Prenatal diagnosis of Fukuyama-type congenital muscular dystrophy by microsatellite analysis

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We applied microsatellite analysis to prenatal diagnosis of Fukuyama-type congenital muscular dystrophy (FCMD), an autosomal recessive severe muscular dystrophy associated with brain malformations. Recent identification of the FCMD gene locus at 9q31–q33 provided the basis for prenatal diagnosis and carrier detection. We recently developed new microsatellite markers which are closer to the FCMD gene and improved the phenotype probability. Nine fetuses in eight unrelated FCMD families, including a twin pregnancy, were analysed using the newly developed markers. Four fetuses showed over 99% probability of being healthy either as normal homozygote (n = 1) or heterozygote carrier (n = 3) and were born without signs of FCMD. The other five fetuses were diagnosed with a probability of FCMD of 99% or greater; all of the latter parents decided to terminate the pregnancies. Brain malformations characteristic of FCMD in one of the aborted fetuses confirmed the diagnosis of FCMD at 19 weeks of gestation.

Key words: brain malformation/Fukuyama-type congenital muscular dystrophy (FCMD)/polymorphism analysis/prenatal diagnosis

Introduction

Fukuyama-type congenital muscular dystrophy (FCMD) is a severe autosomal recessive muscular dystrophy associated with brain malformations, most prominently cerebral and cerebellar cortical dysplasia (Fukuyama et al., 1981). This disorder is the second most common form of muscular dystrophy in Japan, where 1 in 100 persons is presumed to be a heterozygous carrier (Fukuyama and Ohsawa, 1984). Several Caucasian families with typical FCMD have also been reported (Peters et al., 1984). Clinical manifestations of the disease are: generalized hypotonia and weakness are present in infancy, followed by marked muscle atrophy, joint contractures and psychomotor developmental delay in childhood. Upright ambulation, even with support, is attained only rarely. Intellectual, cognitive and communicative functions always are moderately delayed. The clinical course is inexorably progressive, with an average age at death of 16 years. No treatment is available.

Toda et al. (1993) first succeeded in mapping the FCMD locus to 9q31–q33 by genetic linkage analysis and further narrowed the locus to a region of approximately 1 Mb containing a microsatellite marker, D9S306 (Toda et al., 1994). The definition of closely linked, highly informative markers flanking the FCMD locus provided the basis for prenatal diagnosis; successful prenatal diagnosis by polymorphism analyses with those markers including D9S306 was reported recently (Kondo et al., 1996). Toda et al. (1996) further developed new microsatellite markers even closer (<200 kb) to the FCMD gene. Here we report the prenatal diagnosis of nine fetuses from eight families by polymorphism analyses using the newly developed microsatellite markers, attaining at least 99% certainty.

Materials and methods

FCMD families (summarized in Table I)

We were consulted by eight: couples requesting prenatal diagnosis after a second or third child was conceived. Following nondirective genetic counselling according to the Prenatal Diagnosis Guidelines of the Japan Society of Human Genetics, during which the options available were fully discussed, the parents chose prenatal diagnosis. Affected individuals were diagnosed with FCMD according to standard clinical criteria (Fukuyama et al., 1981).

Birthplaces of these FCMD patients and their parents were distributed throughout Japan, except for family 6, in which the father was a Caucasian born in the USA. The FCMD patient in family 4 was the offspring of a consanguineous marriage. In family 6, ultrasonographic examination showed that the fetuses were diamniotic dichorionic twins.

Procedures in prenatal diagnosis

DNA was extracted from peripheral blood leukocytes of FCMD patients, their siblings and parents according to the standard techniques. Amniocentesis was performed under ultrasonography at 14–16 weeks of gestation (Table I). The amniotic fluid obtained was divided in half. DNA was extracted directly from one half and from cultured amniotic cells grown from the other half. Haplotypes of all samples from fetuses and their relatives were analysed using polymorphic microsatellite markers located at 9q31, as described below.

Polymorphism analysis by microsatellite markers

We used four polymorphic microsatellite markers, previously mapped to 9q31. The markers/loci used included D9S127 (Lyall et al., 1992), D9S306 (Weber, 1993), D9S2105 (Toda et al., 1996), D9S2107 (Toda et al.,
et al., 1996), D9S172 (Gyapay et al., 1994), D9S299 (Cooperative Human Linkage Center, 1994), and D9S58 (Kwiatkowski et al., 1992). The FCMD locus is considered to lie within a few hundred kilobases located between D9S2105 and D9S2107 (Kobayashi et al., unpublished data). PCR primer sets for these markers were synthesized. Conditions for PCR and subsequent electrophoresis were as previously described (Toda et al., 1993, 1994).

Based on genotypes, a most likely linkage phase was deduced, haplotypes were constructed, and the fetal phenotypes were estimated.

**Results**

DNA extraction was performed either on uncultured amniotic cells or on cultured cells 2 weeks later. The majority of direct analyses on uncultured amniotic cells were informative. However, in cases 4 and 5, in which amniocentesis was performed at 15 and 14 weeks of gestation, respectively, direct analyses were not successful because of contamination by maternal blood cells.

The haplotypes of the family members and fetuses were analysed with polymorphic microsatellite markers flanking the FCMD locus at 9q31. The markers D9S127, D9S306, D9S299, and D9S58 were used for families 1 and 2 (Figure 1). The FCMD gene is presumed to lie between D9S306 and D9S299 loci. After development of the two closer markers, D9S2105 and D9S2107 (Toda et al., 1996), these were used for prenatal diagnosis in families 3–8 (Figure 1). The FCMD gene is believed to lie between D9S2105 and D9S2107 loci. As summarized in Table I, the fetuses in families 4, 5, and 8 were diagnosed as heterozygote carriers. The fetus in family 7 was diagnosed as a normal homozygote. The parents were informed of the results at 16–18 gestational weeks. The babies in families 4, 5, and 7 were born without signs of FCMD. In family 8, an uneventful pregnancy is ongoing. The fetuses in families 1, 2, 3, and 6 were diagnosed as affected with FCMD, and the parents in families 1, 2, and 6 opted for termination. One pregnancy, in family 3, was complicated with threatened abortion after uneventful amniocentesis at 16 weeks of gestation, resulting in fetal demise at 19 weeks. Maternal gravidity (G) and parity (P) are also included in Table I.

Autopsy of the family 2 fetus was performed with the consent of the parents. On macroscopic examination of the brain, multiple small granular protrusions were noted over the cerebral surface (not shown). A coronal section of the left hemisphere revealed that the parietal cortex was extensively covered by a thick mantle of abnormal tissue (Figure 2A), described as neurogliomesenchymal because it contained neuronal, glial, and pial cellular elements (Nakano et al., 1996). The neuroglial elements in the neurogliomesenchymal tissue appeared to have migrated excessively through multiple breaches in the glia limitans (Figure 2B). Those pathological findings are characteristic of the fetal FCMD brain (Takada et al., 1987; Nakano et al., 1996).

**Discussion**

Technological advances continue to expand the number of genetic disorders that can be diagnosed prenatally. The definition of closely linked, highly informative microsatellite markers flanking a single gene defect can provide the basis for prenatal diagnosis and carrier detection of a disorder in families with at least one affected child (Blumenfeld et al., 1993). Toda et al. (1993) localized the FCMD locus on chromosome 9q31–q33, narrowing the range of possible mutation sites to within a few hundred kilobases between D9S2105 and D9S2107 (Toda et al., 1996). Kondo et al. (1996) first reported successful prenatal diagnosis of two families by haplotype analysis using four polymorphic microsatellite markers flanking the FCMD locus at 9q31. Considering the possibility that recombinations may have occurred at random in the parental alleles, they calculated phenotype probabilities in fetuses as at least 86%. Here we conducted prenatal diagnosis in eight unrelated FCMD families by a similar analysis using markers closer to the FCMD gene. Genetic distances between the FCMD gene and each marker are presumed to be, at most, 1 cM, 0.14 cM, 0.02 cM, and 0.28 cM for D9S306, D9S2105, D9S2107, and D9S172, respectively (Toda et al., 1996). Since the FCMD gene is presumed to lie between D9S2105 and D9S2107, the risk of misdiagnosis through double recombinations between the D9S2105 and FCMD and between FCMD and D9S2107 is calculated as 0.000028%. In other words, fetal phenotype probability is improved as much as >99% by the newly developed markers. Indeed, the fetuses diagnosed...
Figure 1. Application of polymorphic microsatellite markers flanking the Fukuyama-type congenital muscular dystrophy (FCMD) locus to prenatal diagnosis. Pedigrees and haplotype analyses of the eight families are illustrated. Filled shapes indicate affected individuals, and half-filled shapes indicate carriers. Values shown as haplotypes are PCR-product sizes. High-risk chromosomes predicted to be carrying an FCMD-causing mutation are in bold boxes. In families 1 and 2, the four markers used from top (centromere) to bottom (telomere) were D9S127, D9S306, D9S299, and D9S58, and the FCMD gene is presumed to lie between D9S306 and D9S299 loci. In families 3 to 8, the four markers used were D9S306, D9S2105, D9S2107, and D9S172, and the FCMD gene is presumed to lie between D9S2105 and D9S2107 loci.

Figure 2. Brain of family 2 fetus at autopsy. A, coronal section of the left hemisphere. The cerebral cortex in the parietal and temporal lobe was widely covered by a thick mantle of abnormal tissue (arrows). Bar, 10 mm. B, microscopic view of the cerebral cortex. The majority of cortical plate neurones have migrated beyond remnants of the fragmented glia limitans (arrows) in the prominently dysplastic cortex. Haematoxylin and eosin, original magnification ×25.
as heterozygote carriers were born without signs of FCMD, while the fetus diagnosed with FCMD showed brain malformations characteristic of an FCMD fetus (Table I, Figure 2).

The assignment of FCMD to 9q31 also opens a way to isolate the disease gene by the techniques of positional cloning, which ultimately may permit prenatal gene therapy for FCMD. Since pathological findings were remarkable before 20 weeks of gestation (Figure 2), treatment, if any, should be started early in first trimester of pregnancy (Edwards et al., 1995) or in the preimplantation period. Demand for prenatal diagnosis in FCMD families will continue to increase until effective treatment measures are established. In this situation as in others, ethical issues inherent in prenatal diagnosis and treatment should be considered carefully before widespread establishment of prenatal clinical genetics.

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References


