that lacked these lesions there was a thick GFAP-positive layer (Fig. 3C) containing up to hundreds of neurons per section. In many areas this layer was covered by the pia mater (Fig. 3C), resembling the glia-limits of control subjects (Fig. 3D).

This glioneuronal tissue was contiguous with the lateral aspects of the rostral pontine tegmentum and reached the angle between the medial lemniscus (dorsomedial) and the bundle of pontocerebellar fibers (ventromedial) (Fig. 3E, F), where glioneuronal tissue was present in the subpial (Fig. 3E) and LGH-like extra-pial (not shown) locations. In the dorsal aspects of the pontine tegmentum there was occasionally a typical LGH with multiple pial-glial bridges resembling that of the midbrain tectum. TH-immunoreactive neurons were distributed normally in the area of the locus ceruleus (not shown).

**Medullary Neuropathology of FCMD**

All the FCMD cases had a band of gliotic tissue along the lateral surface of the rostral medulla oblongata (Fig. 4A–C, E), which was thicker than the glia-limits of control subjects (Fig. 4D). Except for 1 case that showed neuronal clustering (Fig. 4A, B), the number of neurons in this structure was less than 5 per section, much smaller than in the pontine base lesion. Although extra-pial protrusion of glial tissue was occasionally observed (Fig. 4F), most of the glial band was located beneath the pial surface (Fig. 4G), resembling the glia-limits of the control subjects (Fig. 4H). Dorsally, this subpial band extended over the inferior cerebellar peduncle (Fig. 4C) and reached the lateral recess of the fourth ventricle (Fig. 5A). The pontobulbar body (Fig. 5B) was covered by the glial band in each case and was involved within this band in 2 cases (Fig. 5A).

The pyramidal tracts were either hypoplastic (Fig. 5C), asymmetrical (Fig. 5D), or multi-lobulated (Fig. 5E). The arcuate nucleus showed disarrangement in 3 cases, being either ill-defined (Fig. 5C) or protruded (Fig. 5D). In 3 cases, some clusters of neurons were noted in the area dorsolateral to the inferior olivary nucleus or lateral to the ambiguous nucleus (Fig. 5E). Such neuronal clusters were not observed in control subjects (Fig. 5F).

In contrast, the configuration of other neuronal structures in the lateral medulla oblongata, including the external cuneate, spinal trigeminal, and inferior olivary nuclei, appeared normal. The labeling of TH-immunoreactive neurons in the ventrolateral medulla oblongata was often weak compared to the control subjects, but their distribution was normal (not shown).

**DISCUSSION**

In this study, we observed various dysplastic lesions in the brainstem of FCMD patients, mostly distributed along the surface of the brainstem. In particular, morphogenesis of the pontine nucleus was severely affected, which appeared to be closely related to aberrant development of the pyramidal tract. To understand the implication of these abnormal findings in FCMD, we discuss in the following sections the developmental aspect of their constituents from the viewpoint of neuronal migration.

**Subpial Extramural Pontine Migratory Stream (Fig. 6)**

In all FCMD cases, we found abnormal glial tissue distributed extensively over the basal pontine areas, consistent with previous reports (1). This tissue involved the pontine nucleus, which originates in the precrerebellar neuropithelium, a matrix tissue located ventromedial to the lateral recess of the fourth ventricle in the dorsolateral rhombencephalon (16). Young neurons then migrate ventrally in a circumferential or tangential course (Fig. 6B), forming a subpial strand (the anterior extramural precrererebellar migratory stream) to reach the pontine flexure (17, 18) between 11 and 17 gestational weeks, when the pyramidal tract fibers descend through the pontine level. Packed within this subpial stream, the immature neurons migrate along the tangentially oriented neuronal processes (19), directed by chemoattractants from the floor plate and other unknown cues (20).

Protrusion of the pontine nucleus into the tangential direction, reminiscent of the vertical overmigration of the cortical neurons in FCMD (4, 5), suggested a primary defect in the migration of pontine nucleus neurons. Disarrangement of this nucleus may lead to subsequent disorganization of longitudinal (corticospinal and corticopontine) and transverse (pontocerebellar) fibers in the basal pons. Consistent with this, preliminary experiments
Fig. 4. Neuropathological findings of the FCMD medulla oblongata. A: Medulla oblongata of patient 1. B: Higher magnification of the tissue within the inset 1 in panel (A). An aberrant cluster of neurons is indicated by an arrow. C–E: Thick glioneuronal tissue covers over the lateral surface of patient 5 (C, E), in contrast to the thin glia-limitans of a 16-year-old control subject (D). F: Extra-pial glial tissue in the ventrolateral surface of medulla (patient 8). G, H: Lateral surface of medulla in patient 5 (H, and inset in panel C) and a 6-year-old control subject (H). Abbreviations: DCN: dorsal cochlear nucleus, IO: inferior olivary nucleus, NA: ambiguous nucleus, PBB: pontobulbar body, VCN: ventral cochlear nucleus. A, B: Luxol fast blue-Periodic acid Schiff staining; C–H: GFAP immunostaining. Scale bar = 200 μm.
Fig. 5. Neuropathology of the dorsal and ventral medulla oblongata in FCMD. A: Enlargement of the area shown in inset 2 of Figure 4A. Neurons of the pontobulbar body (PBB) are intermingled in the glial tissue. B: Comparable area in a 2-year-old control subject. C: Higher magnification of the tissue in Figure 4A inset 3. In a consecutive section the area ventral to the dotted line is immunopositive for GFAP (not shown). D: Protuberance of the arcuate nucleus (arrow) in patient 9. E: Neuronal clusters (arrows) dorsolateral to the inferior olivary nucleus in patient 6. F: Medulla oblongata of a 3-year-old control subject. PBB: pontobulbar body. A, B: GFAP immunostaining; C-F: Klüver-Barrera staining. Scale bars: A–C = 200 μm; D–F = 500 μm.
Fig. 6. Radial (black arrows) and tangential (gray arrows) migration of neurons affected in the FCMD brainstem. A: Fetal mesencephalon. B, C: Fetal rhombencephalon. Migration trajectories of neurons in the tectum and the substantia nigra (A), the pontine nucleus (B), and the arcuate nucleus (C) are shown.

have shown fukutin immunoreactivity localized in the pontine migratory stream in the normally developing human brainstem during the early fetal period (unpublished data), suggesting that the fukutin protein may play a role in the migration and settling process of the neurons in this migratory stream.

Medullary Structures Originating from the Precerebellar Neuroepithelium

In the ventrolateral medulla oblongata, we observed superficial glial tissue that was less extensive and contained fewer neuronal components than the basal pontine areas. The external cuneate and lateral reticular nuclei showed normal location and arrangement, although their neurons form a tangential, subpial extramural migratory stream like those of the pontine nucleus (21). These regional differences may reflect different mechanisms of regulation of migration between subpopulations of the “precerebellar system” that originate from the precerebellar neuroepithelium and send afferent (mossy and climbing) fiber projections to the cerebellum (16, 20).

The arcuate nuclei and the pontobulbar body of Essick (22) were either disarranged or involved within the glial tissue. These nuclei also are likely to originate in the precerebellar neuroepithelium (23) (Fig. 6C) and have different projections to the cerebellum (24). Although the pathological findings here were less conspicuous than in the pontine nucleus, fukutin protein may play a role in the development of these nuclei. The neurons in the superficial glial tissue (Fig. 4B) may represent an ectopic settling of the neurons of the arcuate nucleus and the pontobulbar body. However, we have so far been unable to obtain evidence for this by immunohistochemistry of the muscarinic acetylcholine receptor, a marker of the arcuate nucleus (25).

Interestingly, the inferior olivary nuclei appeared normal in FCMD, in contrast to the dysgenesis of this structure in Miller-Dieker lissencephaly (26) and Walker-Warburg syndrome (27). Perhaps LIS-1 (28) and fukutin protein play different specific roles in the guidance of migrating neurons.

Radial and Tangential Migration Pathways in the Developing Midbrain

In the tectum of midbrain, immature neurons migrate radially along the glial fibers and form a laminated structure, as they do in the cerebral cortex (29, 30). We found LGH or abnormal glioneuronal tissue (patient 1) with disorganized lamination and multiple pial-glial bridges in the tectum of FCMD patients. The similarity of these lesions to cerebral micropolygyria suggests a common mechanism of dysplasia.

Dopaminergic neurons of the substantia nigra originate in the ventricular zone of the floor plate, migrate radially along the glial fibers (31) and then tangentially along nerve fibers (32) (Fig. 6A), and accumulate beneath the ventral pial surface before the corticospinal fibers descend to this level (32, 33). The ectopic TH-immunopositive neurons ventral to the cerebral peduncle may be the remnants of this population, although it is unclear whether this pathogenesis is due to disruption of radial or tangential migration.

Subpial Gliotic Band and the Pathogenesis of FCMD

In contrast to LGH in the midbrain tectum and pontine tegmentum, the superficial glioneuronal tissue over the dorsolateral medulla oblongata and the ventrolateral pons were mostly located beneath the pia mater. This pattern of distribution of the subpial gliotic band corresponds to the migration trajectory zone of the precerebellar system. Since neurons of the subpial tangential migratory stream pass through the spaces interior to the glial end-feet (34),
the dysplastic glioneuronal tissue may result from a decreased integrity of the subpial glial structure. Other anomalous structures located in the subpial areas, including the tectum and dopaminergic neurons in the midbrain and the arcuate nucleus in the medulla oblongata, may be explained by a similar mechanism. Alternatively, these may result from some intrinsic change in the migratory neurons, since fukutin protein is expressed predominantly in migratory neurons during fetal period (7, 35). The selective involvement of the pontine nucleus among the precerebellar system may also support this latter possibility.

Although little is known about the function of fukutin, this protein is suspected to be a glycosylation enzyme (36, 37). A possible target molecule of fukutin is dystroglycan, since this molecule is not glycosylated normally in FCMD skeletal muscle (38). Conditional knockout of dystroglycan in mice resulted in gyral fusion and neuronal disarrangement in the cerebral cortex and persistence of the external granular cells in the cerebellar cortex (36). These findings resemble those of FCMD and implicate hypoglycosylation of dystroglycan in the brain malformation in FCMD. Although no brainstem pathology was described in this knockout mouse, neuronal expression of dystroglycan is robust in the brain of adult mice, including the brainstem (39). To further elucidate the pathogenesis of brainstem anomalies in FCMD, future studies should focus on dystroglycan and other possible target proteins in the migrating pontine nucleus neurons (40).

In conclusion, this study provides considerable evidence for perturbation of the radial and tangential migration systems in the FCMD brainstem. With regard to the biochemical function of fukutin protein, it should be further explored to determine whether this aberrant migration results from a decreased integrity of the glial structure or an altered neuronal property with subsequent disruption of glioneuronal interaction.

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