Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular disorder with an autosomal dominant trait, and its frequency is about one in 20,000. It is characterised by weakness and atrophy of the facial, shoulder girdle, and upper limb muscles. The pelvic girdle and lower limbs subsequently also become involved, and, eventually, 20% of patients have to use wheelchairs by the age of 40 years. Most patients develop clinical symptoms in late childhood or adolescence, although the onset of the disease and its clinical severity are heterogeneous.

The FSHD locus was mapped to the subtelomeric region of the long arm of chromosome 4 by genetic linkage analysis. More than 95% of patients with FSHD had a small (<35 kb) EcoRI fragment on chromosome 4q35 on southern blotting analysis with the probe p13E-11 (FSHD1A; MIM 158900). This EcoRI fragment contains tandem repeats of the 3.3 kb Kpnl unit (D4Z4). The number of D4Z4 repeats varies from 11 to 150 in healthy people, although the number is fewer than 11 in patients with FSHD1A. Although no responsible gene has been isolated within the FSHD region, the number of D4Z4 repeats is a critical determinant of the age of onset and clinical severity of the disease. In general, 1–3 D4Z4 repeats are associated with a severe form of the disease that presents in childhood, 4–7 repeats with the most common form of FSHD, and 8–10 repeats with a milder disease and reduced penetrance.

Probe p13E-11 crosshybridises with chromosome 10q26, which contains highly homologous 3.3 kb Kpnl repeat units. As the BglII restriction enzyme site exists exclusively within each unit derived from 10q26, but not in D4Z4 (a unit from 4q35), double enzyme digestion with EcoRI and BglII can discriminate between the 4q35 (BglII resistant) fragments and 10q26 (BglII sensitive) fragments. The highly homologous structure means that the subtelomeric interchromosomal translocation between chromosomes 4 and 10 occurs often (in about 20–30% of people) and has been suggested to contribute to deletion of Kpnl repeats on chromosome 4q35. The frequency of translocation, however, was not significantly different between healthy people and those with FSHD.

More complicatedly, some people have five EcoRI fragments in total, including one additional BglII resistant fragment. These people were suggested to have somatic mosaicism of the 4q35 region and two cell populations with different fragment sizes.

To clarify the frequency of the de novo mutation, the penetrance, and influence of the shortened repeats on clinical symptoms, we performed clinical and genetic analyses on patients with FSHD1A and both parents of each patient.

**PARTICIPANTS AND METHODS**

We extracted genomic DNA from peripheral blood lymphocytes with a standard technique after informed consent was obtained. We analysed 255 DNA samples, including samples from 85 Japanese patients with FSHD1A and both parents of each patient.

We used pulsed field gel electrophoresis (PFGE) and conventional gel electrophoresis to determine the size and chromosomal origin (chromosome 4 or 10) of each of the fragments. The DNA was double digested with EcoRI/HindIII and EcoRI/BglII for PFGE and with EcoRI and EcoRI/BglII for the conventional study. After we transferred the DNA to Hybond N’ (Amersham Biosciences, Tokyo, Japan), we performed overnight hybridisation at 65°C with the probe p13E-11, as described previously. We also used probe pMA13 to identify people with a deletion of the p13E-11 recognition site and with hybrid repeats that consisted of clusters of type 4 and type 10 Kpnl units. pMA13 is a 1.3 kb Stul digested fragment within the Kpnl unit. We scanned the hybridised membranes and stored the image data for densitometric analysis. We estimated the intensity of each fragment with densitometry and BAS2500 (Fuji Photo Film, Tokyo, Japan). For people with somatic mosaicism, we estimated the proportion of cells with a small EcoRI fragment by comparing the labelled intensity with the expected intensity.
We used the BglII/BlnI dosage test with the probe p13E-11, as reported previously, to detect the interchromosomal translocation. This method characterizes the first KpnI repeat as a BlnI resistant 4.0 kb (chromosome type 4q) fragment or a BlnI sensitive 1.8 kb (chromosome type 10q) fragment. We calculated the intensity ratio of the two bands and classified all people in accordance with the number of chromosomes with BlnI resistant (type 4q) KpnI units: nullsomy (N: two type 10q repeats on chromosome 4), monosomy (M: one type 10q repeat on chromosome 4), disomy (D: standard), trisomy (T: one type 4q repeat on chromosome 10), or quatrosoy (Q: two type 4q repeats on chromosome 10).

RESULTS
All 85 unrelated patients had clinical symptoms consistent with FSHD and a small EcoRI fragment of the 4q35 region <35 kb. The fragment sizes were 10–27 (mean 17.5) kb.

In 35/85 (41%) families, only the proband had a small BlnI resistant EcoRI fragment; these cases were suggested to be the result of de novo mutations (fig 1). Nineteen patients were men and 16 women. The size of the small EcoRI fragment varied from 10 to 27 (mean 15.2) kb. All but one proband had four EcoRI fragments (derived from both chromosomes 4 and 10). One patient had five EcoRI fragments that included a faint 10 kb fragment, and this was suggested to be somatic mosaicism. The BglII/BlnI dosage test showed that 5/29 (17%) of the de novo probands, including one patient with mosaicism, had one type 4 repeat on chromosome 10 (trisomy), and others had a standard disomic pattern (data not shown).

The remaining 50 patients were familial cases; one parent had a small EcoRI fragment of the same size as that of the proband. Twenty-three patients inherited a small EcoRI fragment from their father and 27 from their mother.

Surprisingly, 26 (52%) of the parents (11 fathers and 15 mothers) with a small EcoRI fragment had no clinical symptoms (fig 1). Overall, 17/85 (20%) of families had a parent with somatic mosaicism. The age range of parents with a small EcoRI fragment who were unaffected by the disease was 40–75 (mean 57.4) years at the time of examination, and the size of the short EcoRI fragment varied from 10 to 26 (mean 19.3) kb. The other parents who had a small EcoRI fragment (12 fathers and 12 mothers) had clinical symptoms consistent with FSHD. The age range of parents with symptoms of FSHD was 38–67 (mean 52.8) years, and the small fragment sizes ranged from 12 to 25 (mean 18.7) kb. Interestingly, no correlation was seen between the development of clinical symptoms and the size of the small EcoRI fragment (fig 2). One mother who had a 25 kb EcoRI fragment had weakness in the shoulders, but no muscle weakness was observed in one father with a 14 kb fragment.

Southern blotting analysis showed that 17/50 carrier parents with a deleted allele had five EcoRI fragments that included a fainter, small EcoRI fragment (fig 3). Double digestion with EcoRI and BlnI showed one more BlnI resistant fragment than was expected from the BglII/BlnI dosage test (data not shown). Densitometric analysis confirmed that the radiolabelling intensity of these small EcoRI fragments was fainter than expected and varied from 15% to 90%. These results suggest that 17/50 (34%) of parents with a small EcoRI fragment had somatic mosaicism of the 4q35 region and two cell populations with different combinations of EcoRI fragments. Five parents with mosaicism (three fathers and two mothers) had clinical symptoms consistent with FSHD, but 12 parents with mosaicism (six fathers and six mothers) were unaffected (fig 1). The size and intensity of a small EcoRI fragment were variable and did not correlate with the clinical features of the disease. A man with mosaicism with a 20 kb fragment was affected, while a man with a 10 kb fragment with mosaicism was asymptomatic (fig 2). In addition, a man with a 17 kb fragment in 90% of cells was unaffected, but women with the same sized fragment in 46% and 52% of cells had clinical symptoms consistent with FSHD (data not shown).

The interchromosomal translocation between 4q35 and 10q26 was seen in 4/13 (31%) people with somatic mosaicism. Three parents (one father and two mothers) had three type 4 repeats (trisomy), and one father had monosomy of a type 4 repeat (data not shown).

DISCUSSION
Facioscapulohumeral muscular dystrophy is a dominantly inherited common muscular dystrophy with a high occurrence of new mutations. Most patients with FSHD had a deletion of tandem repeated 3.3 kb KpnI units on chromosome 4q35, but the deletion mechanism of the repeat units is not known. Genetic analysis of 85 unrelated Japanese patients with FSHD1A and both parents of each patient by southern blotting analysis found that 41% of cases were the result of de novo mutations. A high proportion of de novo mutations may be caused by the specific structure of the region associated with FSHD on chromosome 4.

A possible consideration with respect to the shortened repeats is interchromosomal translocation between the subtelomeric region of chromosomes 4 and 10, which is observed in 20–30% of the healthy population. Frequently observed recombination implies a role for deletion of the 4q35 region; however, the ratio of translocation is similar between healthy people and patients with the disease. The translocation ratio in the 35 patients with de novo mutations in our study was not significantly different from that reported previously in healthy people. Further studies are needed to elucidate the exact role of interchromosomal translocation for the deletion of repeated units on 4q35.

Another possible cause is somatic mosaicism. Somatic mosaicism of the 4q35 region was seen in 15–20% of the healthy parents of patients with FSHD. Van der Maarel et al reported that the patient or an asymptomatic parent had somatic mosaicism in 40% of families with de novo cases of FSHD. On the other hand, only 3% of random blood donors have somatic mosaicism. In our study, 17/50 (34%) parents

![Figure 1](https://www.jmedgenet.com) Results of southern blotting analysis and clinical symptoms of 85 families of patients with FSHD1A. M, male; F, female.
with a deleted allele had somatic mosaicism of the 4q35 region, and 17/85 (20%) of patients with FSHD1A inherited a small EcoRI fragment from parents with mosaicism. These results suggest that somatic mosaicism is one of the major factors in the development of FSHD1A. A previous study showed that 46% of people with mosaicism had one or more BlnI resistant units on chromosome 10; that is nearly fivefold more frequent than in healthy people.17 In the present study, however, 3/13 (23%) of parents with mosaicism had type 4q BlnI resistant units on chromosome 10; this proportion was similar to that in healthy people.17

In the people with mosaicism, the size and intensity of a small EcoRI fragment were variable and did not correlate with the clinical features of the disease. This result may not be surprising, however, because somatic mosaicism occurs in the early embryonic stage, and the percentage of cells with

![Figure 2](image-url)  
**Figure 2** EcoRI fragment size of parents with a deleted allele.

![Figure 3](image-url)  
**Figure 3** Southern blotting analysis of two families with a proband with FSHD1A and a parent with somatic mosaicism. DNA was digested by EcoRI/HindIII (E/H) and EcoRI/BlnI (E/B) and separated by PFGE. The father in F180 (arrowhead) had three BlnI resistant EcoRI fragments (black arrows) that included a faint small (17 kb) EcoRI fragment. The affected son inherited the small fragment from his father. Fragments derived from chromosome 10 are shown with white arrows. The mother in F143 (arrowhead) had four BlnI resistant EcoRI fragments (black arrows) that included a faint 17 kb fragment and one BlnI sensitive EcoRI fragment (white arrow). This mother was confirmed to have one type 4 repeat on chromosome 10 (trisomy) by the BglII/BlnI dosage test. The affected son carried this 17 kb fragment. Alleles from chromosome Y are marked with asterisks.
deleted alleles will vary between tissues. In our study, no significant difference was seen between the sexes in the clinical symptoms in parents with mosaicism, although asymptomatic female carriers of mosaicism have been reported as predominant. 30–32

When we consider the penetrance of FSHD, our findings are important. Despite the clinical heterogeneity, even in the same family, patients with FSHD usually become symptomatic in the second decade of life. Penetration increases in an age dependent manner and has been estimated to be <5% for children aged 0–4 years, 21% for those aged 5–9 years, 58% for those aged 10–14 years, 86% for those aged 15–19 years, and 95% for those aged ≥20 years. 33

Another study showed that non-penetrance at the age of 60 years has been estimated as 2–5%. 27 From these studies, FSHD was suggested to be a highly penetrant disease. Our retrospective study, however, showed that penetrance was low, being estimated at 59% (excluding somatic mosaicism) and 48% (including somatic mosaicism). The number of mothers without mosaicism who were unaffected by the disease was higher than the number of fathers; however, no significant differences between sexes were seen in the numbers of affected parents and of parents with mosaicism. The size of small EcoRI fragments of unaffected parents was variable; the smallest fragment was 14 kb. This fragment was estimated to contain only two KpnI repeated units (1D4Z4), which generally causes severe phenotypes from childhood. Further clinical follow up studies of parents with a small EcoRI fragment who are unaffected by FSHD are needed. Penetrance, however, seems to be lower than previously reported. Even in random blood donors, 3–6% of people have FSHD sized type 4 repeat arrays. 16–20

The existence of asymptomatic people with a small EcoRI fragment strongly suggests the involvement of additional unknown factors in the development of clinical symptoms. Position effect variegation, which induces allele specific transcriptional repression of genes located centromerically, has been proposed as the molecular mechanism of FSHD. Recently, unexpected gene expression that was related inversely to the number of repeat units was reported in the muscles of patients with FSHD. 21 In addition, one of the two variants of the 4q subtelomere was reported to be associated uniquely with patients with FSHD. 22 Additional studies are needed to clarify the molecular pathomechanism of this complicated disease.

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