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Research article

Chromosome 4q;10q translocations; Comparison with different ethnic populations and FSHD patients

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Abstract

Background: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder characterized by the weakness of facial, shoulder-girdle and upper arm muscles. Most patients with FSHD have fewer numbers of tandem repeated 3.3-kb *Kpn*I units on chromosome 4q35. Chromosome 10q26 contains highly homologous *Kpn*I repeats, and inter-chromosomal translocation has been reported.

Methods: To clarify the influence on the deletion of the repeats, we surveyed three different ethnic populations and FSHD patients using the *Bgl*II/*Bln*I dosage test.

Results: The frequency of translocation in 153 Japanese, 124 Korean, 114 Chinese healthy individuals and 56 Japanese 4q35-FSHD patients were 27.5%, 29.8%, 19.3%, and 32.1%, respectively. The ratio of '4 on 10' (trisomy and quatrosomy of chromosome 4) was higher than that of '10 on 4' (nullsomy and monosomy of chromosome 4) in all populations.

Conclusions: The inter-chromosomal exchange was frequently observed in all four populations we examined, and no significant difference was observed between healthy and diseased groups.

Background

Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular disorder with an autosomal dominant trait. FSHD is characterized by weakness and atrophy of facial, shoulder-girdle and humeral muscles, with occasional subsequent pelvic-girdle and lower limb involvement. More than 95% of patients with FSHD have a smaller (< 35 kb) *Eco*RI fragment on chromosome 4q35 detected by probe p13E-11 and are called 4q35-FSHD [1–3]. This *Eco*RI fragment in normal individuals contains tandem repeated 3.3-kb *Kpn*I units (D4Z4) varying from 11 to 150 in number, while 4q35-FSHD patients showed less than ten units [2,3]. No responsible gene has been isolated within the FSHD gene region.

Probe p13E-11 cross-hybridizes with chromosome 10q26, which contains highly homologous 3.3-kb *Kpn*I repeated units. Since the *Bln*I restriction enzyme site exists

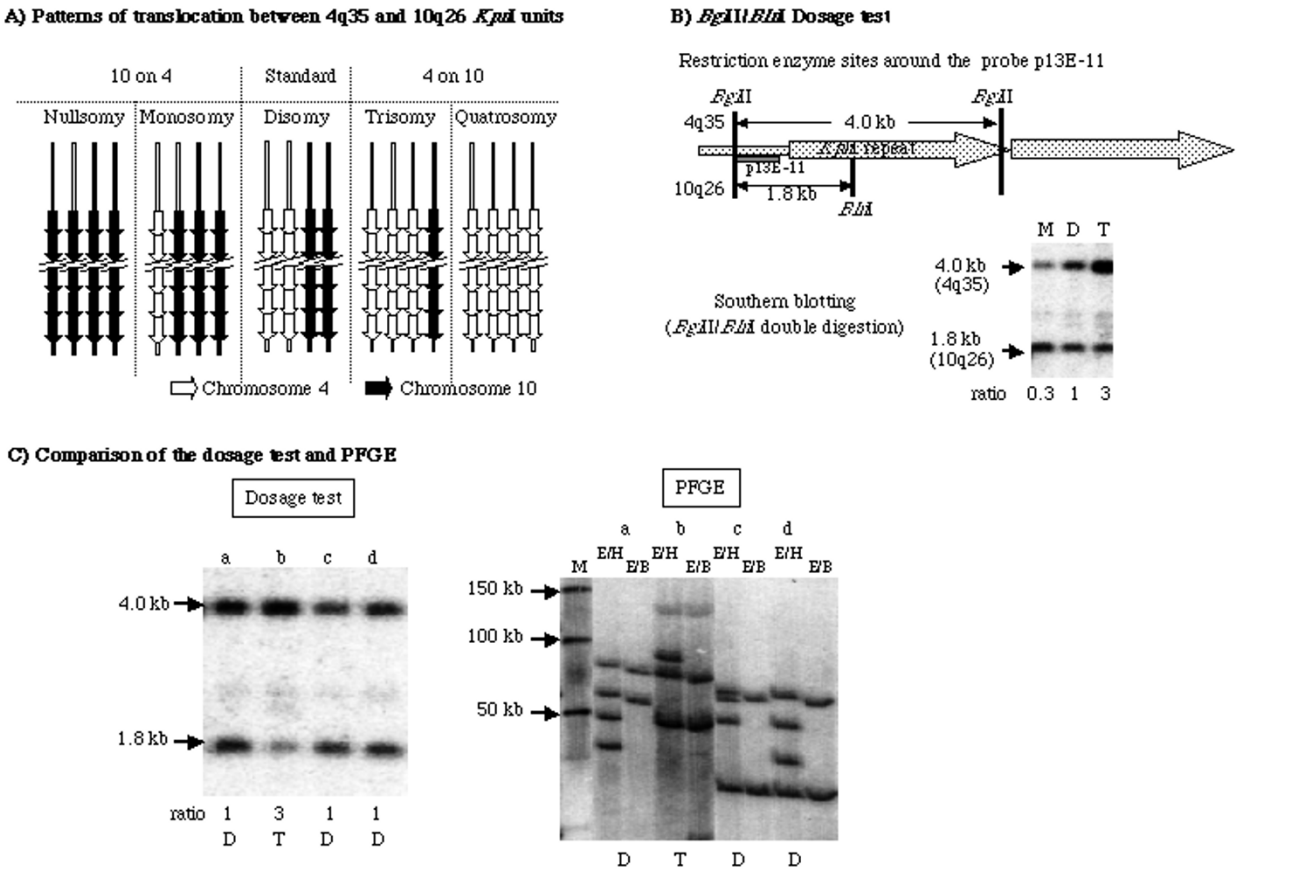

Figure 1

PFGE and the *Bgl*II/*Bln*I dosage test A) Patterns of translocation between chromosome 4q35 and 10q26 *Kpn*I units. Subtelomeric translocation changes the number of *Bln*I-resistant (from chromosome 4q35) and *Bln*I-sensitive (from chromosome 10q26) fragments. According to the number of units from chromosome 4, each individual is classified as nullsomy, monosomy, disomy, trisomy or quatrosomy. B) *Bgl*II/*Bln*I dosage test. Double enzyme digestion with *Bgl*II and *Bln*I characterizes the first *Kpn*I unit as a 4.0-kb fragment from chromosome 4q35 or a 1.8-kb fragment from chromosome 10q26. The ratio estimated from the intensity of the two fragments are; nullsomy (N) = 0 (0/4), monosomy (M) = 0.3 (1/3), disomy (D) = 1 (2/2), trisomy (T) = 3 (3/1), and quatrosomy (Q) = infinity (4/0). C) Comparison of the dosage test with PFGE. Thirty Japanese individuals were examined using both PFGE and the dosage test. The ratio from the dosage test was consistent with the results of PFGE in all samples. D: disomy, T: trisomy E/H: *Eco*RI/*Hind*III, E/B: *Eco*RI/*Bln*I, M: Marker

exclusively within each unit derived from 10q26, but not in D4Z4 (an unit from 4q35), double enzyme digestion using *Eco*RI and *Bln*I can discriminate as 4q35 (*Bln*I-resistant) and 10q26 (*Bln*I-sensitive) units [4]. In a Dutch control population, subtelomeric translocations between chromosomes 4 and 10 were seen in about 20% of individuals [5–7]. Furthermore, somatic mosaicism was found in 40% of *de novo* FSHD families and 46% of these individuals had *Bln*I-resistant units on chromosome 10 [8]. These findings imply that frequent recombination between the subtelomeric region of chromosomes 4 and 10 has some roles for deletion of the FSHD region. In this study, we examined the frequency of subtelomeric translocation among three different ethnic populations of Japanese, Korean and Chinese, and compared with Japanese 4q35-FSHD patients.

Methods

Blood samples were obtained with informed consent. Genomic DNA was extracted from peripheral blood lymphocytes using a standard technique.

The *Bgl*II/*Bln*I dosage test was accomplished using the probe p13E-11 as previously reported [9]. We examined 153 Japanese, 124 Korean and 114 Chinese healthy individuals, and unrelated 56 Japanese 4q35-FSHD patients.

Table: Results of the dosage test

	10 on 4		Standard	4 on 10			Exchange ratio (%)
	N	M	D	T	Q	Total	
Japanese	1	11	111	29	1	153	42/153 (27.5)
Korean	0	5	87	32	0	124	37/124 (29.8)
Chinese	0	6	92	16	0	114	22/114 (19.3)
4q35-FSHD	0	5	38	13	0	56	18/56 (32.1)
Dutch*	0	18	158	23	2	201	43/201 (21.4)

N: nullsomy, M: monosomy, D: disomy, T: trisomy, Q: quatrosomy * The findings from the Dutch population were estimated from the results of PFGE [7].

All individuals were classified into five groups according to the number of chromosomes with *Bln*I-resistant 4q-type *Kpn*I units; nullsomy (N: 0), monosomy (M: 1), disomy (D: 2), trisomy (T: 3) and quatrosomy (Q: 4) (Figure 1A). Less than 5% of individuals showed unexpected ratios of chromosome 4q;10q, and excluded from this study. The frequency of translocation in each group was estimated and statistical analysis was performed using the chi-square test. We also tested 30 Japanese healthy individuals using pulsed field gel electrophoresis (PFGE) to certify the results obtained by the dosage test. For PFGE, we used agarose embedded "plug" DNA samples [10].

Results

Results of PFGE and the dosage test

The results of PFGE were completely identical to those of the *Bgl*II/*Bln*I dosage test in 30 Japanese individuals examined (Figure 1C), and we used the dosage test for further analysis.

The frequency of 4q;10q translocations

The frequency of individuals having translocated *Kpn*I units in Japanese was 27.5% (42/153), Korean 29.8% (37/124), Chinese 19.3% (22/114), and 4q35-FSHD patients 32.1% (18/56) (Table). There was no significant difference between each population and the Dutch population reported previously [7]. No gender difference was observed in any population (data not shown).

The ratio of chromosome 10 with 4-type repeats (4 on 10) in Japanese was 19.6%, Korean 25.8%, Chinese 14.0% and 4q35-FSHD 23.2%. On the other hand, the ratio of chromosome 4 with 10-type units (10 on 4) in Japanese was 7.9%, Korean 4.0%, Chinese 5.3%, and 4q35-FSHD 8.9% (Figure 2). The frequency of '4 on 10' was higher than that of '10 on 4' in all populations examined, although the ratio of '4 on 10' and '10 on 4' were similar in the Dutch population [5,7]. We could not find any differences between healthy populations and Japanese 4q35-FSHD patients.

Discussion

The molecular size of *Eco*RI fragments on chromosomes 4 and 10 detected by probe p13E-11 varies markedly from 10 to 300 kb. Since fragments longer than 50 kb are difficult to detect by conventional Southern blot analysis, PFGE analysis using agarose embedded plug DNA is often necessary to identify all four fragments from chromosome 4 and 10. However, we cannot always obtain such high quality DNA samples. The *Bgl*II/*Bln*I dosage test used in this study is a useful and easy method to reveal translocation between chromosome 4 and 10, which characterizes the first *Kpn*I repeat unit as a *Bln*I-resistant 4.0-kb (chromosome 4-type) or a *Bln*I-sensitive 1.8-kb (chromosome 10-type) fragment. It should be noted, however, the dosage test cannot detect all inter-chromosomal exchanges, i.e., if one had exchanged *Kpn*I repeats at the distal portion following the standard repeats, this exchange will be missed and judged as standard. van Overveld *et al*. reported that approximately 4.3% (9 among 208) of individuals with standard first repeat showed a hybrid consisting of both *Bln*I-resistant and -sensitive repeats [7]. Therefore, the dosage test may underestimate the exchange ratio, although the present results of the PFGE and dosage tests were identical in 30 Japanese individuals. In the present study, less than 5% of individuals showed unclassified ratios of chromosome 4q;10q. These individuals may have complicated chromosomal rearrangements, or a deletion of the probe p13E-11 region as previously described [9]. The limitation of the densitometric analysis should be also considered. We are currently examining in detail on these individuals.

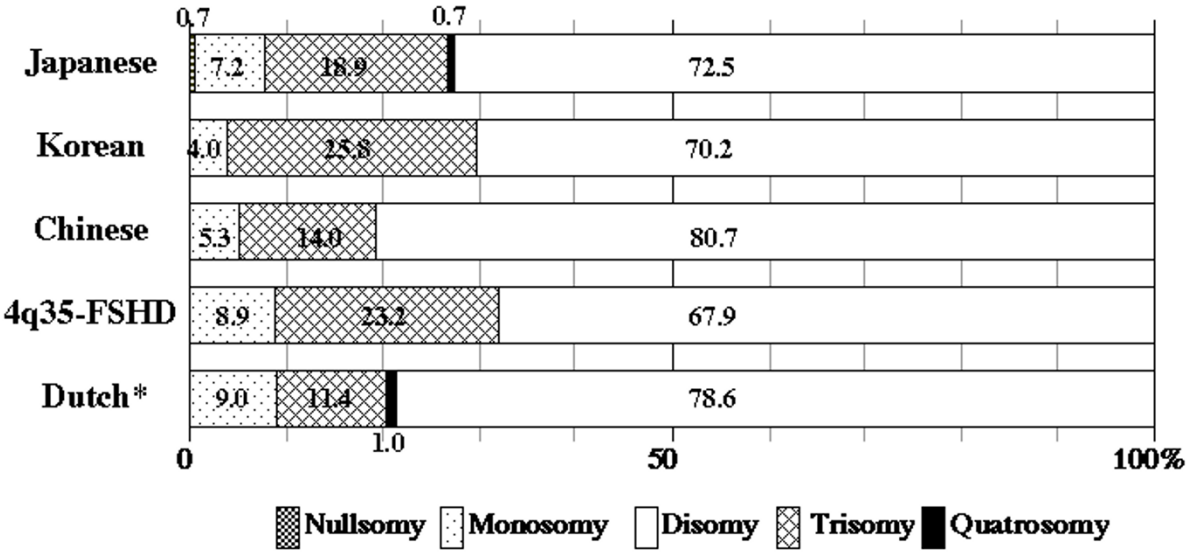

Figure 2
Frequency of the translocation between chromosomes 4q35 and 10q26 The 4 on 10 (trisomy and quatrosomy) is more frequently observed than 10 on 4 (nullsomy and monosomy) in all populations examined, although these were similar in the Dutch population. The findings from the Dutch population were estimated from the results of PFGE [7].

The subtelomeric exchange between chromosomes 4 and 10 was frequently observed in all four populations we examined, and their ratios were similar to the Dutch population previously reported [5,7]. The inter-chromosomal exchange may contribute to the deletion of*Kpn*I repeats on chromosome 4q35, although there was no difference between healthy and diseased individuals. The ratio of '4 on 10' (trisomy and quatrosomy of chromosome 4) was higher than that of '10 on 4' (nullsomy and monosomy) in all populations we examined. Translocations of chromosome ends have been reported to cause several disorders, such as alpha-thalassemia mental retardation syndrome, Wolf-Hirschhorn syndrome and Miller-Dieker syndrome. Further studies will be needed to clarify the influence of the subtelomeric exchange to the deletion of the repeated units on FSHD.

Conclusions

The frequency of translocation was 27.5% (Japanese), 29.8% (Korean), 19.3% (Chinese), and 32.1% (Japanese 4q35-FSHD patients). The ratio of '4 on 10' (trisomy and quatrosomy of chromosome 4) was higher than that of '10 on 4' (nullsomy and monosomy of chromosome 4) in all populations. In our study, there was no difference between healthy and diseased groups. Further studies will be needed to clarify the influence of the subtelomeric exchange to the deletion of the repeated units on FSHD.

Competing interests

None declared.

Authors' contributions

TM participated in the design of the study and performed the statistical analysis. KG and GY carried out the molecular genetic studies including the dosage test and PFGE. JHL and CZ obtained control DNA samples of Korean and

Chinese. YKH and KA conceived of the study, and participated in its design and coordination.

All authors read and approved the final manuscript.

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