Spectrum of Brain Changes in Patients With Congenital Muscular Dystrophy and FKRP Gene Mutations

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Objectives: To report the spectrum of brain magnetic resonance imaging findings in 13 patients with congenital muscular dystrophy and FKRP gene mutations and to explore possible genotype-phenotype correlations.

Design: We retrospectively reviewed brain magnetic resonance imaging in patients with congenital muscular dystrophy and FKRP gene mutations.

Patients: Thirteen patients with congenital muscular dystrophy and mutations in the FKRP gene.

Results: Five of the 13 patients had the typical phenotype originally described for congenital muscular dystrophy (MDC1C) with normal intelligence and normal brain magnetic resonance imaging while 3 other patients had isolated cerebellar cysts and mental retardation without any other sign of posterior fossa of supratentorial abnormalities. In the remaining 5 patients cerebellar cysts were associated with structural brain changes involving the posterior fossa and the cortex, ranging from focal unilateral periventricular nodular heterotopia to marked cerebellar dysplasia and pontine hypoplasia. In 2 of these 5 patients the severity and distribution of changes resembled muscle-eye-brain disease in 1 patient who had mild Walker-Warburg syndrome. The distribution of FKRP gene mutations identified in this group of patients did not reveal any obvious association with the severity of central nervous system involvement.

Conclusions: The severity of central nervous system involvement observed in our patients in contrast broadly reflected the severity of the disruption of α-dystroglycan glycosylation. In particular, dystroglycan expression was almost absent in the patients with muscle-eye-brain diseaslike phenotype and less severely reduced in the patients with congenital muscular dystrophy (MDC1C) with or without cerebellar cysts. This study further highlights the central role that dystroglycan has in neuronal migration.

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Mutations in the Fukutin-related protein (FKRP) gene result in a wide spectrum of clinical conditions ranging from a form of congenital muscular dystrophy (MDC1C) to a milder and common form of limb girdle muscular dystrophy (LGMD2I), often associated with onset into adult life. FKRP is a putative glycosyltransferase and although its precise function has not been established yet, abnormal glycosylation of α-dystroglycan expression on skeletal muscle biopsy specimens has been reported in both MDC1C and LGMD2I, lending to the speculation that this protein is a substrate for FKRP. The degree of abnormal glycosylation of α-dystroglycan broadly correlates with disease severity: patients with MDC1C have a much more severe depletion of high-molecular-weight α-dystroglycan compared with cases with later-onset LGMD2I. The reduction in glycosylated dystroglycan expression in muscle observed in FKRP-related disorders is also the hallmark of many other forms of congenital muscular dystrophy (CMD), which are collectively named “dystroglycanopathies.” These include Fukuyama CMD (FCMD Online Mendelian Inheritance in Man [OMIM] 253800) caused by mutations in fukutin; muscle-eye-brain disease (MEB [OMIM 236670]) due to mutations in POMGnTI; Walker-Warburg syndrome (WWS [OMIM 236670]) due to mutations in POMT1; and MDC1D secondary to mutations in LARGE. The underlying genetic defects in these disorders are mutations in known or putative glycosyltransferase enzymes, with a demonstrated or putative action on α-dystroglycan glycosylation. Regarding POMT1 and POMGnTI gene defects, this speculation has been confirmed by the finding of a direct role of these 2 enzymes in O-linked mannosylation of this protein.
Brain involvement is a frequent but not constant feature in patients with dystroglycanopathies. While FCMD, MEB, and WWS are invariably associated with structural brain abnormalities, brain involvement in patients with mutations in FKRP is variable. Both intelligence and brain structure have been reported as normal in patients having LGMD2I and in those originally described as having MEB-like features. However, to date only a few cases have been reported and both the frequency and the detailed pattern of regional structural brain involvement has not been reported in patients with FKRP mutations.

The aims of this study are to report details of brain MRI findings in 13 patients with CMD and FKRP gene mutations and to explore possible genotype-phenotype correlations.

**METHODS**

**PATIENTS**

Sixty-eight patients having a diagnosis of CMD, high levels of creatine kinase, and clinical and biochemical features suggestive of a dystroglycanopathy were screened for FKRP mutations. Seven of the 68 patients screened had normal brain MRIs and 61 had changes on brain MRIs. The range of the brain involvement present in these patients ranged from nonspecific white matter changes (n=5), cerebellar hypoplasia (n=10), cerebellar cysts (n=9), and MEB-like brain changes (n=9), to WWS-like phenotype (n=28). In patients with MEB- and WWS-like phenotype, mutations in POMGnT1 and POMT1 had been excluded.

**PROCEDURES**

Genetic analysis was performed after obtaining informed consent (HH Trust protocol 00/5802). Mutation analysis was performed by amplifying a 1.7-kilobase fragment of genomic DNA containing the entire FKRP coding sequence using Advantage-GC Genomic Polymerase Mix (Clontech Laboratories Inc, Palo Alto, Calif) and primers FKRP-1F (AAAGGGAATTGAGAAAGAGC) and FKRP-5F (GCTCACACAGAGCTTCTCC). Polymerase chain reaction products were separated by agarose gel electrophoresis, purified (Qiagen, Venlo, the Netherlands), and used for direct sequencing. Sequencing reactions were carried out using an ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, Calif) and primers FKRP-1R (GACGGAAGAGCTTACCG, FKRP-2R (CCTCAGGTTGAAGGT), FKRP-3F (CGTTGTCGCCCCATGACCC), FKRP-4R (CCTTCTCCCATAGAAG), and FKRP-5R. Sequencing products were separated on an ABI 377 automated sequencer (Applied Biosystems) and analyzed using SeqEd (Applied Biosystems).

**BRAIN MRI**

All of the patients already had a brain MRI when mutations in the FKRP gene were found. All MRI studies were reviewed retrospectively by 2 investigators (E.M. and M.R.) who were blinded to clinical information, including genetic testing. The MRIs were analyzed looking for normal or abnormal anatomy and in particular for the following abnormalities: cerebellar abnormalities (vermian and/or hemispheric involvement, paying particular attention to the possible presence or absence of cerebellar cysts or other signs of cerebellar dysplasia), cortical malformation (type and location), white matter changes, and ventriculomegaly. Any additional abnormalities detected on the MRIs were also recorded.

**RESULTS**

Mutations in FKRP were identified in 13 (6 males and 7 females) of 56 patients with CMD screened. Seven of the 13 patients had already been previously described. Ten of the 13 patients had a clinical phenotype compatible with MDC1C with leg hypertrophy, upper limb wasting, and high levels of creatine kinase. One had a phenotype compatible with WWS; 2 had a phenotype compatible with MEB. The **Table** lists details of the mutations in the FKRP gene and of their phenotypes.

**BRAIN MRI**

The age at which the children had brain MRIs ranged from 22 months to 11 years. Five patients (cases 1-5) had normal brain MRIs with no evidence of cortical, subcortical, or white matter involvement. Reports of 4 of these 5 patients have already been published but a brain MRI was available for only 3 of 4 patients. All attend mainstream schools and their
cognitive abilities seem to be adequate; there has never been any concern about their academic achievements. Two of the 5 have been formally tested and their IQs are normal (IQ 95 and 101). None have epilepsy.

Three patients (cases 6-8) had isolated cerebellar cysts without any cortical involvement. Reports of 2 of 3 have already been published.8 The cysts were bilateral (Figure 1) and 1 of the 3 also showed white matter changes. All 3 patients are mentally challenged (IQ 50, 56, and 70). One of the 3 had microcephaly.

Another 5 patients (cases 9-13) had cerebellar cysts associated with other structural brain changes. In 1 of the 5 (case 9) the cerebellar cysts were associated with unilateral left frontotemporal nodular heterotopia and bilateral frontal pachygyria with no obvious abnormalities of the white matter and normal ventricles (Figure 2). The child has a normal IQ and no microcephaly.

In another child (case 10) there was also pontine hypoplasia and a marked dysplasia of cerebellar hemispheres and vermis, and ventricular dilatation, with white matter loss and increased extracerebellar space. There also was abnormal appearance of cortical folding in the frontal and occipital lobes but this was interpreted as secondary to the severe white matter loss (Figure 3). The patient also had absent septum pellucidum. The child has microcephaly and while the patient’s IQ has not been formally tested, the child is able to speak and read well and is functioning at an average level in secondary school.
Patient 11 had microcephaly, thin corpus callosum, extensive white matter changes with high T2 signal, and cerebellar hypoplasia with a few cerebellar cysts (Figure 4). This child has microcephaly and although not formally tested, is mentally challenged. Reports of patient 12 (already published10) showed features suggestive of more severe cobblestone lissencephaly and a Dandy-Walkerlike malformation with hypoplasia of brainstem and cerebellar vermis, moderate hypoplasia, dysplasia of cerebellar hemispheres, and multiple cerebellar cortical and subcortical cysts. In addition, a thickened (pachygyric) cortex over the frontal pole and medial aspect of the anterior frontal pole was seen.

The MRI of patient 13 (already published10), obtained at 3 days of age showed an absence of cortical sulci with a thick 1-cm cortex, indicating lissencephaly, discontinuous laminar heterotopia just beneath the cortex, and a large cyst within the posterior fossa of the brain, with splaying of the cerebellar hemispheres. There was absence of the cerebellar vermis, pons and brainstem hypoplasia with an enlarged tectum, mild kinking at the midbrain–pons junction, marked dilatation of the lateral and third ventricles, and absence of the corpus callosum. The white matter signal was increased throughout the supratentorial regions. This child had absent psychomotor development and died at 3 years.

In this study we report details of various patterns of brain involvement in patients with CMD onset and mutations in the FKRP gene, ranging in severity from MDC1C to WWS-like conditions. Only 5 of the 13 patients, who were initially seen with congenital onset of weakness and had confirmed FKRP gene mutations, had normal brain MRIs and normal IQs. Three of 5 had already been described in our original article on phenotype-genotype correlation in patients with MDC1C and FKRP gene mutations.7 While at the time of the original description of the phenotypes associated with FKRP gene mutations we reported that the common phenotype of patients with MDC1C had no associated CNS features, we have been able to identify only a single new case with a normal brain MRI since 2001.1,7 None of the other cases of severity comparable to MDC1C discussed by us in this article and recently reported by others have normal brain MRIs,8,11 suggest-
arding that brain involvement is a more common feature than originally anticipated in patients with MDC1C.

Patients with cerebellar cysts, either isolated or associated with vermis hypoplasia and white matter abnormalities, were, in contrast, the most common group. Loui-chi et al9 recently described 7 Tunisian families with MDC1C, although in 6 of 7 families an identical single mutation was identified, suggesting a local founder effect. Brain MRI was available for 6 of these patients and the brain MRIs showed cerebellar cysts, associated with vermis hypoplasia, in 5 patients. Five of 6 patients also showed white matter abnormalities, although in a patient in whom the MRI was performed a second time when the patient was older the white matter changes had almost completely disappeared. A similar trend of transient dysmyelination has been previously reported in FCMD and MEB, and the transient dysmyelination probably represents a common phenomenon in dystroglycanopathies. The case of another patient with MDC1C and cerebellar cysts has been recently reported24 with no obvious white matter abnormalities. In our cohort white matter changes were found in 1 of 3 patients with cerebellar cysts and in 3 of 5 patients with structural brain changes. In one of these patients, characterized by cerebellar cysts without cortical dysplasia, the white matter changes consisted of large cystic areas in the parietal white matter; in another patient with cerebellar dysplasia and pons hypoplasia, the abnormal signal was associated with white matter loss secondary to associated ventricular dilatation. The severity and distribution of these changes, and those reported in most of the previously reported cases of MDC1C, are different from those observed in patients with MDC1A (merosin-deficient CMD), the form of MDC1C, although in 6 of 7 families an identical single mutation was identified. Most patients with FCMD show an anteroposterior gradient as also seen in MDC1C. In a large series reported in 1996,20 all 21 patients had polymicrogyria involving the frontal lobe; the parietotemporal lobe was involved in 6 patients, while 12 also had occipitotemporal pachygyria-agryria. In other studies the prevalence of the occipitotemporal pachygyric areas was more common, with invariably associated polymicrogyric changes in the frontal and parietal lobes.21-23

In 5 of 13 patients described in this study, cerebellar cysts were associated with structural brain changes involving the posterior fossa, cortex, or both. The cases of 2 patients with severe changes resembling MEB and a WWS-like condition have already been reported,19 but we also found FKRP gene mutations in another 3 cases with various degrees of structural brain abnormalities, including focal unilateral periventricular nodular heterotopia to marked cerebellar dysplasia and pons hypoplasia. Our findings not only suggest further heterogeneity in the spectrum of brain changes associated with FKRP gene mutations but also a hierarchy of severity of such changes. On brain MRI the cerebellum seems to be the most vulnerable structure affected in patients with FKRP-related conditions. Indeed, several patients with MDC1C who experienced the least severe CNS involvement had cerebellar cysts unassociated with any other structural change in the posterior fossa or in the supratentorial regions. In several of these patients, however, there were also associated supratentorial white matter abnormalities. None of these patients had structural eye involve-

ment and mental retardation was either absent or mild. With increasing clinical and radiological severity, the cerebellar cysts seem to be associated with other structural abnormalities of the posterior fossa, such as pons and brainstem hypoplasia and more extensive cerebellar dysplasia or hypoplasia. In patients with more severe involvement we observed, in addition to the cerebellar and brainstem abnormalities, structural involvement of the cortex that appears to follow an anteroposterior gradient. The cortical structural abnormalities can be best appreciated over the frontal pole and medial aspect of the anterior frontal pole and in the parietal lobes that appear polymicrogyric. The occipital lobes appear involved only in the most severe case in which the whole cortex has an agyric/lissencephalic aspect.

A similar anteroposterior gradient is also present in the reported cases of MEB due to mutations in POMGnTI gene, all of which show cerebellar involvement associated with local areas of cobblestone cortex localized in the frontal lobes.4,13-16 However, in a pathological study17 bilateral agyric areas were reported at post mortem, affecting the lateral convexity of the occipital lobes in 2 siblings, while the rest of the brain showed a pachygyric appearance. The agyric appearance of the occipital cortex resembled, in these cases, that observed in some patients affected by MDC1A (merosin-deficient CMD). In MDC1A the cortical dysplasia, when present, affects the occipital, occasionally the temporal, but not the frontal lobes.12,18 In patients with FCMD, the pattern of brain involvement can vary in severity considerably and this has been more recently associated with the mutation identified. Most patients with FCMD show an anteroposterior gradient as also seen in MDC1C. In a large series reported in 1996,20 all 21 patients had polymicrogyria involving the frontal lobe; the parietotemporal lobe was involved in 6 patients, while 12 also had occipitotemporal pachygyria-agryria. In other studies the prevalence of the occipitotemporal pachygyric areas was more common, with invariably associated polymicrogyric changes in the frontal and parietal lobes.21-23

The increasing severity of CNS involvement observed in patients with FKRP gene mutations broadly reflects the severity of the disruption of α-dystroglycan glycosylation. In particular the patient with MEB-like phenotype had almost-absent α-dystroglycan expression, in contrast to those patients with MDC1C with or without cerebellar cysts in whom α-dystroglycan expression was severely reduced compared with normal but still clearly present. This is in contrast to the mild to moderate reduction in α-dystroglycan expression that characterized patients with the milder LGMD2I allelic variant, in which brain is never affected.24

The defective glycosylation of α-dystroglycan is likely to be central to the development of the CNS structural defects in MDC1C and other glycosylation disorders belonging to this category. Dystroglycan is a heavily glycosylated peripheral membrane protein that binds to extracellular ligands such as laminin, agrin, perlecain, neurexin, and biglycan. O-linked glycosylation mediates binding of α-dystroglycan to the globular or G domain found in its various ligands (with the exception of biglycan) and differences in the level of its glycosylation

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have been demonstrated to affect the affinity of this interaction. α-Dystroglycan is expressed during development in basement membranes. During CNS development the formation of a continuous meningeal layer with its associated pial basement membrane is essential for the proper brain development. Indeed, during CNS development cell proliferation occurs in the ventricular zone, where dystroglycan is highly expressed. Postmitotic neurons migrate toward the developing cortical plate on radial glia with end feet attached to the pial basement membrane. The discontinuity of the pial-glial limitans, which is the result of the abnormal glycosylation of α-dystroglycan, determines the migration of neurons and glia beyond the pial basement membrane. This eventually gives rise to the macroscopic appearance of the cobblestone cortex. That α-dystroglycan is the major culprit for the observed abnormal neuronal migration disorder is supported by the observation that targeted disruption of dystroglycan in brain results in disrupted pial basement membrane formation and disorganized cortical layering with migration of cortical neurons and glial cells beyond the pial basement membrane in mice. Similar abnormalities have also been demonstrated at the pathological level in the LARGE-deficient mouse, in the fukutin chimeric null mice; but also in WWS, FCMD, and MEB brains at post mortem. In addition to the disrupted radial migration defect, morphological studies of the brainstem in FCMD indicate perturbed tangential migration. Although perturbed neuronal migration secondary to defective basement membrane clearly plays a significant role in the observed CNS defect, other mechanisms are also likely to be involved. In particular, disrupted neoglial interactions in specific populations of migrating neurons could play a role in corticogenesis. Using an elegant model of neuronal migration in cerebellar slice cultures, Qu and Smith in 2004 demonstrated that antibodies directed against glycosylated α-dystroglycan inhibited granule neuron migration in this system. Interesting experiments performed in the past in vivo, injecting antibodies against HNK-1 (a glycoepitope present on α-dystroglycan) in chicken embryos also resulted in perturbation of cranial neural crest migration. These observations, together with the recently demonstrated expression of fukutin and POMGnT1 in migrating neurons, not only in the meningeal cells that secrete extracellular matrix component, suggest a role of dystroglycan, fukutin, and POMGnT1 in the process of interaction with glial cells and neuronal migration. Analysis of the MRI obtained in MDC1C might also indirectly suggest the involvement of multiple mechanisms in the generation of neuronal migration defects. In particular, the identification of a subcortical lamination heterotopia in the patient with WWS, and of periventricular nodular heterotopia in one patient with MDC1C who had cerebellar cysts, suggests a defect of the migratory properties of neurons, not simply a defect of overmigration, which is the hallmark of the cobblestone-type lissencephalies. Similar abnormalities have been previously described in patients with WWS and FCMD. Further pathological studies in FKRP-deficient brain, and in situ studies focused on the pattern of regional and developmental expression of FKRP, will help clarify this matter.

Regarding genotype-phenotype correlation, no patient was found to carry 2 FKRP nonsense mutations, and we suspect that this might be incompatible with life. We analyzed the distribution of mutations identified in patients with MDC1C with and without brain involvement and those of patients with MEB and WWS. No recognizable pattern emerged; all these mutations appear to be located in the putative catalytic domain of the molecule, but it is impossible to assign, a priori, the severity that an individual mutation has. FKRP is a putative glycosyltransferase and the mechanism of disease in these patients likely results from disruption of this enzymatic function. This could occur either as a result of mislocalization of FKRP from the Golgi apparatus, where it resides, or of its enzymatic activity. Although a recent report using an in vitro assay suggested that the main mechanism of disease in MDC1C is the mislocalization of mutant FKRP, we have recently been able to show using FKRP-specific antibodies that this protein is normally localized in the Golgi apparatus in patients with MDC1C, including cases with brain involvement such as one of the children with MEB-like phenotype discussed in this article. This suggests, therefore, that the main mechanism of disease in MDC1C is related to a direct effect that the mutations have on the FKRP functional activity.

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**References**


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