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Evidence for subtelomeric exchange of 3.3 kb tandemly repeated units between chromosomes 4q35 and 10q26: implications for genetic counselling and etiology of FSHD1

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy, clinically characterized by asymmetric weakness of muscles in the face, shoulder girdle and upper arm. Deletion of an integral number of 3.3 kb repeated units within a highly polymorphic *Eco*RI fragment at chromosome 4q35, generating a relatively short *Eco*RI fragment (<35 kb), has been shown to cause FSHD1. Probe p13E-11 detects these short fragments in FSHD1 patients, and has therefore been used for diagnostic DNA analysis. However, the reliability of this analysis has been hampered by cross-hybridization of p13E-11 to chromosome 10q26-linked *Eco*RI fragments of comparable size, which also contain a variable number of 3.3 kb repeated units. Recently, a *Bln*I restriction site was identified within each of the repeated units derived from chromosome 10q26, which enables differentiation of the two polymorphic p13E-11 loci in most cases without haplotype analysis. Remarkably, applying the differential analysis to screen DNA of 160 Dutch cases referred to us for FSHD1 diagnosis, we obtained evidence for subtelomeric exchange of 3.3 kb repeated units between chromosomes 4q35 and 10q26 in affected and unaffected individuals. Subsequently, analysis of 50 unrelated control samples indicated such exchange between chromosomes 4q35 and 10q26 in at least 20% of the population. These subtelomeric rearrangements have generated a novel interchromosomal polymorphism, which has implications for the specificity and sensitivity of the differential restriction analysis for diagnostic purposes. Moreover, the high frequency of the interchromosomal exchanges of 3.3 kb repeated units suggests that they probably do not contain (part of) the FSHD1 gene, and supports position effect variegation as the most likely mechanism for FSHD1.

INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) is clinically characterized by progressive weakness of the facial, shoulder-girdle and upper arm muscles, and in some cases, also the abdominal, foot extensor and pelvic girdle muscles (1,2). The autosomal dominant inherited myopathy shows a variable phenotypic expression with a high penetrance of 95% at the age of 20, and an estimated prevalence of 1 in 20 000 (1,3).

Linkage analysis assigned the major FSHD locus (FSHD1) to chromosome 4q35 (4,5) distal to D4S139 (6–8). However, genetic heterogeneity was demonstrated for a small number of FSHD families, not linked to 4q35 (9–12). In patients, the FSHD1 locus on chromosome 4q35 is characterized by a DNA rearrangement within a highly polymorphic *Eco*RI fragment detectable using probe p13E-11 (D4F104S1) (13). In patients, these FSHD1-associated rearrangements generate *Eco*RI fragments which are shorter than 35 kb (13–17), while the size of the 4q35-linked *Eco*RI fragments in unaffected individuals varies from 35 to 300 kb (13). Analysis of the p13E-11 *Eco*RI fragments of four unrelated FSHD1 patients and controls revealed that the polymorphic fragment contains a variable number of 3.3 kb repeated units (D4Z4), a critical number of which is deleted in patients (18).

As short *Eco*RI fragments (<35 kb) linked to 4q35 are associated with FSHD1, probe p13E-11 has been used for diagnostic DNA analysis (17). However, a straightforward analysis is hampered by cross-hybridization of p13E-11 to another polymorphic locus which has recently been assigned to

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chromosome 10q26 (12,19). This locus is similarly characterized by *Eco*RI fragments containing a variable number of 3.3 kb repeated units. The size of these chromosome 10 fragments can be up to 300 kb, but also less than 35 kb in 10% of the population (12,17). Hence, short *Eco*RI fragments derived from 10q26 may incorrectly be interpreted as chromosome 4q35 deletion fragments. Haplotype analysis in FSHD families using chromosome 4q35 and 10q26 markers had to be performed to determine the chromosomal origin of a short (<35 kb) p13E-11 fragment (17). However, due to the presence of small families and isolated cases, only about 50% of short p13E-11 fragments could unambiguously be assigned to chromosome 4q35, and subsequently used for diagnostic purposes (17).

Recently, comparative sequence analysis of 3.3 kb repeated units derived from chromosomes 4q35 and 10q26 revealed the specific presence of a *Bln*I site within each repeated unit from chromosome 10q26. As this restriction site is not present in the repeated units from chromosome 4q35, differential restriction analysis using *Bln*I enabled direct differentiation of the chromosomal *Eco*RI fragments without haplotype analysis (19). By double digestion of genomic DNA with *Eco*RI and *Bln*I (or *Avr*II), the p13E-11 *Eco*RI fragments from 4q35 are reduced in size by 3 kb, while the *Eco*RI fragments derived from chromosome 10q26 are fragmented completely. This differential restriction analysis enabled presymptomatic and prenatal diagnosis to be performed with an improved efficiency and reliability.

Here, we report the screening of 160 independent Dutch familial or isolated cases referred to us for FSHD1 diagnosis, using the differential restriction analysis. In most cases an unequivocal diagnosis was achieved. Remarkably, a few individuals appeared to be 'monosomic' or 'trisomic' for the 4q35-linked p13E-11 fragment. Further analysis of these cases suggested the occurrence of subtelomeric exchanges of 3.3 kb tandemly repeated units between chromosomes 4q35 and 10q26, generating hybrid *Eco*RI fragments. As interchromosomal exchanges would have implications for the specificity and sensitivity of the diagnostic DNA testing, the frequency of such events in the population was determined by screening 50 unrelated control DNA samples.

RESULTS

Diagnostic application of differential restriction analysis

*Eco*RI and *Eco*RI/*Bln*I digested DNA of FSHD patients and family members was analyzed by conventional agarose gel electrophoresis and subsequent Southern blot hybridization using probe p13E-11. To illustrate the potency of the differential restriction analysis for diagnostic purposes, the results of family 87 (17) are shown in Figure 1. After *Eco*RI digestion only, probe p13E-11 detected a fragment of approximately 30 kb in affected as well as in some unaffected individuals. Haplotype analysis using chromosome 4q35 and 10q26 markers revealed that the 30 kb p13E-11 *Eco*RI fragment does not cosegregate with either the chromosome 4q35 or 10q26 haplotype (17) (Fig. 1). However, recently, differential restriction analysis using *Eco*RI and *Bln*I indicated heterogeneity of the 30 kb *Eco*RI fragments. In the affected individuals, the 30 kb *Eco*RI fragment was reduced in size by 3 kb indicating a chromosome 4 origin (Fig. 1, haplotype 'A'), whereas in the unaffected individuals the 30 kb *Eco*RI fragment was completely fragmented, revealing a chromosome 10 origin. The chromosomal origin of the 30 kb *Eco*RI fragments as suggested by the differential restriction analysis, was confirmed by haplotype analysis performed previously (17). Accordingly, an unequivocal diagnosis was achieved in this family.

In total, 160 independent Dutch familial or isolated FSHD index cases were screened using the differential restriction analysis. In 46 of 160 cases long (>35 kb) *Eco*RI fragments were observed only. Almost all of these 46 cases were clinically unclear, and referred for exclusion of FSHD1. So far, no unambiguously diagnosed FSHD patient without short *Eco*RI fragment was observed, which might carry a mutation in the FSHD1 gene itself, or be linked to a second FSHD locus. In 114 of 160 cases, *Eco*RI digestion revealed a short (<35 kb) p13E-11 fragment. By double digestion with *Eco*RI and *Bln*I, this short fragment was found to originate from chromosome 4 in 102 of 114 cases, indicating a high probability of FSHD1. Short *Eco*RI fragments completely fragmented by *Bln*I digestion, were detected in 12 of 114 individuals. Accordingly, these fragments were assumed to originate from chromosome 10q26, suggesting a lowered probability of FSHD1. However, at least three 4q35-linked familial and three isolated cases carrying such short, apparently chromosome 10q26 derived, *Eco*RI fragments, were clinically unambiguously diagnosed with FSHD (GWP). Although differential restriction analysis and clinical diagnosis implied that these cases either contain a mutation in the FSHD1 gene itself or are linked to a second FSHD locus, further analysis suggested another explanation (see below).

Subtelomeric exchange of repeated units

In applying the differential restriction analysis we observed several FSHD index cases and unaffected spouses which appeared to be either 'monosomic' or 'trisomic' for the p13E-11 fragment on chromosome 4q35. To demonstrate such aberrant *Bln*I restriction patterns, a very interesting family (family 28, Fig. 2a) was selected. In this family, a 25 kb *Eco*RI fragment was detected which cosegregates with FSHD1 and which was reduced in size by 3 kb after *Bln*I digestion (Fig. 2b). Therefore, a clear diagnosis was achieved. Remarkably, the father (individual 28.1) who is asymptomatic, also carries a second short *Bln*I-resistant *Eco*RI fragment (22 kb). As the father is transmitting both his chromosomes 4 and both his chromosomes 10 whereas the 22 kb fragment is not inherited by any of his children (Fig. 2a), he is considered somatic mosaic for this 22 kb fragment. In addition, the father strikingly showed two other *Bln*I-resistant, 4q35-like fragments. In his offspring, three *Bln*I-resistant *Eco*RI fragments were also detected in sons 28.4 and 28.5, while one *Bln*I-resistant *Eco*RI fragment only was observed in the youngest son 28.6. Moreover, the unaffected mother (individual 28.2) also shows one *Bln*I-resistant *Eco*RI fragment only. Pulsed-field gel electrophoresis was performed (Fig. 2b), which confirmed the apparent 'mono- and trisomy' observed by conventional gel electrophoresis. We hypothesized that an exchange of 3.3 kb repeated units between chromosomes 4q35 and 10q26 might have occurred, generating hybrid p13E-11 fragments (Fig. 3). In cases 28.1, 28.4 and 28.5, one of the chromosome 10q26 fragments contain repeated units without *Bln*I sites, probably derived from chromosome 4q35. In cases 28.2 and 28.6 one of the chromosome 4q35 fragments contains 10q26-like repeated units including *Bln*I sites.

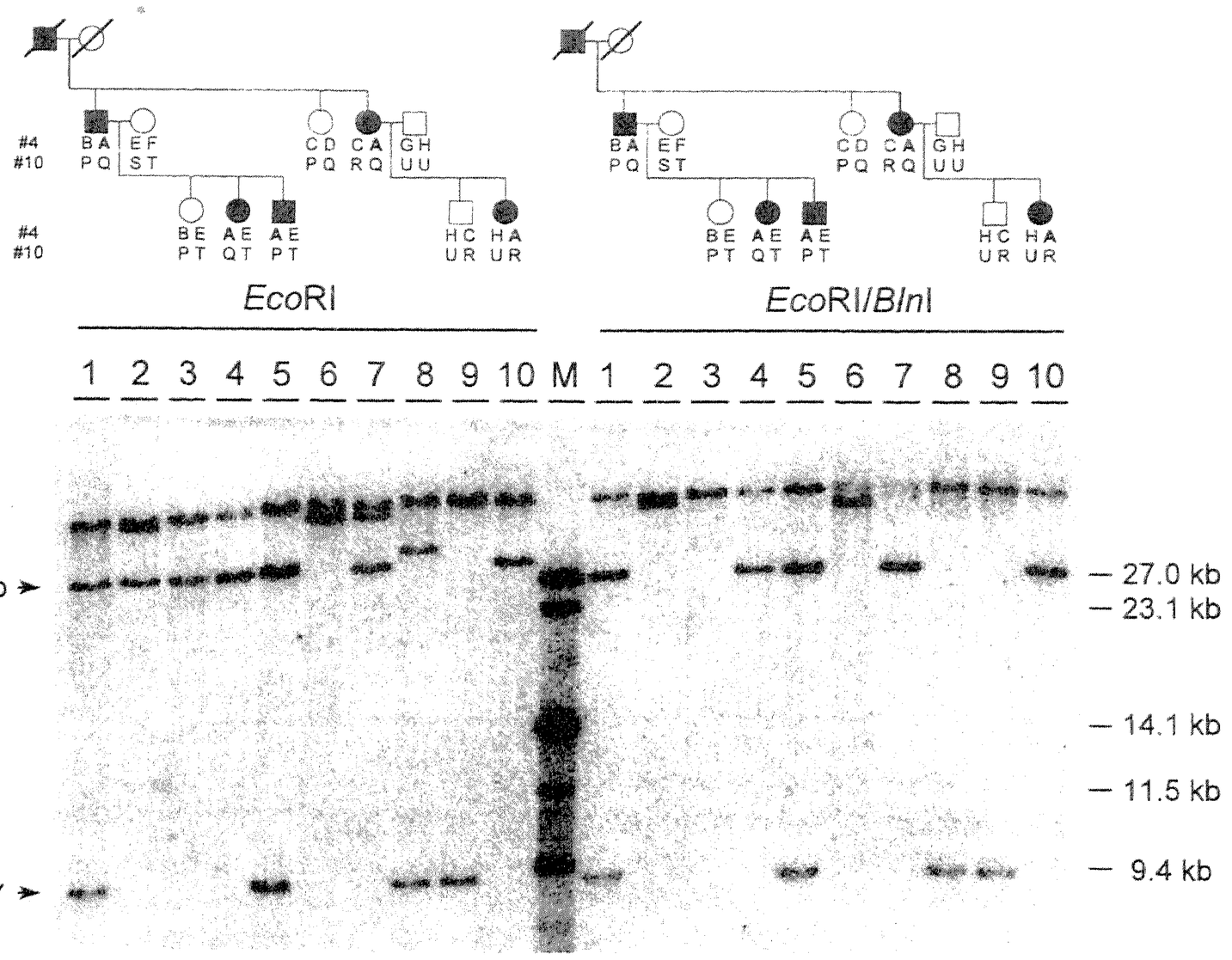

Figure 1. Differential restriction analysis of family 87. Conventional gel electrophoresis of *Eco*RI and *Eco*RI/*Bln*I digested DNA and subsequent Southern blot analysis using probe p13E-11. The 30 kb *Eco*RI fragment observed in affected and unaffected family members appears to be heterogeneous after *Bln*I digestion. The fragment is 3 kb reduced in size, indicating a chromosome 4q35 origin, in the affected members only. The chromosome 4 (#4) haplotypes are indicated by 'A-H', whereas the chromosome 10 (#10) haplotypes are indicated by 'P-U'. The FSHD1-linked haplotype A is depicted in bold. The marker (M) is lambda DNA digested with *Hin*dIII and *Pst*I. The Y-specific fragment detected by p13E-11 is indicated.

Haplotype analysis using chromosome 4q35 and 10q26 markers (D4S163–D4S139 and D10S555–D10S590, respectively) was performed to differentiate the chromosomes 4 and 10 segregating in this family (Fig. 2a). One of the 4q35-like *Eco*RI/*Bln*I fragments observed in individuals 28.1, 28.4 and 28.5 indeed segregates with a chromosome 10q26 haplotype indicating it to be a hybrid p13E-11 fragment. In addition, one of the 10q26-like fragments observed in individuals 28.2 and 28.6 segregates with a chromosome 4q35 haplotype, also supporting the interchromosomal exchange hypothesis.

Subtelomeric exchange of 3.3 kb repeated units between chromosome 4q35 and 10q26 has implications for the specificity and sensitivity of the differential restriction analysis as a method for diagnostic DNA analysis. As illustrated in Figure 3, short *Eco*RI fragments linked to chromosome 4q35 may be either *Bln*I-sensitive or *Bln*I-resistant. Healthy individuals may carry short hybrid *Eco*RI fragments linked to chromosome 10q26, while a chromosome 4q35 origin and therefore a high probability of FSHD1 would be suggested by the differential restriction analysis. In parallel, cases carrying short, 4q35-linked *Eco*RI fragments exhibiting *Bln*I-sensitive repeated units, might incorrectly be diagnosed with a low probability of FSHD1. Examples of such cases are mentioned in the previous paragraph: in six of 114 cases which were unambiguously diagnosed with FSHD, a short chromosome 10q26-like *Eco*RI fragment was observed. One of these cases is member of a well-studied 4q35-linked FSHD1 family (family 25) (18,20) in which a short 15 kb *Eco*RI fragment is cosegregating with the disease. This 15 kb fragment was cloned from the index case (patient 250301) in a previous study (18). Detailed Southern blot analysis using probes p13E-11 (D4F104S1), 9B6A (D4Z4) and pLAM1 (D4Z3) (Fig. 4), indicated this fragment to contain repeated units each carrying a *Bln*I site, incorrectly suggesting a chromosome 10q26 origin.

To determine the frequency of subtelomeric exchanges of repeated units between chromosomes 4q35 and 10q26 in the population, 50 male control individuals were screened using pulsed-field gel electrophoresis of *Eco*RI/*Bln*I double digested DNA. In five cases (10%) apparent 'monosomy' was observed, while in five other cases (10%) 'trisomy' was detected. One 'monosomic' case and one 'trisomic' case are shown in Figure 5. Accordingly, the frequency of interchromosomal exchanges was estimated to be 20% (10 of 50). However, the frequency is probably higher given the higher frequency of recombination events in females, not included in this control study. Moreover, ~1% of cases may contain two types of hybrid fragments. These cases appear to be 'disomic' and will remain unnoticed (see Fig. 5, control 2).

DISCUSSION

The differential restriction analysis of FSHD cases is based on the presence of a *Bln*I restriction site in each of the 3.3 kb repeated units derived from chromosome 10q26 (19). As this *Bln*I site is not present in the repeated units of chromosome 4q35, differentiation between the two polymorphic p13E-11 loci can easily be achieved in most cases. Therefore, especially in isolated cases suspected of having FSHD, the differential restriction analysis accounts for an important improvement of the diagnostic DNA analysis. In addition, in some familial cases haplotype analysis may not reveal clear differentiation between chromosome 4q35

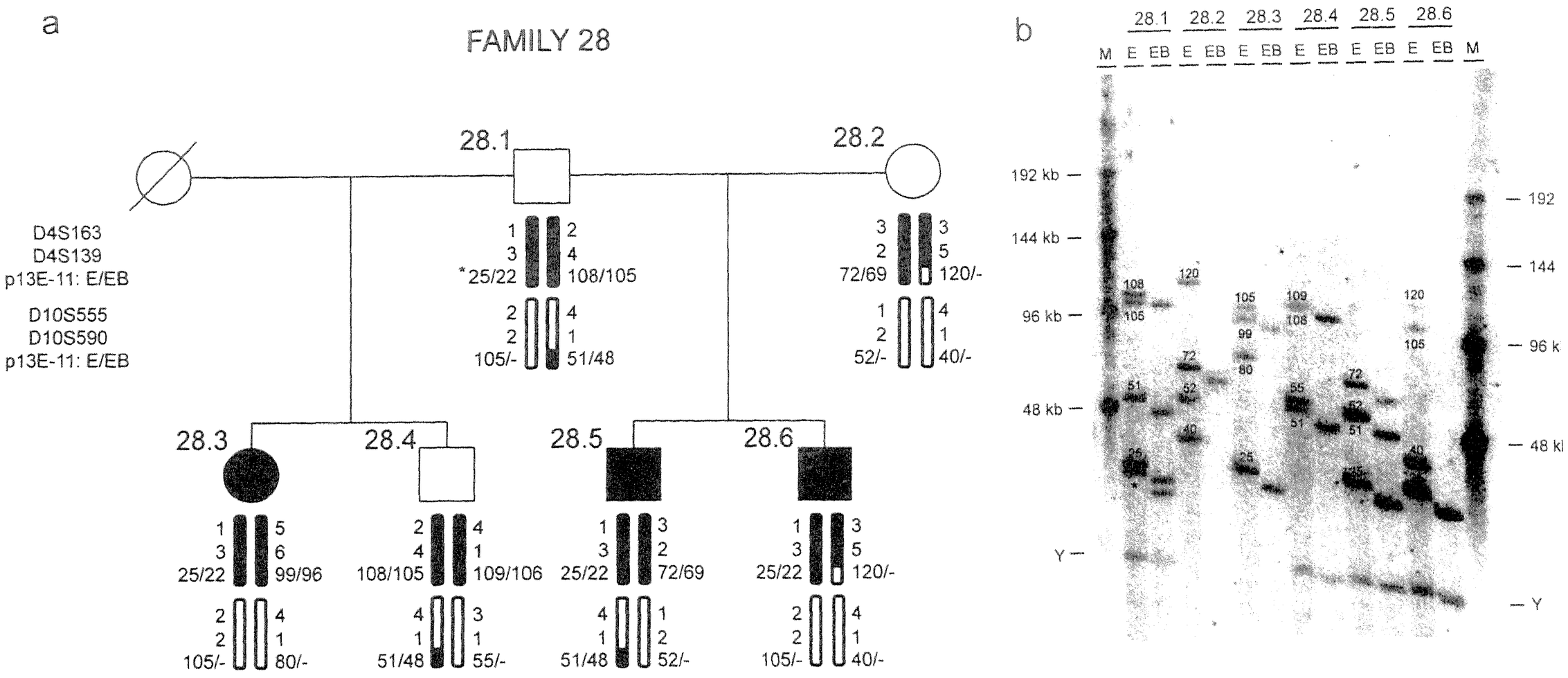

Figure 2. (**a**) Haplotype analysis of family 28 using chromosome 4q35 and 10q26 markers. The sizes of the p13E-11 fragments after *Eco*RI (E) and *Eco*RI/*Bln*I (EB are shown. *Eco*RI fragments containing repeated units derived from chromosome 10q26 are completely digested by *Bln*I (–). Haplotypes including hybrid p13E-11 *Eco*RI fragments are depicted by filled (chromosome 4q35) or open (chromosome 10q26) bars with swopped ends. The asterisk indicates that the father (28.1) is mosaic, carrying an additional 22 kb fragment possibly derived from the 25 kb fragment (b). (**b**) Differential restriction analysis of family 28. Pulsed-field gel electrophoresis of *Eco*RI (E) and *Eco*RI/*Bln*I (EB) digested DNA and subsequent Southern blot analysis using probe p13E-11, revealed that cases 28.1, 28.4 (upper band contains two fragments) and 28.5 are apparently 'trisomic' and cases 28.2 and 28.6 'monosomic'. The chromosomal origins of the *Eco*RI fragments were confirmed by haplotype analysis (a). The sizes (in kilobases) of the *Eco*RI fragments are depicted above or below the fragments. The marker (M) is a 48 kb lambda DNA ladder. The Y-specific fragment detected by p13E-11 is indicated.

and 10q26 derived *Eco*RI fragments due to either non-informative marker data or short *Eco*RI fragments of similar size segregating in the family. In this paper, we describe one such family (family 87, Fig. 1) which remained diagnostically inconclusive after haplotype analysis, but which could reliably be diagnosed after *Eco*RI/*Bln*I double digestion. Applying the differential restriction analysis, most of 160 index cases clinically suspected of having FSHD and referred for DNA analysis, were diagnosed unequivocally.

Although the diagnostic value and the specificity and sensitivity of the differential restriction analysis were found to be high, we observed several FSHD index cases and unaffected spouses exhibiting unanticipated *Bln*I restriction patterns. In family 28 (Fig. 2) apparent 'monosomy' and 'trisomy' for chromosome 4q35-linked p13E-11 fragments was detected. We hypothesized that an exchange of 3.3 kb repeated units between chromosomes 4q35 and 10q26 might have occurred. This exchange would generate hybrid p13E-11 *Eco*RI fragments: chromosome 4q35-linked *Eco*RI fragments containing chromosome 10q26-like or *Bln*I-sensitive repeats and conversely, chromosome 10q26-linked *Eco*RI fragments containing chromosome 4q35-like or *Bln*I-resistant repeats. Further analysis of family 28 using pulsed-field gel electrophoresis to separate the individual chromosomal *Eco*RI and *Eco*RI/*Bln*I restriction fragments, and subsequent haplotype analysis using markers from the chromosome 4q35 and 10q26 regions, supported this subtelomeric exchange hypothesis.

As subtelomeric exchange of the 3.3 kb repeated units between chromosomes 4q35 and 10q26 would have implications for the specificity and sensitivity of the differential restriction analysis, we estimated the frequency of interchromosomal exchange in the population. Apparent 'trisomy' or 'monosomy' for a 4q35-linked *Eco*RI fragment was observed in at least 20% of the population. The specificity of the differential restriction analysis is determined by the percentages of unaffected individuals carrying short 10q26-linked *Eco*RI fragment which contains *Bln*I-resistant repeated units (mostly 'trisomics'), whereas the sensitivity is determined by the percentages of FSHD patients carrying a short 4q35-linked *Eco*RI fragment which contains *Bln*I-sensitive repeated units (mostly 'monosomics'). Differential restriction analysis of 50 unrelated controls revealed two 'trisomic' samples and one apparent 'disomic' sample (Fig. 5, control 2), exhibiting a short (<35 kb) chromosome 10q26-linked *Eco*RI fragment containing *Bln*I-resistant repeated units. Therefore, at present the specificity of the differential restriction analysis is estimated to be 94% (47 of 50). In addition, differential restriction analysis of 160 Dutch FSHD index cases revealed in 12 of 114 Dutch index cases a short (<35 kb) *Eco*RI fragment containing *Bln*I-sensitive repeated units. Based on clinical signs, at least six of these cases were unambiguously diagnosed with FSHD. The other six cases were either not analyzed by our neurologist (GWP) and therefore considered to be 'clinically uncertain', or possibly misdiagnosed. As an *Eco*RI fragment which is completely fragmented by *Bln*I suggests a chromosome 10q26 origin, the six clinically certain FSHD cases would have been predicted to have a low probability of FSHD1. However, pulsed-field gel electrophoresis revealed that these cases are 'monosomic' after *Eco*RI/*Bln*I double-digestion, and carry a short hybrid chromosome 4q35-linked *Eco*RI fragment containing *Bln*I-sensitive repeated units. Therefore, the sensitivity of the differential restriction analysis is estimated to be 95% (108 of 114).

In conclusion, the differential restriction analysis facilitates an efficient and unequivocal diagnosis in approximately 95% of cases. However, index cases which show clear clinical FSHD signs but carry a short *Eco*RI fragment completely fragmented by

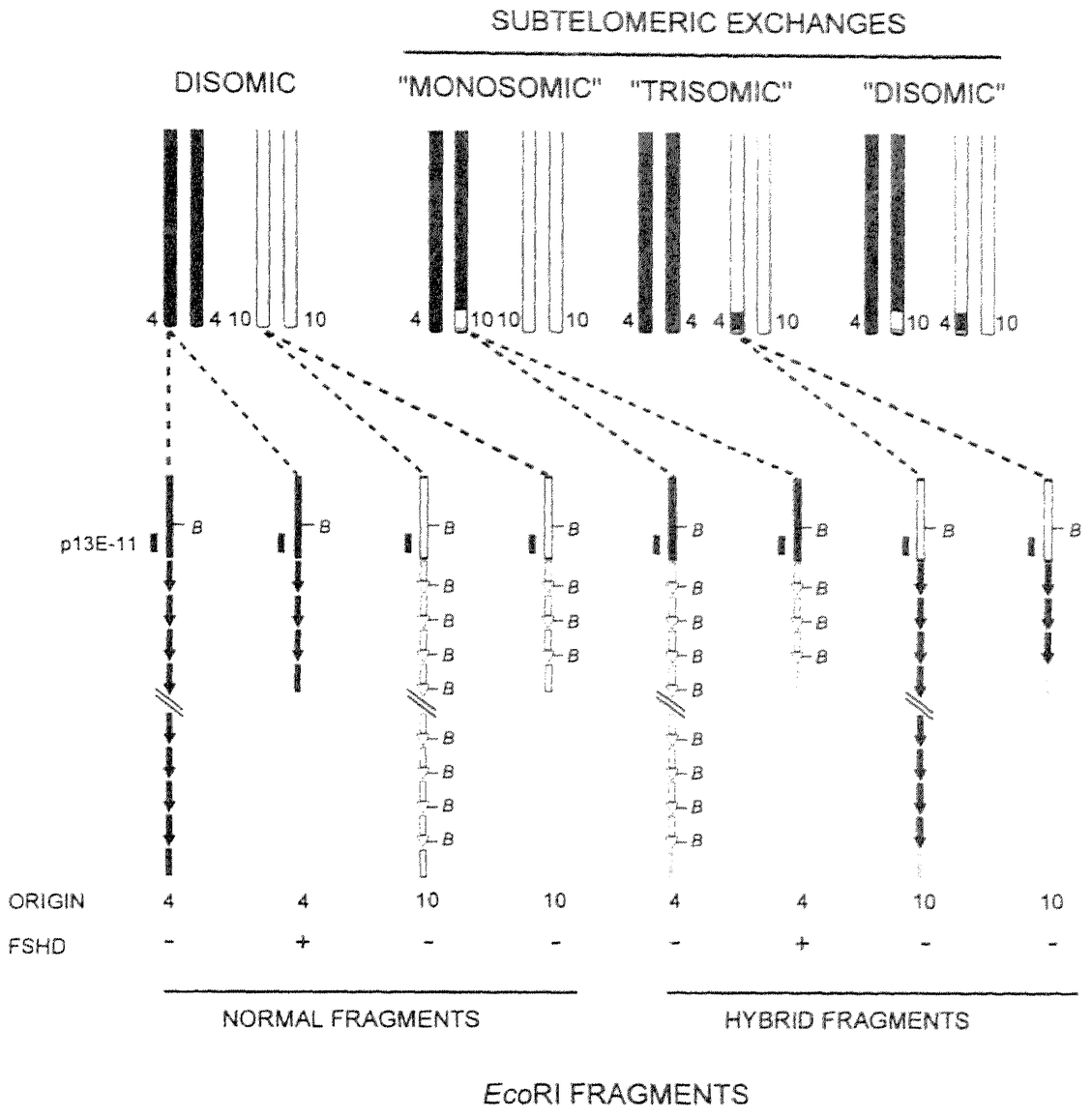

Figure 3. Model to explain the aberrant *Bln*I restriction patterns observed after differential restriction analysis, which suggest some cases to be 'monosomic' or 'trisomic' for the chromosome 4q35 *Eco*RI fragment. On top, the subtelomeric regions of chromosomes 4q35 (filled bars) and 10q26 (open bars) and potential subtelomeric exchanges are depicted. Below, normal and hybrid and long or short *Eco*RI fragments derived from chromosome 4q35 or 10q26, are shown. As the origin of the telomeric regions of the hybrid fragments might be either chromosome 4q35 or 10q26, they are indicated by grey bars. Note that short, 4q35-linked, hybrid *Eco*RI fragments containing *Bln*I-sensitive repeated units are also diagnostic for FSHD1, but might lead to misdiagnosis when based on differential restriction analysis only.

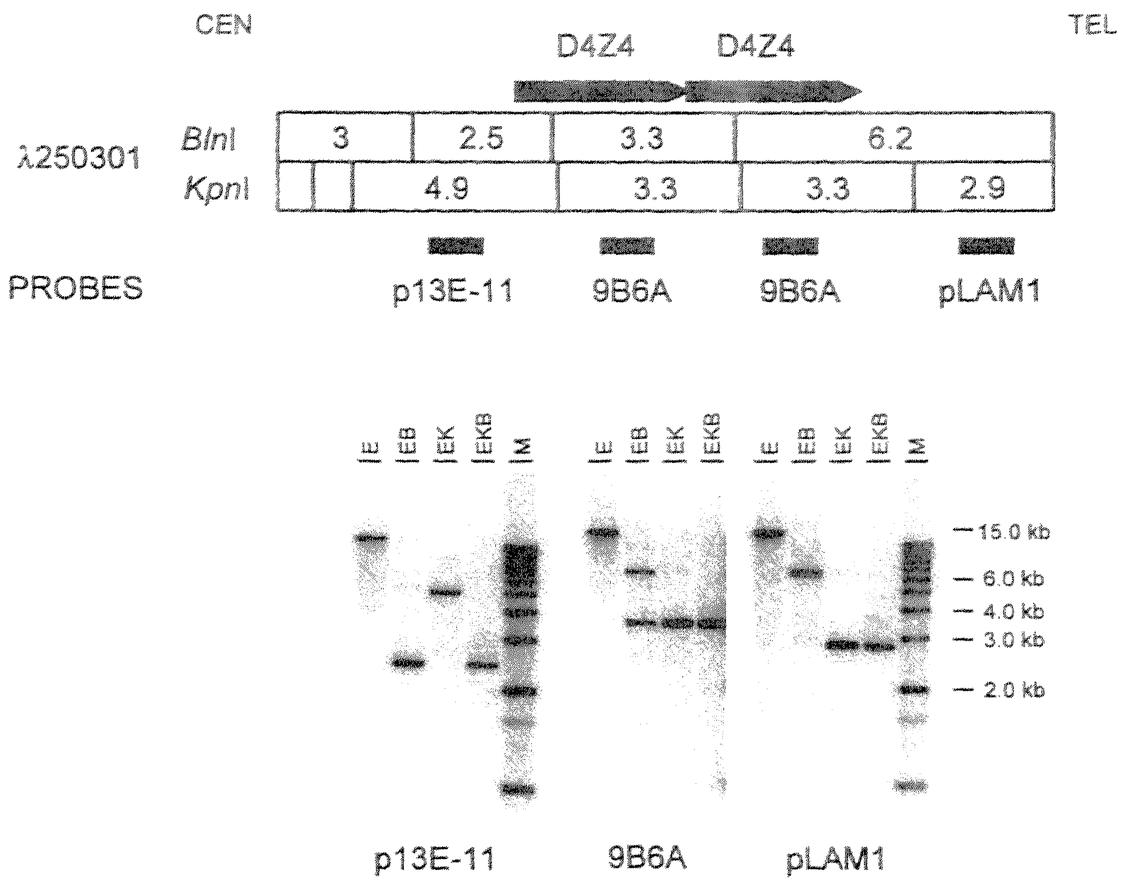

Figure 4. Detailed analysis of the 15 kb *Eco*RI fragment (λ250301) cloned from FSHD patient 250301. On top, the *Kpn*I and *Bln*I restriction maps are shown. Sizes of fragments are indicated in kilobases. The 3.3 kb repeated units (D4Z4) are depicted by arrows. Below, Southern blot analysis of the *Eco*RI (E) fragment digested by *Bln*I (EB), *Kpn*I (EK), or by *Kpn*I and *Bln*I (EKB) using probes p13E-11 (D4F104S1), 9B6A (D4Z4) and pLAM1 (D4Z3). The marker (M) is a 1 kb lambda DNA ladder. Both repeated units were found to contain a *Bln*I site. The chromosomal origin of the telomeric part of the *Eco*RI fragment could not unambiguously be determined.

*Bln*I suggesting a chromosome 10q26 origin, should be further analyzed by pulsed-field gel electrophoresis and haplotype analysis to confirm the chromosomal origin of the short fragment, and to prevent misdiagnosis.

The molecular mechanism behind the subtelomeric exchanges between chromosomes 4q35 and 10q26 remains to be elucidated. Chromosomal rearrangements occur more often in (sub)telomeric regions than in other parts of the genome. Translocations of chromosome ends have recently been reported as the cause for idiopathic mental retardation syndrome (21), α-thalassemia mental retardation syndrome (22), Wolf–Hirschhorn syndrome (23), Miller–Dieker syndrome (24) and cri-du-chat syndrome (25). However, in this paper we report an interchromosomal polymorphism without phenotype, which is to our knowledge a remarkable novelty in human genetics. Owing to high homology between the chromosome 4q35 and 10q26 subtelomeric regions, it is plausible that there is an interchromosomal 'cross-talk' during meiosis. Two types of chromosomal rearrangements might have generated the exchange of 3.3 kb repeated units: either translocations including the particular telomeres, or gene conversions limited to the repeated units only. As the respective telomeric regions of chromosomes 4q and 10q contain non-unique sequences only, it will be difficult to determine whether the telomeres are involved in the interchromosomal exchanges. In addition, in most cases analyzed the p13E-11 *Eco*RI fragments revealed *Bln*I restriction patterns as expected; either completely fragmented, or reduced in size by 3 kb. Therefore, probably complete arrays of repeated units have been exchanged, suggesting that in case of telomeric translocations the proximal breakpoint might even be proximal to the repeated units. However, in rare cases the *Eco*RI fragment was found to be reduced in size by more than 3 kb after *Bln*I digestion, indicating that hybrid p13E-11 *Eco*RI fragments may also contain a mixture of *Bln*I-resistant and *Bln*I-sensitive repeated units. Finally, whether the hybrid *Eco*RI fragments are due to a recurrent event of interchromosomal exchange in the population, or part of a polymorphism which is due to one unique interchromosomal exchange event in evolution, remains to be elucidated.

Independent of the molecular mechanism, the interchromosomal exchanges of repeated units have implications for the etiology of FSHD1; a short 4q35-linked *Eco*RI fragment will give FSHD1, irrespective of origin of the repeated units. A *de novo* exchange generating a short 4q35-linked fragment containing *Bln*I-sensitive repeated units from chromosome 10q26, will cause FSHD1 in the same way as a chromosome 4q35 intra-fragment deletion. However, the frequency of such an event is bound to be low given the low percentage (<10%) of short chromosome 10q26-linked *Eco*RI fragments (12,17). Interchromosomal exchanges of the 3.3 kb repeated units between chromosomes 4q35 and 10q26 with a rather high frequency (20%) suggest that it is unlikely that the FSHD1 gene (partially) resides within the repeated units at chromosome 4q35. Moreover, an Italian family was reported recently, in which an abnormal chromosome 4, a derivative of an unbalanced translocation of 4q35 with an acrocentric chromosome, segregates through three generations in phenotypically normal individuals (26). The resulting monosomy of the 4q35 region in unaffected individuals supports the

hypothesis that the 3.3 kb repeated units do not contain (part of) the FSHD1 gene. These observations favour the position effect variegation (PEV) model to be the most likely underlying molecular mechanism of FSHD1.

MATERIALS AND METHODS

Patient and control material

Familial and isolated cases are referred to us through one of the seven Clinical Genetics Centres in the Netherlands. They are referred by neurologists after being diagnosed clinically as possibly having FSHD. In this study, 160 FSHD index cases are included. Further analysis was performed on two particular 4q35-linked families. In family 87 (17) a 30 kb *Eco*RI fragment, and in the Turkish family 28 a 25 kb *Eco*RI fragment segregates with the disease. DNA isolated from 50 unaffected male individuals was used in the control study.

Southern blot analysis

Genomic DNA was isolated from freshly collected blood as previously described (27). DNA was digested with restriction enzymes *Eco*RI and *Bln*I according to the manufacturer's instructions (Pharmacia). Conventional gel electrophoresis was performed as reported by Bakker *et al.* (17). DNA was transferred to a Hybond N^+ membrane (Amersham). The inserts of probes p13E-11(D4F104S1) (13), 9B6A (D4Z4) (13) and pLAM1 (D4Z3) (18) were labelled by random priming with α^{32}P-dCTP, using the megaprime DNA labelling system (Amersham). Hybridizations were performed for 16 h at 65°C as described (28). The blots were washed at 65°C and at a stringency of 2×

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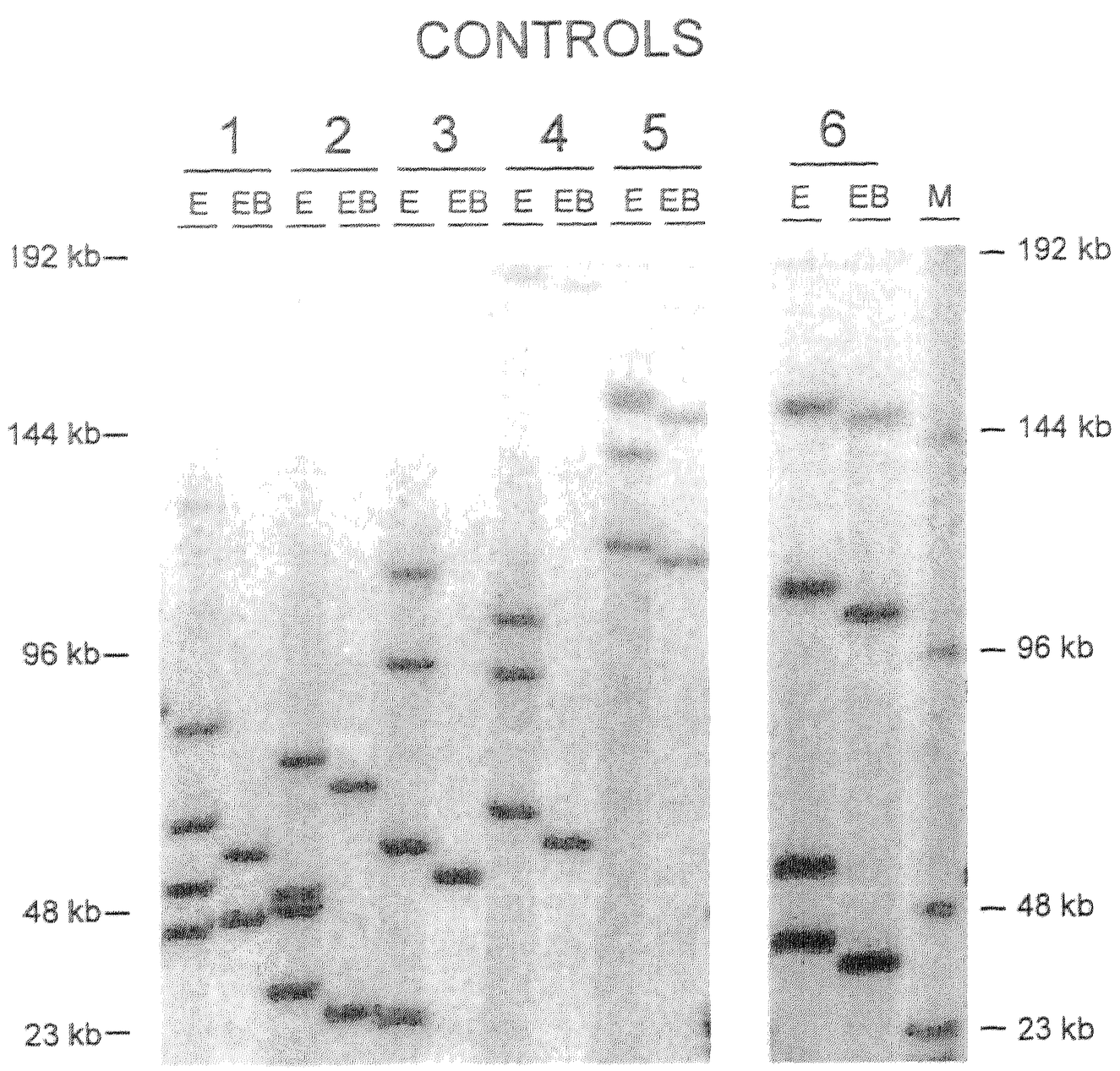

Figure 5. Differential restriction analysis of six of 50 unaffected male control individuals. Pulsed-field gel electrophoresis of *Eco*RI (E) and *Eco*RI/*Bln*I (EB) digested DNA and subsequent Southern blot analysis using probe p13E-11. Control 3 appears to be 'monosomic', whereas control 6 appears to be 'trisomic'. Although control 2 is unaffected, he exhibits a short (28 kb), *Bln*I-resistant *Eco*RI fragment. Presumably, this short fragment is linked to chromosome 10q26. As control 2 is 'disomic', he probably carries another hybrid fragment which is linked to chromosome 4q35. The marker (M) is a 48 kb lambda DNA ladder.

SSC/0.1% SDS, followed by autoradiography for 16–36 h at –70°C using Konica AX film with an intensifying screen.

Pulsed-field gel electrophoresis

Electrophoresis was performed in 0.5×TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA, adjusted to pH 8.0) at 18°C. *Eco*RI and *Eco*RI/*Bln*I double digested DNA in solution was run for 30 h at an electric field of 8.5 V/cm. In four identical cycles, switch times were increased linearly from 1 s at the beginning and 20 s at the end of each cycle. A pause interval of 2% of the forward switch time was included. After electrophoresis, gels were stained with ethidium bromide. The high molecular weight DNA molecules were fragmented by a UV-Cross-Linker (Stratagene) at 180 000 J/cm^3.

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