1. Introduction

Alpha-dystroglycanopathies are a clinically and genetically heterogeneous group of muscular dystrophies characterized by the reduced or absent glycosylation of alpha-dystroglycan (Muntoni et al., 2002). The hypoglycosylation of alpha-dystroglycan leads to decreased binding of its ligands, including laminin, agrin and perlecan in skeletal muscle and neurexin in the brain. The only known target for this type of glycosylation is alpha-dystroglycan, and together with other proteins of the dystrophin-glycoprotein complex it forms a link between extracellular matrix proteins and actin cytoskeleton. The clinical manifestations of alpha-dystroglycanopathies are extremely variable, leading to a broad spectrum of phenotypes with limb-girdle muscular dystrophy (LGMD) without mental retardation delineating the milder end, and Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB) and Fukuyama type congenital muscular dystrophy (FCMD) the severe end (Muntoni & Voit, 2004) (Fig. 1). In most of the severe disorders, the eyes and the brain are affected in addition to congenital muscular dystrophy (CMD). Here, CMD is defined as onset of weakness prenatally or within the first 6 months of life, and LGMD is defined by later onset weakness, specifically after having acquired ambulation. The brain abnormalities are described as cobblestone lissencephaly; available pathological studies have demonstrated breeches of the glia limitans and over-migration of cortical neurons into the pial spaces. In WWS, the lifespan of patients is severely reduced and brain and eye abnormalities extremely severe (Dobyns et al., 1989); MEB and FCMD patients generally survive beyond infancy, ocular manifestations are usually milder in FCMD than in MEB (Fukuyama et al., 1981, Santavuori et al., 1989). To date, mutations in six genes which encode putative or confirmed glycosyltransferases have been identified in these autosomal recessively inherited disorders: Protein-O-mannosyl transferase 1 and 2 (POMT1 and POMT2), Protein-O-mannose 1,2-N-acetylglucosaminyltransferase 1 (POMGnT1), Fukutin-related protein (FKRP), Fukutin (FKTN), and LARGE. Initially, each gene was associated with one syndrome (original phenotype): POMT1 and POMT2 mutations giving rise to WWS; POMGnT1 mutations in patients with MEB; FKRP mutations in patients with congenital or late-onset muscular dystrophies (MDC1C and LGMD 2I); FKTN mutations in patients with FCMD; LARGE mutations in a patient with congenital muscular dystrophy type 1D (MDC 1D). Subsequently, mutation analysis in patients with milder or more severe syndromes within the dystroglycanopathy spectrum demonstrated allelic heterogeneity for different mutations in each of the dystroglycanopathy genes (Fig. 1). Null mutations in POMT1, POMT2, POMGnT1, FKRP,
FKTN and LARGE are associated with the most severe end of the clinical spectrum (WWS) of dystroglycanopathy, although not an absolute rule.

Fig. 1. Gene mutations and clinical phenotypes of alpha-dystroglycanopathy

<table>
<thead>
<tr>
<th>Gene</th>
<th>mutation</th>
<th>LGMD 2M</th>
<th>CMD</th>
<th>CMD-MR</th>
<th>FCMD</th>
<th>WWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FKTN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POMGnT1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>POMT1</td>
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<tr>
<td>POMT2</td>
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<td></td>
</tr>
<tr>
<td>FKRP</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LARGE</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>WWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDC 1D</td>
<td></td>
</tr>
<tr>
<td>CMD-MR : CMD with mental retardation</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Gene mutations and clinical phenotypes of alpha-dystroglycanopathy

2. Broader clinical spectrum and worldwide distribution of FKTN mutations

A wide clinical spectrum is also evident for the FKTN mutations that were first reported in patients with FCMD and later in patients with WWS and in patients with LGMD 2M without mental retardation. FCMD is the second most common form of muscular dystrophy in Japanese population after Duchenne muscular dystrophy, but is seen very rarely in other population. The incidence of FCMD is 3-10 per 100,000 or nearly half that of Duchenne muscular dystrophy in the Japanese population, with a carrier frequency of one in 80 and is one of the most common autosomal recessive disorder in Japan (Fukuyama & Ohsawa, 1984). FCMD patients have muscular dystrophy with severe mental retardation and a neuronal migration abnormality. Epilepsy and eye abnormalities are also frequently associated with FCMD. Toda et al. (1993) localized the FCMD locus to chromosome 9q31-33 using genetic linkage analysis. They further defined the FCMD locus within a much smaller segment and also found evidence for strong linkage disequilibrium. Haplotype analysis using the markers D9S2105, 2107, and D9S172 indicated that most FCMD-bearing chromosomes in Japanese pedigrees were derived from a single ancestral founder (Toda et al., 1996). Most Japanese FCMD patients are homozygous for an ancestral founder mutation.
in FCMD gene, which arose from the insertion of a 3 kb retrotransposon element into the 3’ untranslated region (UTR). Some patients are compound heterozygous, carrying another mutation in addition to the founder insertion, and leading to a more severe FCMD variant (Kobayashi et al., 1998a). The absence of patients with two non-founder mutations in Japan led to the hypothesis that this may be lethal, however since 2003 compound heterozygosity for many different mutations have been described in non-Japanese populations, including homozygosity for nonsense mutations. To date, at least 24 different FKTN mutations have been described in non-Japanese patients and phenotypes of these patients cover the entire range of alpha-dystroglycanopathies (Yis et al., 2011).

We performed clinical studies in 41 families with FCMD examined between 1972 and 1992 (Yoshioka & Kuroki, 1994). These patients were diagnosed on the standard criteria of FCMD described by Fukuyama et al. (1960). After the discovery of FCMD gene, we investigated gene mutations of these FCMD families (Yoshioka et al., 2008). Here, we at first describe the clinical studies performed until 1992 and later analysis of the genotype-phenotype relationship in FCMD. We then present Japanese CMD patients with alpha-dystroglycanopathy with other gene mutations than FKTN and without any known gene mutation. In addition, we describe FKTN mutations outside Japan and compare them with Japanese FCMD patients.

### 2.1 Japanese FCMD patients diagnosed on standard criteria between 1972 and 1992

We performed clinical and genetic studies in 41 families with FCMD examined by us between 1972 and 1992 (Table 1) (Yoshioka & Kuroki, 1994). The diagnosis in these patients was established according to standard criteria described by Fukuyama et al. in 1960, which were, briefly, early onset hypotonia, joint contractures, severe mental retardation with occasional convulsions, and dystrophic abnormalities detected in the muscle biopsy specimen. Nine families (22%) had multiple affected children (“familial” FCMD). Unfortunately, two siblings in nine families had already died at the time of examination, and detailed clinical data other than their clinical diagnosis were therefore not available. The other 32 families had only one affected child (“sporadic” FCMD). Parental consanguinity was documented in 5 sporadic FCMD families and none of the familial cases. In total, 48 patients, including 7 sib pairs, were evaluated with regard to maximum motor ability, mental and convulsion states, cranial CT or MRI findings, and EEG and ophthalmological data.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Familial</th>
<th>Sporadic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of families</td>
<td>41</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Consanguineous marriage</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Number of patients</td>
<td>50</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td>Male : female</td>
<td>22 : 28</td>
<td>5 : 13</td>
<td>17 : 15</td>
</tr>
</tbody>
</table>

Table 1. Patients with Fukuyama-type Congenital Muscular Dystrophy (FCMD) [diagnosed according to standard criteria between 1972 and 1992, reproduced from Yoshioka & Kuroki, 1994]
Fig. 2. Maximum motor ability in sib pairs and in familial and sporadic FCMD cases. The familial cases are numbered from 1 to 9. The members of each sib pair are connected by a line (Reproduced from Yoshioka & Kuroki, 1994).

Three patients in our familial group but only one in our sporadic group showed no head control (“bedridden”), whereas a few ambulatory patients were seen in both groups (Fig. 2).
The familial FCMD patients showed relatively more severe motor disability than that in the sporadic FCMD patients. The maximum motor ability in most patients in both groups consisted of sitting without help. Although the speech ability varied between sibs and between families (Fig. 3), all patients showed moderate to severe mental retardation. As for convulsion states, about half of the patients had febrile or afebrile convulsions in both familial and sporadic groups. The convulsion state in 7 sib pairs was the same; both sibs in 3 families had afebrile or febrile convulsions, while in 4 other families neither had convulsion. EEG showed paroxysmal discharges in three sibling pairs with convulsions, while in two of the other sibling pairs without convulsion a difference between siblings in EEG findings was apparent. Ophthalmologically, myopia, weakness of the orbicularis oculi, nystagmus, and optic nerve atrophy were common findings.

![Fig. 3. Speech ability in sib pairs and in familial and sporadic FCMD cases. Familial cases are numbered from 1 to 9, and the members of each sib pair are connected by a line (Reproduced from Yoshioka & Kuroki, 1994).](image)

Typical CT or MRI findings seen in FCMD were pachgyria or polymicrogyria of the fronto-temporo-occipital regions, moderate dilatation of the lateral ventricles, especially posteriorly (colpocephaly), the abnormal signal in the cerebral white matter, and cerebellar cysts closely related to polymicrogyria (Fig. 4). However, in one family (S-family), the elder brother had the typical CT findings of FCMD, while the younger brother had marked dilatation of the lateral ventricles and an occipital encephalocele (Fig. 5). In addition, retinal detachment was present in the younger brother at birth, whereas in the elder brother it developed at 3 years. Our study revealed that FCMD patients ranged from ambulatory to bedridden, and some were able to form sentences while others uttered no meaningful words. Convulsions were found in about half of the patients. Hydrocephalus, encephalocele and retinal detachment were rare but true findings in FCMD.
Fig. 4. Typical MR imaging in a FCMD patient aged 16 years. T1-(upper) and T2-(lower) weighted axial magnetic resonance images of the cerebellum (left) and cerebrum (right). Cerebral cortical dysplasia is mild, and numerous cysts closely related to polymicrogyria are seen in the cerebellum (Reproduced from Yoshioka et al., 2008).

Based on these observations, we considered the clinical spectrum of FCMD to be much broader than previously described and to overlap with that of “mild” WWS and of MEB.

2.2 Genetic study of S-family and further analyses of FCMD haplotype

The FCMD locus was initially localized to chromosome 9q31-33 by genetic linkage analysis and homozygosity mapping (Toda et al., 1993, 1994).

We analyzed one Japanese family (S-family) in which three siblings were affected with severe cerebral malformations in association with ocular anomalies and muscle disease (Yoshioka et al., 1992). Both parents were healthy and nonconsanguineous. The elder brother showed pachygyria on computed tomographic scan (Fig.5), retinal detachment in both eyes at the age of three years and dystrophic findings on a muscle biopsy. He was diagnosed clinically as having FCMD. The second pregnancy resulted in a male infant with anencephaly who survived for five minutes. Anencephaly was regarded as WWS with extreme brain abnormality. The third son exhibited at birth such characteristic features as
pachygyria, encephalocele, hydrocephalus, retinal detachment in both eyes, elevated serum creatine kinase activity, and arthrogryposis multiplex congenita which were consistent with WWS (Fig. 5).

Fig. 5. Cranial computed tomographic scans of two siblings of S-family. Left: the first son at the age of 5 months. Moderate dilatation of the lateral ventricles, especially posteriorly (colpocephalic) and pachygyria in the temporo-parietal region are shown. The low density area in the white matter is apparent. Right: the third son at birth. Marked dilatation of lateral ventricles and occipital encephalocele are evident (Reproduced from Yoshioka & Kuroki, 1994).

Genetic analysis of this family was performed using polymorphic microsatellite markers flanking the FCMD locus (Toda et al., 1995). Genomic DNA was extracted from peripheral blood leukocytes of the parents, the first and the third siblings. Both patients (FCMD and WWS) shared exactly the same haplotype at seven marker loci spanning 16 cM and surrounding the FCMD locus. This suggests that both affected siblings should carry the same combination of FCMD alleles, each with a mutation. Since the patients of FCMD and WWS carry the identical combination of mutations on either allele of the FCMD locus, these clinical conditions are caused by the mutations in the same gene. The difference in clinical manifestations between FCMD and WWS may reflect the pleiotropy or variation of expressivity of the FCMD gene.

Later, it was found that one specific haplotype was shared by 82% of FCMD chromosomes (Kobayashi et al., 1998b). These data supported the hypothesis of a single founder of this disease in the Japanese population. Moreover, eight haplotypes different from the founder’s were observed in FCMD chromosomes, indicating that eight different FCMD mutations in addition to the founder’s have occurred in Japan. Thereafter, it was clarified that a
Muscular Dystrophy retrotransposal insertion exists within this candidate-gene interval in all FCMD chromosomes carrying the founder haplotype. Two independent point mutations confirm that a mutation of this gene is responsible for the condition (Kobayashi et al., 1998a).

Using new polymorphic microsatellite markers, we genotyped five CMD patients from four families including the S-family who had severe eye and brain anomalies, such as retinal dysplasia and hydrocephalus (Yoshioka et al., 1999). All patients were heterozygous for the founder haplotype of the FCMD gene. In S-family, the Japanese founder haplotype of the FCMD gene was derived from the patients’ mother and the haplotype, which cosegregate with nonsense mutation on exon 3 of the FCMD gene, was derived from their father (Fig. 6). Thus, two siblings were compound heterozygotes for FCMD. This showed severe eye anomalies such as retinal dysplasia or detachment and hydrocephalus could be included in the clinical spectrum of FCMD. The clinical spectrum of FCMD is much broader than previously presumed.

Fig. 6. Genotypes of S-family including two brothers at polymorphic microsatellite loci flanking the FCMD locus. This family is heterozygous for the founder insertion allele. The founder haplotype 138-192-147-183 for markers D9S2105-D9S2170-D9S2171-D9S2107 was derived from the patients’ mother and the haplotype 130-201-157-183, which cosegregated with nonsense mutation on exon 3 of the FCMD gene, was derived from their father. Thus, two brothers are compound heterozygotes for the disease (Reproduced from Yoshioka et al., 1999).

To investigate the distribution and origin of the founder insertion of FCMD, Watanabe et al. (2005) screened a total of 4,718 control DNA samples from Japanese and other Northeast
Asian population. Fifteen founder chromosomes were detected among 2,814 Japanese individuals. Heterozygous carriers were found in various regions throughout Japan, with an averaged ratio of 1 in 188, although previous reports have estimated the carrier frequency to be as high as 1 in 80 and the incidence to be 3-10/100,000 births (Fukuyama & Ohsawa, 1984). In Korean populations, they detected one carrier in 935 individuals. However, they were unable to detect any heterozygous alleles in 203 Mongolians and 766 Mainland Chinese populations. These data largely rule out the possibility that a single ancestor bearing an insertion-chromosome immigrated to Japan from Korea or Mainland China and appear to confirm that FCMD carriers are rare outside of Japan.

2.3 Phenotype-genotype relationship in FCMD after discovery of FCMD gene, FKTN

Between 1994 and 2006, we diagnosed thirty-five patients with FCMD because they had the founder mutation homozygously or heterozygously, in Kobe City Pediatric and General Rehabilitation Center for the Challenged, Utano National Hospital, Shiga Medical Center for Children and Shizuoka Children’s Hospital (Yoshioka et al., 2008). Among these 35 patients, we found 18 patients (eight boys and 10 girls) carrying a homozygous founder mutation (homozygous patients or homozygotes) and 17 patients (eight boys and nine girls) with a compound heterozygous mutation (heterozygous patients or heterozygotes). The range of follow-up was between one and 30 years (average 16.5 years) in homozygotes and between two and 19 years (average 12.8 years) in heterozygotes (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Homozygous patients</th>
<th>Heterozygous patients</th>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Male : Female</td>
<td>8 : 10</td>
<td>8 : 9</td>
</tr>
<tr>
<td>Follow-up period :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16.5 years</td>
<td>12.8 years</td>
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<tr>
<td>Range : years :</td>
<td>1 : 2~30 : 8</td>
<td>2 : 5~19 : 5</td>
</tr>
<tr>
<td>Maximum motor ability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedridden</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Head control</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sit without help</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Walk without help</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mental status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No meaningful words</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Speak single words</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Speak in sentences</td>
<td>11</td>
<td>1</td>
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<tr>
<td>Radiological findings</td>
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<td>Pachygyria/polymicrogyria</td>
<td>16</td>
<td>16</td>
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<tr>
<td>Hydrocephalus</td>
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<tr>
<td>Cephalocele</td>
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<td>1</td>
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<tr>
<td>Ophthalmological findings</td>
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<tr>
<td>Retinal dysplasia</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Clinical characteristics of FCMD patients diagnosed between 1994 and 2006 (Reproduced from Yoshioka et al., 2008)
Genomic DNA was extracted from patients' peripheral blood leukocytes. After digestion of genomic DNA with PvuII, Southern hybridization was performed using fEco8-I as a probe to detect the 3 kb founder insertion in the DNA. If a patient had only one founder insertion (compound heterozygotes), we then screened all exons and flanking introns of the FKTN gene by polymerase chain reaction direct sequencing to detect nonsense or missense mutations. Some members had been genotyped with polymorphic microsatellite markers as described previously (Kobayashi et al., 1998b). Each chromosome containing the 3 kb retrotransposonal insertion was concordant with the founder haplotype represented as 138-192-147-183 for markers D9S2105-D9S2170-D9S2171-D9S2107.

Clinical characteristics in both groups are summarized in Table 2. Most homozygotes could sit without help and speak in sentences, while half of the heterozygotes were bedridden and most spoke no meaningful words. Typical CT or MRI findings of FCMD were seen in almost all cases. These included polymicrogyria within the cerebral cortex that was primarily in the frontal lobes (Figs.4 and 5). In the cerebellum, numerous intraparenchymal cysts closely related to the polymicrogyria were seen at the hemispheres (Fig.4). More severe cortical dysplasia was usually found in heterozygotes than in homozygotes. Hydrocephalus and cephalocele were found only in heterozygotes. In particular, ophthalmological findings showed a clear difference between the two groups; retinal dysplasia and detachment were only found in heterozygotes.

In comparison with our study, systematic analysis of the FCMD gene in 107 unrelated patients by Kondo-Iida et al. (1999) revealed that 80 probands (75%) were homozygous for the 3 kb insertion, 25 (23%) were heterozygous, and two did not show the 3 kb insertion on either allele. In our study, however, the number of homozygotes and heterozygotes was almost the same. Although both groups included some sib pairs in our study, the number of probands was 16 for the homozygotes and 15 for the heterozygotes, which was also almost the same number. This result might be due to the small number of patients examined or a regional inclination in our study, as most of our patients lived in the western part (Kansai district) of Japan.

According to the report by Kondo-Iida et al. (1999), among patients homozygous for the founder mutation, 91.5% showed milder (stand or walk with or without support) or typical (able to sit unassisted or to slide on buttocks) phenotypes, and only 2.5% of cases were classified as severe (could sit only with support or had no head control), while among patients with heterozygous for the founder mutation, 92% showed severe phenotypes. This was true in our study, as most homozygotes could sit without help, while half of the heterozygotes were bedridden. It was speculated that because the 3’-UTR of a gene affects the stability of its mRNA, the 3 kb sequence inserted in that portion of the FCMD gene may alter the secondary structure of FCMD mRNA and render it unstable. This notion is supported by RT-PCR analysis that revealed low levels of the expected amplification product occurred in patients who were homozygous for the founder mutation and lower than normal in patients heterozygous for the insertion and another mutation. In other words, chromosomes carrying the 3 kb insertion may merely produce a lower level of mature fukutin than normal and generate a relatively mild phenotype. On the other hand, nonfounder mutations, which include nonsense and missense mutations within the coding region, cause major structural changes in the fukutin protein and thus are likely to produce severe effects.
2.4 Seizure-genotype relationship in FCMD patients diagnosed between 1994 and 2006

Mutational analysis of 35 patients with FCMD is shown in Table 3. Each chromosome containing the 3 kb retrotransposon insertion was concordant with the founder haplotype represented as 138-192-147-183 for markers D9S2105-D9S2170-D9S2171-D9S2107. Eighteen patients were homozygous for the 3 kb insertion and 17 were heterozygous. Mutations other than the 3 kb insertion were identified seven of the 12 heterozygous patients examined. These included five patients with a nonsense mutation in exon 3, one patient with a missense mutation in exon 5 and one patient with a nonsense mutation in exon 8. Among five patients with mutation in exon 3, afebrile seizures were found in three patients. One of them showed intractable seizures and the other developed infantile spasms at age six months. Two of five patients with a mutation in exon 3 had no seizures during follow-up as they died at ages 2 and 5 years, respectively, raising the possibility that seizures could

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Location</th>
<th>Mutation</th>
<th>Type of mutation</th>
<th>No. of patients</th>
<th>Seizure status</th>
</tr>
</thead>
</table>
| Homozygous for the founder haplotype (138-192-147-183) | 3’ Untranslated region | 3 Kb insertion | Instability of mRNA | 18 | Afebrile seizures : 8  
Febrile seizures : 3  
No seizure : 7 |
| Heterozygous for the founder haplotype* |                   |          |                  | 17 | Afebrile seizures : 9  
Febrile seizures : 5  
No seizure : 3 |
| 130-201-157-183                   | Exon 3            | R47X     | Nonsense         | 5  | Afebrile seizures : 3  
(Intractable : 1, infantile spasms : 1)  
No seizure : 2  
(died aged 2 and 5 years) |
| Not examined                      | Exon 5            | M133T    | Missense         | 1  | Afebrile seizures : 1  
(Intractable : 1) |
| Not examined                      | Exon 8            | R307X    | Nonsense         | 1  | Febrile seizures : 1  
(Intractable : 2) |
| 139-201-155-183                   | Unknown**         | Unknown  | Unknown          | 4  | Afebrile seizures : 3  
(Intractable : 2) |
| 148-196-153-183                   | Unknown           | Unknown  | Unknown          | 1  | Febrile seizures : 1 |
| 128-199-155-183                   | Not done***       | Not done | Not done         | 1  | Afebrile seizures : 1 |
| 138-194-155-183                   | Not done          | Not done | Not done         | 1  | Febrile seizures : 1 |
| 138-199-147-191                   | Not done          | Not done | Not done         | 1  | No seizure : 1 |
| 138-196-147-191                   | Not done          | Not done | Not done         | 1  | Febrile seizures : 1 |
| Not examined                      | Not done          | Not done | Not done         | 1  | Afebrile seizures : 1 |

* Haplotypes other than the founder’s haplotype are shown below.  
** Sequence analyzed, but no mutation found.  
*** Sequence analysis was not done yet.  

Table 3. Relationship between genotypes and seizures in FCMD patients (Reproduced from Yoshioka et al., 2008).
develop later. All five patients also showed severe clinical manifestations suggestive of WWS. One patient with a missense mutation in exon 5 had intractable complex partial seizures and severe psychomotor retardation. However, one patient aged six years with a nonsense mutation in exon 8 developed a febrile seizure at the age of four years four months and he had no EEG paroxysmal discharges. On the other hand, five of twelve patients who underwent sequence analysis of chromosome without the 3 kb insertion revealed no mutation within the coding region of FKTN. It is probable that the mutations in these alleles lie in regulatory regions such as promoter sequences or intronic sequences critical for alternative splicing. Among them, four patients showed the same haplotype, 139-201-155-183, for markers D9S2105-D9S2170-D9S2171-D9S2107 and showed severe phenotypes. In addition, two of them had intractable seizures.

It is interesting that in this study seven of 18 (39%) homozygous and three of 17 (18%) heterozygous patients had no seizures during follow-up. Although two of our heterozygotes without seizures died at ages 2 and 5 years, respectively, all homozygotes without seizures were older than four years and two patients were over 30 years of age.

In addition, some had only febrile seizures throughout their life. Antiepileptic drugs were prescribed for half of the FCMD patients and no intractable seizures were observed in homozygotes. These facts showed that seizures occurring in FCMD patients were not always severe. Milder cortical dysplasia was suggested in FCMD, especially in homozygotes.

From these observations, it was concluded that mutational analysis of the FCMD gene could predict seizure prognosis. Heterozygotes usually developed seizures earlier than homozygotes and some heterozygotes showed intractable seizures. Special attention is necessary when treating epilepsy in heterozygotes. Mutational analysis other than the 3 kb insertion and haplotype analysis may also help to predict seizure prognosis.

2.5 Worldwide distribution of Fukutin mutation

A Turkish geneticist who had read our paper (Yoshioka et al., 1999) asked us to analyze her CMD patient. This Turkish boy had characteristics of WWS. Parents were first cousins, and their first son was unaffected. The infant was born by cesarean section and weighed 2,700 gm (25th percentile); his height was 50cm (50th percentile), and head circumference was 47 cm (>97th percentile). Physical examination showed respiratory difficulties, central cyanosis, generalized hypotonia, hydrocephaly, bilateral buphthalmus, and cataracts. Rieger’s anomaly with iris atrophy and peripheral corneal adhesions was noticed. After cataract extraction of the right eye, ocular examination showed that the optic disc and the retina were hypoplastic. Cranial computed tomography showed hydrocephalus and cortical atrophy. After birth, this patient was supported by mechanical ventilation and died on the 10th day. Neuropathological examination showed agyric hemispheres with polymicrogyria in several cortical segments and severe cortical disorganization in other segments. The ventricles released 600ml of cerebrospinal fluid. CMD was also seen, with variation in fiber size, and fibrosis. Immunohistochemical analysis showed greatly reduced staining for alpha-dystroglycan, but normal immunoreactivity for beta-dystroglycan in the skeletal muscle membrane. Serum creatine kinase levels were greatly elevated.
Genetic analysis of this family was performed. As expected, the patient had no Japanese founder insertion. We then screened all exons and flanking introns of the fukutin gene in the patient by polymerase chain reaction direct sequencing. We detected a homozygous 1bp insertion mutation, nt504(insT), in exon 5 of FKTN. This mutation causes a frameshift, resulting in a premature termination at codon 157. Both parents and the brother were heterozygous for this mutation. This is the first case worldwide in which a FKTN mutation has been found outside the Japanese population (Silan et al., 2003).

Later, another Turkish boy with WWS phenotype was found to have a homozygous nonsense mutation in FKTN by the research group in the Netherlands (Beltran-Valero de Bernabe et al., 2003). The homozygous nonsense mutations within the coding region identified in two Turkish patients are predicted to cause a total loss of FKTN and are likely to produce a more severe phenotype which closely resembles WWS.

Manzini et al. (2008) assembled a large cohort of patients with typical WWS (43 affected individuals from 40 families), drawn from Middle Eastern consanguineous families (16 patients from 14 families) and from consanguineous and nonconsanguineous families from Europe and the Americas (27 cases). They found that 40% (16/40 families) of patients in their cohort carried mutations in the coding sequence of POMT1, POMT2, FKRP or FKTN with no POMGnTI or LARGE mutations detected. FKTN and FKRP mutations in particular were much more common than previously suggested and were mostly identified in non-consanguineous patients of European descent (6/27 cases). All Ashkenazi Jewish patients in their group shared an identical haplotype at the FKTN locus and the same homozygous mutation c.1167_1168insA in exon 9 suggesting a founder effect in this population. They identified the carrier frequency of this mutation to be 0.7% in the Ashkenazi population in Israel, which will be extremely informative for genetic testing. A striking difference was observed in the geographic distribution of mutations, as Middle East families were mostly carriers of POMT1 mutations (35.7%, 5/14 families), while the most common cause of European/American cases was FKTN mutations (18.5%, 5/27 cases). An additional four USA Ashkenazi Jewish families with WWS were found to have a founder mutation in FKTN in this population (Chang et al., 2009).

The cohort consisted of 92 unrelated individuals who showed hypoglycosylation of alpha-dystroglycan at the sarcolemma by immunolabelling of skeletal muscle sections (80 patients) or had the clinical phenotype being highly suggestive of a alpha-dystroglycanopathy (12 patients) was analyzed the mutation of genes related to alpha-dystroglycanopathy (Godfrey et al., 2007). Homozygous and compound heterozygous mutations were detected in a total of 31 probands (34 individuals from 31 families). Mutations in FKTN, typically associated with FCMD in Japan were found in six patients, none of whom are of Japanese origin. Only two of these patients had structural brain involvement; one patient affected by WWS and one by a MEB-FCMD phenotype. The remaining patients had no structural brain involvement; one case had CMD-no mental retardation and never acquired the ability to walk but has normal IQ and five individuals from three families have entirely normal intellect and a mild LGMD phenotype (LGMD 2M). Interestingly in the latter two of these families, a dramatic response to steroid therapy was noted (Godfrey et al., 2006).

Vuillaumier-Barrot et al. (2009) reported four newly diagnosed Caucasian patients with FKTN mutations with a broad spectrum of phenotypes ranging from CMD associated with mental retardation to LGMD without central nervous system involvement. Two patients (two sisters) presented with CMD, mental retardation, and posterior fossa malformation...
including cysts, and brain atrophy at brain MRI. The other two patients had normal intelligence and brain MRI. Sequencing of the \textit{FKTN} gene identified three previously described mutations and two novel missense mutations.

In contrast to studies in Middle East families and European/American cases, a 10-year-old Korean boy with clinical features of FCMD was found to have homozygous Japanese insertion mutation (Lee et al., 2009). His parents were heterozygous carriers of the same mutation. He is the first genetically confirmed FCMD patient in Korea and the first non-Japanese patient carrying homozygous Japanese founder mutation. According to a large northeast Asian population study (Watanabe et al., 2005), the carrier frequency of 3-kb insertion mutation in Korean population is 1 in 935. Based on this observation, the incidence of FCMD by 3-kb insertion mutation is as low as 1 in 3,496,900 in Korea. On the other hand, the Japanese founder mutation was not detected in 766 mainland Chinese individuals (Watanabe et al., 2005). However, the first FCMD case was reported in the Chinese population with a Japanese founder 3-kb insertion and the other copy with a known c.139C>T mutation (Xiong et al., 2009). These Asian case reports emphasize the importance of considering the \textit{FKTN} founder mutation for diagnostic purposes outside of Japan and suggest that segments of the Chinese, Korean and Japanese populations may have a recent common ancestor.

### 2.6 Milder phenotype of FCMD

The first indication that \textit{FKTN} mutations may also cause a much less severe phenotype came from Murakami et al. (2006). They reported that six Japanese patients, all of whom were compound heterozygotes for \textit{FKTN} founder mutation and a point mutation, had minimal muscle weakness, normal intellect and dilated cardiomyopathy. No mutation was found in the other responsible genes for alpha-dystroglycanopathy including \textit{FKRP}, \textit{POMGnTI}, \textit{POMTI}, \textit{POMT2}, and \textit{LARGE} in these patients. Pathological findings in the biopsied skeletal muscles showed only minimal dystrophic changes, but have altered glycosylation of alpha-dystroglycan and reduced laminin binding ability. Cardiac involvement is the most remarkable finding in these patients. All the patients showed dilated cardiomyopathy, and two of them had life-threatening, rapidly progressive cardiac insufficiency. Cardiac involvement is rarely described in patients with alpha-dystroglycanopathy except for some patients with LGMD 2I.

Recently, milder cases of muscular dystrophy associated with \textit{FKTN} mutations have also been reported in non-Japanese populations. Godfrey et al. (2006, 2007) reported on five non-Japanese children from three families with normal intelligence and limb-girdle phenotype, caused by heterozygous point mutations in the \textit{FKTN} gene. Puckett et al. (2009) reported an additional two brothers with a LGMD phenotype due to compound heterozygous \textit{FKTN} mutation. These two brothers had elevated CK, mild muscle weakness and normal cognition. They lack any cardiac or ocular abnormalities. In addition to their mild clinical presentation, patients were also unique from an ethnic and molecular standpoint. Their father was of European descent and their mother, Japanese. The children, however, did not possess the common Japanese founder mutation. Rather, the brothers had two \textit{FKTN} missense mutations, one of which, c527T>C, had not been previously reported. This is significant, as the vast majority of patients reported have been either homozygous or heterozygous for the common retrotransposon insertion. Despite the milder skeletal muscle phenotype of these patients and those reported by Murakami et al. (2006), muscle biopsies show a reduction in fully
glycosylated alpha-dystroglycan similar to severe forms of CMD, such as FCMD and MEB. This emphasizes that immunophenotype may correlate poorly with clinical severity.

3. Japanese patients with alpha-dystroglycanopathy with other gene mutations than FKTN

In Japan, FCMD is the most common form of CMD, whereas MEB, WWS, MDC1C and MDC1D were rarely seen. WWS has been observed in many population groups with a worldwide distribution (Dobyns et al., 1989). In contrast, both MEB and FCMD show striking founder effects. MEB was first described in Finland, where it is most prevalent, owing to a strong founder effect following by genetic drift (Santavuori et al., 1989, Haltia et al., 1997). Consequently, most MEB patients have come from a small, geographically isolated population in Finland, with few Caucasian exceptions. Taniguchi et al. (2003) examined 30 patients from various countries, including Japan and Korea, who were diagnosed as WWS, severe FCMD or MEB. Two Japanese patients were identified as compound heterozygotes of POMGnT1 mutations. Severe hydrocephalus was observed prenatally by an ultrasonograph in both patients, and in one of them hydrocephalus required a ventriculo-peritoneal shunt at one year of age. Therefore, MEB patients may exist with a broader distribution than previously expected. Later, Matsumoto et al. (2005) performed detailed genetic and clinico-pathological analyses on 62 Japanese patients whose limb-muscle specimens showed altered glycosylation of alpha-dystroglycan. FKTN mutations were found in 54 patients (86%) examined, reflecting the most common form of CMD in Japan. In this study, the first patient with MDC1C (FKRP mutation) in oriental countries was found. Clinically, this patient showed severe muscle weakness from early infancy, marked elevation of serum CK level, calf hypertrophy, and normal intelligence; those are consistent with MDC1C. Further, the structural abnormality in the cerebellum was seen on brain MRI including disorganized folia and multiple cysts, those are commonly observed in FCMD/MEB. In addition, two MEB (POMGnT1 mutations) and one WWS (POMT1 mutation) were genetically confirmed. These studies show that patients with alpha-dystroglycanopathy in Japan have not only FKTN mutations but also have mutations of other genes such as POMT1, POMT2, POMGnT1 and FKRP. Molecular genetic studies have been helpful in defining subgroups of CMD.

4. CMD patients without known gene mutations of alpha-dystroglycanopathy

We studied a Japanese CMD patient with brain abnormalities without FKTN mutation using immunohistochemical analysis of dystrophic muscle and full mutational analysis of POMGnT1 and FKRP genes (Yoshioka et al., 2004).

Hypotonia and generalized muscle weakness became apparent during the first year of life. Consanguineous marriage was not noted. He obtained head control at 4 months, rolled over at 9 months, learned to sit unsupported at 12 months, crept at 19 months and stood with support at 26 months of age. At 4 years he could walk with the short leg braces using walker and speak two-word’s sentences. Serum creatine kinase levels were markedly elevated (2,776 IU/L, normal range <130 IU/L). Brain MRI showed thick and bumpy cortices with shallow sulci and abnormal white matter changes (Fig. 7). Ophthalmologically, he had no abnormalities. Immunohistochemical analysis showed reduced staining of alpha-dystroglycan, while expression of merosin and beta-dystroglycan was normal (Fig. 8). Sequence analysis of POMGnT1 and FKRP revealed no mutation.
Fig. 7. MR imaging at 15 months of age (the upper line) and 26 months of age (the lower line) on T2-weighted sequences. The cerebral white matter shows symmetric high intensity, which decreases with age (Reproduced from Yoshioka et al., 2004).

Fig. 8. Immunohistochemistry. Transverse serial frozen sections of skeletal muscle biopsies from the patient were immunostained with antibodies against alpha-dystroglycan (A), beta-dystroglycan (B), and laminin alpha-2 (merosin) (C). Note that beta-dystroglycan and laminin alpha-2 were present but alpha-dystroglycan was absent in sarcolemma of muscle fibers. Scale bar=20 micrometer (Reproduced from Yoshioka et al., 2004).
From these observations, this CMD patient seemed to belong to alpha-dystroglycanopathy. However, sequence analysis of FKTN, POMGnT1 and FKRP showed no mutations. Although analyses of POMT1, POMT2 and LARGE are necessary, there seems to be still many CMDs whose causative genes are unknown. We previously reported these patients including this case as a variant of CMD (Yoshioka et al., 2002).

Among their 62 Japanese patients with alpha-dystroglycanopathy, Matsumoto et al. (2005) found four patients with no mutation in the known genes associated with glycosylation defects of alpha-dystroglycan. They were clinically diagnosed to have MEB or WWS. All four patients showed severe mental retardation, hypotonia from early infancy, and eye involvements. Brain MRI displayed type II lissencephaly, enlarged lateral ventricles, and hypoplastic brainstem and cerebellum. In the skeletal muscles, three patients who were clinically diagnosed as WWS showed severe dystrophic changes with marked fibrous tissue involvement. However, one patient who was clinically diagnosed as MEB showed only mild myopathic changes in his muscle.

It was true in non-Japanese patients with alpha-dystroglycanopathy. Although 31 among 92 probands (34%) with alpha-dystroglycanopathy had homozygous and compound heterozygous mutations in the known genes, a large number of remaining patients with clinico-pathological features indistinguishable from the ones with mutations were not found to have mutations in any of the genes studied (Godfrey et al., 2007). More, as yet undefined, genes are likely to be involved in the pathogenesis of the alpha-dystroglycanopathies. The identification of these genes may provide additional information on the pathway of glycosylation of alpha-dystroglycan.

5. Conclusion

Defects in genes responsible for altered glycosylation of alpha-dystroglycan cause a group of muscular dystrophies that are variably associated with central nervous and eye abnormalities, known as alpha-dystroglycanopathies. These comprise FCMD, MDC1C and 1D, WWS, and MEB. Mutations have been reported in six putative or demonstrated glycosyltransferases; FKTN, FKRP, LARGE, POMT1, POMT2, and POMGnT1. Although each disorder was initially associated with one gene, it has recently been shown that the spectrum of phenotypes is broader than previously thought, and all these syndromes can be associated with mutations in any of the six genes known to be involved in alpha-dystroglycan glycosylation (Fig. 1).

A wide clinical spectrum is also evident for the FKTN mutations that were first reported in patients with FCMD, and later also in patients with WWS, and in patients with LGMD. FCMD is most frequent in Japan, and relatively homogenous phenotype. The strikingly high prevalence of FMD among the Japanese appears to result from the initial founder effect, whose expansion occurred in relative isolation. Most FCMD-bearing chromosomes in Japan are derived from a single ancestral founder who lived a few thousands years ago. Seventy-five percent of Japanese patients are homozygous for the ancestral mutation and have a relatively milder phenotype than patients who are compound heterozygous for the ancestral mutation and another mutation. The Japanese founder 3’ insertion mutation is therefore...
regarded as a relatively mild mutation. The most common form of FCMD in Japan presents clinically with a combination of generalized muscle weakness, congenital structural brain malformations, seizures, decreased vision, and cardiomyopathy. Most patients are never able to walk independently and have moderate to severe cognitive delay. Japanese patients, who are compound heterozygous for the founder and another mutation, have much more severe WWS-like manifestations including hydrocephalus and microphthalmia.

However, an increasing number of FKTN mutations are being reported outside Japan. To date, at least 24 different FKTN mutations have been described in non-Japanese patients and phenotypes of these patients cover the entire range of alpha-dystroglycanopathies (Yis et al., 2011). Among 23 patients with CMD and mutations in the FKTN gene in non-Japanese populations, 10 patients had a WWS-like phenotype including severe brain and eye abnormalities, the remainder had a milder FCMD, MEB or LGMD phenotype. In addition to the Japanese founder mutation, the mutation c.1167insA in exon 9 has been found to be homozygous in seven non-consanguineous Ashkenazi Jewish families, with an estimated carrier frequency of the mutation of 0.7% in the Ashkenazi population in Israel. Affected patients in these Jewish families all had a severe WWS-like phenotype.

The WWS phenotype caused by FKTN mutations is associated with the presence of two loss-of-function mutations, although not an absolute rule. The relatively few reports of FKTN mutations in patients with mild phenotypes may be a result of ascertainment bias and the recent increase in the number of reports of such findings may result in increased mutation analysis in FKTN for such patients in the future.

In summary, these results confirm that outside Japan, muscular dystrophy due to FKTN mutations is not as rare as initially supposed, and could be associated with a large spectrum of phenotypes, compared to the relatively homogenous phenotype in the Japanese population.

Although alpha-dystroglycanopathy is still largely unknown, comprehensive mutation analysis in patients and genotype/phenotype correlation across the spectrum of disease caused by these genes may provide clues to gene function. Functional analysis in animal models will determine how these mutations affect the proteins.

6. References


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With more than 30 different types and subtypes known and many more yet to be classified and characterized, muscular dystrophy is a highly heterogeneous group of inherited neuromuscular disorders. This book provides a comprehensive overview of the various types of muscular dystrophies, genes associated with each subtype, disease diagnosis, management as well as available treatment options. Though each different type and subtype of muscular dystrophy is associated with a different causative gene, the majority of them have overlapping clinical presentations, making molecular diagnosis inevitable for both disease diagnosis as well as patient management. This book discusses the currently available diagnostic approaches that have revolutionized clinical research. Pathophysiology of the different muscular dystrophies, multifaceted functions of the involved genes as well as efforts towards diagnosis and effective patient management, are also discussed. Adding value to the book are the included reports on ongoing studies that show a promise for future therapeutic strategies.

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