

ONLINE MUTATION REPORT

Very low penetrance in 85 Japanese families with facioscapulohumeral muscular dystrophy 1A

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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.¹ 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.²⁻⁴ 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).⁵⁻⁸ This *EcoRI* fragment contains tandem repeats of the 3.3 kb *KpnI* 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.⁶⁻⁸ 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.⁸⁻¹²

Probe p13E-11 crosshybridises with chromosome 10q26, which contains highly homologous 3.3 kb *KpnI* repeated units. As the *BlnI* 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 *BlnI* can discriminate between the 4q35 (*BlnI* resistant) fragments and 10q26 (*BlnI* sensitive) fragments.¹³ 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 *KpnI* repeats on chromosome 4q35.¹⁴⁻¹⁶ The frequency of translocation, however, was not significantly different between healthy people and those with FSHD.¹⁷

More complicatedly, some people have five *EcoRI* fragments in total, including one additional *BlnI* resistant fragment. These people were suggested to have somatic mosaicism of the 4q35 region and two cell populations with different fragment sizes.^{10 18-21}

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

Key points

- Facioscapulohumeral muscular dystrophy (FSHD) is a common autosomal dominant muscular dystrophy.
- Most patients with FSHD have fewer numbers of tandem repeated 3.3 kb *KpnI* units on chromosome 4q35 (FSHD1A), and southern blotting analysis with the probe p13E-11 shows a small *EcoRI* fragment (<35 kb).
- To clarify the deletion mechanism and influence of shortened repeats on clinical symptoms, we examined 85 Japanese unrelated patients with FSHD1A and both parents of each patient.
- In 35 (41%) families, only the proband had a small *EcoRI* fragment and these were suggested to be *de novo* mutations. In the remaining familial cases, somatic mosaicism of the 4q35 region was seen in 17/50 (34%) parents with a small *EcoRI* fragment. This suggests that deletion of the 4q35 region of the chromosome was generated often during mitosis and transmitted to the next generation.
- Although almost complete penetrance of FSHD is known, no clinical symptoms were seen in 26 (52%) parents who carried a small *EcoRI* fragment (including people with mosaicism) in this study.
- The high frequency of parents without the disease but with deletion of the 4q35 region implies the role of additional factors in the development of the clinical symptoms of FSHD.

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/BlnI* for PFGE and with *EcoRI* and *EcoRI/BlnI* 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.⁸ 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 *KpnI* units. pMA13 is a 1.3 kb *StuI* digested fragment within the *KpnI* 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.²² This method characterises 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 quatsomy (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

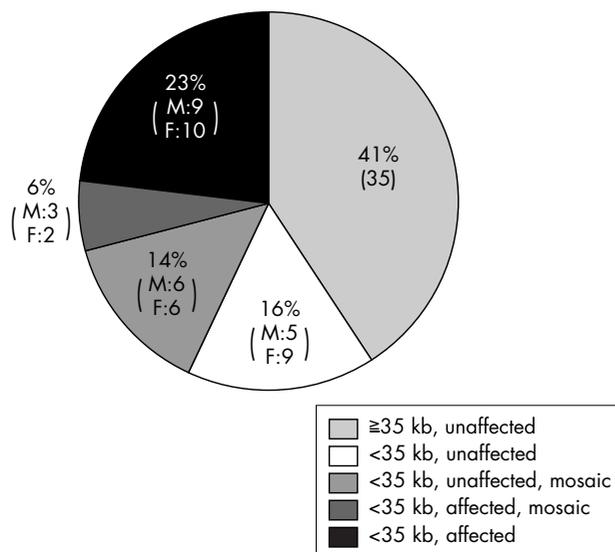


Figure 1 Results of southern blotting analysis and clinical symptoms of 85 families of patients with FSHD1A. M, male; F, female.

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). Densitometrical 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.^{1 10 11 23 24} 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.^{14 17} 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.¹⁷ 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.¹⁷ 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.^{1 20 23–25} 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.²¹ On the other hand, only 3% of random blood donors have somatic mosaicism.¹⁶ In our study, 17/50 (34%) parents

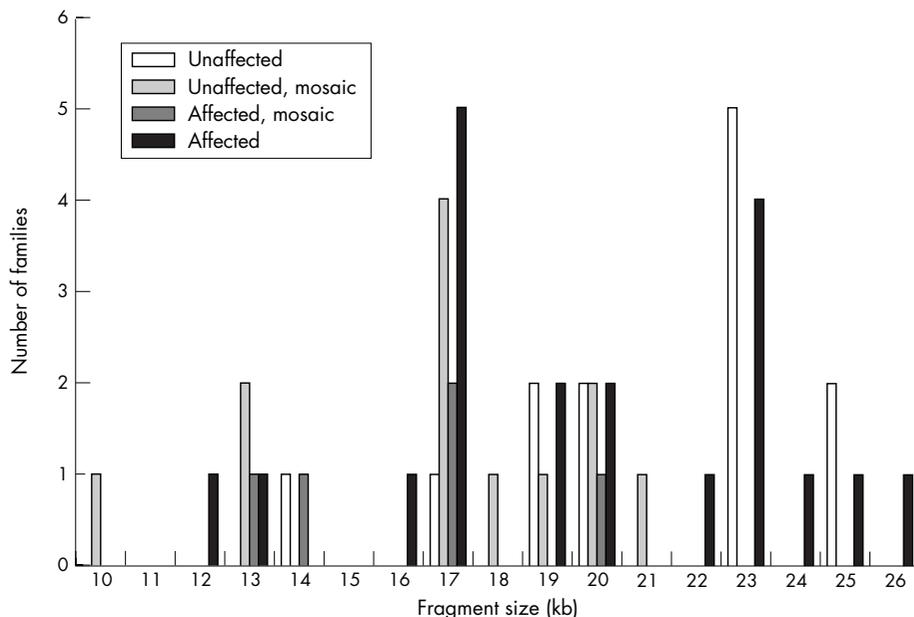


Figure 2 EcoRI fragment size of parents with a deleted allele.

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.²¹ 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

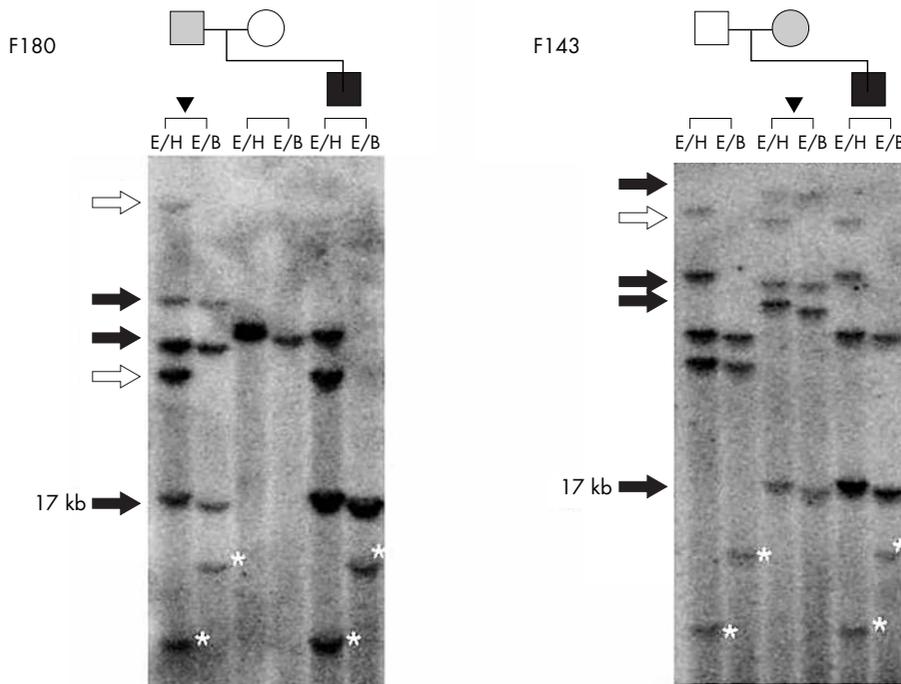


Figure 3 Southern blotting analysis of two families with a proband with FSHD1A and a parent with somatic mosaicism. DNA was digested by *EcoRI/HindIII* (E/H) and *EcoRI/BlnI* (E/B) and separated by PFGE. The father in F180 (arrowhead) had three *BlnI* resistant *EcoRI* fragments (black arrows) that included 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.^{10 21 25}

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. Penetrance 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.²⁶ 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 (D4Z4), 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.^{8 16 28} 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.²⁹ In addition, one of the two variants of the 4q subtelomere was reported to be associated uniquely with patients with FSHD.³⁰ Additional studies are needed to clarify the molecular pathomechanism of this complicated disease.

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