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Possible influences on the expression of X chromosome-linked dystrophin abnormalities by heterozygosity for autosomal recessive Fukuyama congenital muscular dystrophy

(Duchenne muscular dystrophy/Becker muscular dystrophy/gene interaction/unlinked noncomplementation)

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ABSTRACT Abnormalities of dystrophin, a cytoskeletal protein of muscle and nerve, are generally considered specific for Duchenne and Becker muscular dystrophy. However, several patients have recently been identified with dystrophin deficiency who, before dystrophin testing, were considered to have Fukuyama congenital muscular dystrophy (FCMD) on the basis of clinical findings. Epidemiologic data suggest that only 1/3500 males with autosomal recessive FCMD should have abnormal dystrophin. To explain the observation of 3/23 FCMD males with abnormal dystrophin, we propose that dystrophin and the FCMD gene product interact and that the earlier onset and greater severity of these patients' phenotype (relative to Duchenne muscular dystrophy) are due to their being hemizygous for Duchenne muscular dystrophy, a genotype that is predicted to occur in 1/175,000 Japanese males. This model may help explain the genetic basis for some of the clinical and pathological variability seen among patients with FCMD, and it has potential implications for understanding the inheritance of other autosomal recessive disorders in general. For example, sex ratios for rare autosomal recessive disorders caused by mutations in proteins that interact with X chromosome-linked gene products may display predictable deviation from 1:1.

Interactions between different genetic loci have long been appreciated and studied in experimental animal models. Modification (e.g., suppression or enhancement) (1, 2) and unlinked noncomplementation (3–7) are examples of gene interaction that have been defined in yeast, Drosophila, and mice, to name a few. In humans, on the other hand, most genetic diseases appear to follow simple Mendelian inheritance patterns [e.g., Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), cystic fibrosis, etc.] or are clearly multifactorial in nature (e.g., cancer, cardiovascular disease). Owing to the rarity of most human genetic diseases, as well as the obvious inability to perform classical genetic breeding experiments, interactions between different human disease genes are difficult to study. Here we present evidence for gene interactions between mutant dystrophin alleles, which generally cause DMD or BMD, and the as yet unidentified mutant allele for Fukuyama congenital muscular dystrophy (FCMD).

DMD is one of the most common human neuromuscular genetic diseases of young males with an incidence of 1/3500 (8, 9). Both DMD and the milder allelic variant BMD are caused by mutations in the X chromosome-linked gene for dystrophin, a membrane-associated cytoskeletal protein that is generally undetectable in boys with DMD and is altered in patients with BMD (reviewed in ref. 10). Dystrophin is expressed in skeletal, cardiac, and smooth muscle as well as in Purkinje cells in the cerebellar cortex and cerebral cortical pyramidal neurons (11, 12). Based on sequence homology to α-actinin and the spectrins, dystrophin has a putative actin-binding domain at the N terminus (13) and a C-terminal domain that may also interact with other components of the membrane cytoskeleton (14).

Dystrophin abnormalities are considered specific for DMD/BMD; however, in the process of screening patients with various neuromuscular diseases, a few unusual cases of dystrophin abnormalities were found (15–17). One patient with a clinical diagnosis of FCMD was previously shown to have no detectable dystrophin in his skeletal muscle biopsy (16). Since then, two more FCMD patients with dystrophin abnormalities have been found (ref. 17; this work), suggesting that these coincident findings are not merely the result of chance.

FCMD is a rare autosomal recessive disorder endemic to Japan with an incidence of 6.9–11.9 per 100,000 births (reviewed in ref. 18). Both FCMD and DMD result in muscular weakness with dystrophic muscle pathology; however, the phenotypes of these diseases are quite distinct. The former presents with hypotonia and weakness at birth or early infancy and patients often never walk well (18). In contrast, DMD usually first manifests between the ages of 4 and 5 years and patients usually walk until they are 11–13 years old (8). Furthermore, FCMD is characterized by profound mental retardation and polymicrogyria of the cerebrum and cerebellum. In contrast, while up to one-third of DMD patients suffer from some cognitive deficits (8), no consistent central nervous system (CNS) pathology has been detected (19) despite the presumed absence of dystrophin in the postsynaptic membranes of cerebral and cerebellar cortical neurons (12).

In this report, we present a hypothesis to explain why some patients with the clinical phenotype of FCMD have underlying dystrophin abnormalities. We propose that these patients are FCMD heterozygotes who also have DMD and that, in the absence of dystrophin, reduced levels or activity of the FCMD gene product are detrimental, resulting in a phenotype more severe than expected for this genotype.

MATERIALS AND METHODS

Patient Selection and Analysis. FCMD patients in this study were referred to the National Institutes of Neuroscience,
Japan, where clinical diagnoses were made by standard criteria (18, 20). Significant features include either sporadic occurrences or a family history compatible with autosomal recessive inheritance; CNS manifestations, including moderate to severe mental retardation and occasionally seizures (&gt;50% of patients); and muscle hypotonia and weakness since early infancy with delayed motor milestones and dystrophic muscle pathology, including fiber necrosis and regeneration with dense fibrosis. Facial muscles are frequently involved and joint contractures appear in ~70% of cases. Of &gt;3500 biopsies available, samples from 37 patients with an original clinical diagnosis of FCMD (23 male, 14 female) were assayed for dystrophin by indirect immunofluorescence and/or Western blotting as described (15-17). DNA analysis was performed by polymerase chain reaction (21) and/or Southern blotting and hybridization with dystrophin cDNA probes (22, 23).

Case Histories of Dystrophin-Negative FCMD Patients. Patient 52 is a 5-year-old male product of a normal pregnancy who presented with hypotonia at birth and growth and developmental delay in the postnatal period. There was no family history of neuromuscular disease. He first walked at 2.8 years of age, at which time his developmental quotient was 65. Currently, he is mildly hypotonic with facial muscle involvement consistent with FCMD, has hip contractures, and cannot walk or sit alone. His serum creatine kinase level was 8387 units/liter (normal = 12-75), an electromyogram was consistent with myopathy, and muscle biopsy showed dystrophic changes. A computerized tomography scan revealed diffuse corticomedullary atrophy and ventricular dilatation. Polymicrogyria, agyria, and pachygyria were not identified on a magnetic resonance imaging scan, and an electroencephalogram demonstrated background slowing.

Patient 109 is a 6-year-old male with no family history of neuromuscular disease who was noted at birth to be hypotonic. Good head control was delayed until 9 months of age, standing was at 18 months, and walking was at 20 months. He lost the ability to walk by 5 years of age due to muscular weakness and hip and ankle contractures. Currently, he is hypotonic, unable to stand, has facial muscle involvement, and is unable to speak in sentences. His serum creatine kinase was 9650 units/liter, an electromyogram was consistent with myopathy, and muscle biopsy showed features of muscular dystrophy. A computerized tomography scan revealed diffuse cortical atrophy, and an electroencephalogram revealed background slowing but no epilepticiform activity.

Patient 137 is a 15-year-old male who was hypotonic at birth with subsequent developmental delay. Walking was delayed until 3 years of age and he required a wheelchair by the time he was 7 years old. His first words were at 3.5 years and he used sentences at 4.5 years of age. Currently, he has generalized hypotonia, muscular atrophy and weakness with slight facial muscle involvement and multiple joint contractures. Serum creatine kinase levels were 799 and 1061 units/liter, an electromyogram was consistent with myopathy, and a biopsy (at 13 years of age) showed severe dystrophic changes. A computerized tomography scan revealed corticomedullary atrophy and ventricular dilatation. Magnetic resonance imaging showed no evidence of polymicrogyria, agyria, or pachygyria, and an electroencephalogram revealed a slow background. An elder brother with similar signs and symptoms died at 14.3 years of age. Postmortem examination showed no polymicrogyria, agyria, or pachygyria. Although these brothers fit the clinical criteria for FCMD, their pathological diagnosis is atypical FCMD or some other congenital muscular dystrophy with CNS manifestations.

Incidence Calculations. Frequencies of alleles, genotypes, phenotypes, and sex ratios were calculated assuming Hardy-Weinberg equilibrium, an incidence of dystrophin abnormalities of 1/3500 males (9), and a FCMD incidence of 1/10,000 (18). These calculations disregard the observation of a high rate of consanguineous marriage among the parents of patients with FCMD (18), which would cause us to slightly overestimate the allele frequency and carrier rate in the general population. Assuming interaction between an autosomal and an X-linked locus, the frequency (incidence) of a disease phenotype \( f_0 \) that is presumed to have an autosomal recessive mode of inheritance is equal to the sum of the frequency of homozygosity at the autosomal locus and the frequency of heterozygosity at the autosomal locus and hemizygosity for the X-linked disease-associated allele.

Therefore,

\[
f_D = f_a^2 + 2f_a(1 - f_a)(f_x/2),
\]

where \( f_x \) is the frequency of the autosomal disease-associated allele and \( f_a \) is the frequency of the X-linked disease-associated allele. For example, given that the incidence of FCMD \( f_0 \) is \( 1 \times 10^{-4} \) and the incidence of DMD in males \( f_a \) is \( 2.86 \times 10^{-4} \), the allele frequency for the FCMD mutation \( f_0 \) is estimated to be \( 9.86 \times 10^{-5} \). The proportion of male FCMD patients who are affected because they are FCMD heterozygotes and DMD hemizygotes is

\[
2f_0(1 - f_a)(f_a/2)/(f_0 - f_x/2),
\]

or \( \approx 5.4% \).

RESULTS

Dystrophin Abnormalities in Patients with FCMD. In the course of screening patients with various neuromuscular diseases for dystrophin abnormalities, it was discovered that a single patient (patient 52), of five with clinical diagnoses of FCMD, had no detectable dystrophin by either indirect immunofluorescence or Western blotting of his muscle biopsy (16). Subsequently, biopsies from 31 more patients were examined and another (patient 109) was found to have no detectable dystrophin (17). We have since examined one more patient (patient 137) who also had no detectable dystrophin on Western blots despite having clearly detectable myosin levels on the posttransfer gels (Fig. 1). Indirect immunofluorescence for dystrophin in patient 137 was remarkable for low levels of patchy staining with antibodies against the central portion of the protein and no detectable staining when antibodies against the C terminus were used (data not shown). Due to the extensive fibrosis that is a hallmark of FCMD (18), biopsies from many patients with FCMD appear to have reduced levels of dystrophin, but after normalizing for muscle-specific myosin, most FCMD patients clearly have significant levels on Western blots (e.g., patients 53 and 54 in Fig. 1). Furthermore, immunocytochemical analysis of these dystrophin-positive biopsies demonstrated that both dystrophin and spectrin were present in an

![Fig. 1](https://example.com/fig1.png)

Western blot analysis of dystrophin in muscle biopsies from selected patients with FCMD. Numbers refer to patient numbers from text; N, normal controls. (Upper) Western blot stained with anti-30-kDa antibodies (11). The band corresponding to dystrophin is indicated (Dys.). (Lower) Portion of the posttransfer polyacrylamide gel illustrating relative levels of muscle-specific myosin.
occasionally patchy and fluffy pattern with many positive and some negative fibers, suggesting nonspecific alterations due to general membrane defects (17). All the dystrophin-deficient patients are male (e.g., 3/23), while none of the 14 females studied had any detectable dystrophin abnormalities.

To examine whether the lack of dystrophin in these patients was a primary or secondary effect of their disease, we studied their dystrophin genes directly. Patient 52 has an intragenic deletion of exons 51–54 (Fig. 2), which is predicted to cause a frameshift of protein translation resulting in no detectable dystrophin (23). No deletion was detected by polymerase chain reaction analysis of DNA from patient 137 (data not shown); however, this is not surprising in light of the fact that only 65% of DMD patients have detectable deletions (22, 24). DNA from patient 109 was unavailable for study.

**DISCUSSION**

The absence of dystrophin in some patients with clinical diagnoses of FCMD was intriguing because dystrophin deficiency is considered specific for DMD and BMD (reviewed in ref. 10). The simplest explanation for these findings is that the three dystrophin-deficient patients have both DMD and FCMD. This might be expected to occur with a frequency of 1 in 3.5 × 10^7 males in the Japanese population or 1 in 3500 males with FCMD. However, the observed frequency of dystrophin abnormalities in our sample (3/23 males with FCMD) appears to be too high to accept this proposal. The probability of obtaining 3 or more dystrophin abnormalities in 23 FCMD patients by chance alone is ≈1.1 × 10^-7.

A second possible explanation involves the difficulty of establishing an accurate diagnosis without a biochemical or genetic marker for FCMD. Maternal, environmental, genetic, or chance factors could cause variable expressivity of dystrophin abnormalities such that our three patients represent extremes in the DMD/BMD spectrum. Although difficult to address, we believe this hypothesis unlikely given the high observed incidence of unusual presentations in our sample (3/37 FCMD patients) and the lack of similar findings among non-Japanese patients with congenital muscle disorders (ref. 15; E. Hoffman and L.M.K., unpublished data).

Since the clinical presentations of patients 52, 109, and 137 bear a striking resemblance to FCMD, a disorder that is relatively common among the Japanese, yet have the biochemical features of DMD, we suggest a third explanation—namely, that they may be heterozygous for FCMD and hemizygous for dystrophin abnormalities. This genotype could conceivably result in a phenotype that has an earlier onset and is more severe than that usually associated with dystrophin deficiency such that it resembles FCMD. Given an incidence of 10^-3 for FCMD, the carrier frequency is predicted to be 2 × 10^-2 (or = 1.3 × 10^-2 when consanguinity is accounted for) (18). Thus, as many as 1/50 Japanese males with DMD/BMD will also be carriers for FCMD. If we assume that FCMD heterozygotes with DMD/BMD have the phenotype of FCMD, then ≈5.4% of male FCMD patients will have dystrophin abnormalities (see Materials and Methods and Fig. 3A). Given our observation that 3/23 FCMD males have abnormal dystrophin, the relative odds in favor of this frequency (i.e., 5.4% or 1/18.5) over the null hypothesis (1/3500) are ≈2.2 × 10^-3. If the high rate of consanguinity [up to 27% (18)] is taken into account, the estimated carrier rate is lower, thus increasing the estimate of the proportion of male FCMD patients with dystrophin abnormalities.

The three patients with dystrophin abnormality all carried an original clinical diagnosis of FCMD, yet two have some feature that is atypical for FCMD. Patient 52 has an atrophic brain, as assessed by computerized tomography scan; however, magnetic resonance imaging examination did not reveal evidence of corticogyrally defects. Patient 137 had a similarly affected brother whose brain did not have pachygyria or polymicrogyria on autopsy. Although the muscular dystrophy was congenital in these cases, the CNS involvement is apparently milder than typical FCMD; yet it is clearly more severe than found in DMD. Furthermore, a previous report (25) described a Japanese family in which one brother had classical FCMD and another had DMD with unusually severe CNS manifestations, including severe mental retardation, slight cortical atrophy on computed tomography scan, and rare sporadic polyspike wave complexes in both frontal areas on electroencephalogram. These observations are consistent with the idea that dystrophin deficiency, combined with heterozygosity for FCMD, might yield an intermediate phenotype. Thus, the presence of these dystrophin-deficient FCMD patients (estimated to be 1/175,000 Japanese males) may explain some of the clinical and pathological variability among patients with presumptive diagnoses of FCMD (18).

Our explanation for the high frequency of dystrophin abnormalities in patients with FCMD is somewhat analogous to the phenomenon of unlinked noncomplementation that has been demonstrated in *Drosophila melanogaster* (5, 6), *Caenorhabditis elegans* (3), Saccharomyces cerevisiae (4), and, most recently, *Mus musculus* (7). In both *Drosophila* and yeast, one mechanism for noncomplementation involves interacting proteins as proven by the demonstration that recessive mutations in α-tubulin fail to complement recessive β-tubulin mutations in double heterozygotes (4, 5). In other words, the combination of heterozygosity at both loci gives a phenotype similar to that caused by homozygosity at either locus. Johnson (26) has also proposed a similar mechanism in humans, termed metabolic interference, to explain unusual patterns of inheritance. In this model, heterozygosity at one or more loci may be more harmful than homozygosity because of deleterious interactions between normal and mutant proteins. One well characterized example of gene interaction in human disease is the effect that α-globin gene mutations have on patients with β-globin mutations. β-Thalassemia homozygotes who are heterozygous for α-thalassemia have a more balanced ratio of globin chain synthesis and a milder phenotype (27), while α-globin gene triplications exacerbate

**FIG. 2.** Dystrophin gene deletion analysis in patient 52. Southern blots of genomic DNA from a control (lanes 1 and 3) and patient 52 (lanes 2 and 4) digested with HindIII and probed with dystrophin cDNA probes 7–8 (lanes 1 and 2) and 9–10 (lanes 3 and 4). Relevant exon numbers are indicated on the right.
FIG. 3.  Relationship between disease frequency of an apparent autosomal recessive disorder and the proportion of male patients who are affected because they are heterozygous for the recessive disorder and hemizygous for an X-linked defect (assuming a model of intergenic interaction as described in the text). (A) The frequency of X-linked abnormalities in males affected with an apparent autosomal recessive disorder is inversely related to the frequency of the recessive disorder. Shown is the relationship assuming the frequency of the X-linked disorder to be 1/3500 male births as in DMD. (B) Varying the frequency of the X-linked disorder demonstrates that the proportion of males affected because of X-linked disease is directly related to the incidence of the X-linked disorder. The four curves represent different X-linked disease frequencies in males chosen to be comparable to representative diseases; ○, 1/60,000 (factor IX deficiency); △, 1/7500 (factor VIII deficiency); ●, 1/3500 (DMD); □, 1/1500 (fragile X).

The otherwise benign phenotype of β-thalassemia heterozygotes (28).

By analogy to the unlinked noncomplementation seen for α- and β-tubulin alleles (4, 5), we suggest that dystrophin (also a cytoskeletal protein) and the normal FCMD gene product may physically interact in the myofibrillar and/or neuronal cytoskeleton. Dystrophin is localized at the inner surface of the plasma membrane and is associated with an integral membrane glycoprotein complex (14). In light of our hypothesis, it is interesting that freeze-fracture electron microscopy has shown that orthogonal arrays in the plasma membranes are smaller and contain fewer subunit particles in both DMD and FCMD (29, 30). Thus, both dystrophin and the FCMD gene product apparently play a role in maintaining these subcellular structures.

One interesting implication of our model is that phenotypes produced by other X-linked recessive genes may be modified by heterozygosity for mutations in any autosomally encoded proteins that interact with them, and, in fact, this may be one mechanism contributing to the clinical heterogeneity often attributed to differences in genetic background. The frequency of this phenomenon is related to the frequencies of the interacting genes. The proportion of males who are affected because they are hemizygous for an X-linked defect and heterozygous for an autosomal mutation is inversely proportional to the frequency of the autosomal disease and directly proportional to the frequency of the X-linked disease (Fig. 3B).

Another prediction is that these interactions will skew the sex ratio for disorders that are considered autosomal recessive traits. For example, for a relatively common disorder, such as FCMD in Japan, with an incidence of ~1/10,000, we would predict that approximately 51.4% of the FCMD patients would be male (Fig. 4A), and, in fact, this proportion would be higher if the high rate of consanguinity were taken into account (18). The reported male/female ratio of 1.1:1 (18) is therefore consistent with our hypothesis. For rarer disorders, we would expect a larger proportion of the patients to show X-linked defects and, therefore, more unbalanced sex ratios. For instance, if an autosomal recessive disorder with a frequency of 1/10⁶ interacts with DMD, we would expect 62% of these patients to be male. As the frequency of

FIG. 4. Effect of disease frequency on the sex ratio of the affected population assuming a model of intergenic interaction as discussed in the text. (A) The degree of male preponderance is inversely related to the incidence of the recessive disorder. The X-linked disease frequency is 1/3500 as in DMD. (B) The degree of male preponderance is directly related to the incidence of the X-linked disorder. The four curves represent different X-linked disease frequencies as described in Fig. 3B.
the X-linked disorder increases, the proportion of affected males also goes up (Fig. 4B). This model also implies that if more than one autosome locus is associated with the autosomal recessive phenotype, a larger proportion of patients would be expected to be male, and more of these males would have dystrophin abnormalities.

There have been suggestions of a male predominance in some forms of autosomal recessive spinal muscular atrophy (SMA) (31, 32), a disease with an incidence of 4–6 × 10−5 (9). Further support for the idea that there may be some interaction between dystrophin abnormalities and the SMA gene product is our previous observation of a smaller dystrophin protein in a male patient with an ambiguous clinical picture intermediate between SMA type III and BMD (patient 4 in ref. 15). This patient had an older brother who carried the diagnosis of SMA, type III, suggesting that one brother was homozygous for SMA while the other may have been a heterozygote with BMD.

Finally, our hypothesis that dystrophin and other disease-related gene products (e.g., FCMD) may interact can be tested by mating dystrophin-deficient mice with various autosomal recessive mouse neuromuscular mutants. This approach has already been successfully used to identify interacting muscle genes in Drosophila (33). In this way, we hope to identify mice with mutations in proteins that interact with dystrophin by the increased severity of the phenotype associated with a hemizygous dystrophin mutation. Hopefully, the eventual identification of the FCMD gene defect will allow direct confirmation of our hypothesis.

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