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Identification and regional localization of a highly polymorphic dinucleotide repeat D11S614 to the interval in 11q23.3 flanked by recurrent translocation breakpoints

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SUMMARY

A highly informative dinucleotide repeat polymorphism has been identified at the D11S614 locus on chromosome 11q23. Ten different alleles have been observed at this locus, and the heterozygosity frequency is approximately 85%. Physical localization of this marker in a panel of somatic cell hybrids containing chromosome 11 translocations showed that it maps to 11q23.3, within the interval between the recurrent t(4;11) leukaemia breakpoint and the t(11;22) Ewing's sarcoma breakpoint. This physical mapping data is consistent with the genetic mapping which indicates tight linkage to other markers in the q23.3 region including PBGD, CD3D and D11S29. Regional localization of highly informative markers such as D11S614 will facilitate integration of the genetic and physical maps.

INTRODUCTION

Human chromosome region 11q22–24 contains several important disease loci as well as at least three recurrent translocation breakpoints (Geurts van Kessel, 1985; Griffen *et al.* 1986; Sacchi *et al.* 1986). Identification of highly polymorphic markers which map within defined intervals of 11q22–24 will greatly facilitate precise localization of these disease genes and fine mapping around them. We have characterized a highly informative dinucleotide repeat sequence, identified in a cosmid contig containing D11S614 (Sugiyama *et al.* 1991), and have regionally localized it to the interval in 11q23.3 between two breakpoints, the leukaemia-associated t(4;11) and the Ewing's sarcoma-associated t(11;22).

MATERIALS AND METHODS

D11S614 CA repeat

The cosmid 8D11 (D11S614, Sugiyama *et al.* 1991) was originally isolated as one of a series of human clones present in a library constructed from a somatic cell hybrid line containing human chromosome 11q13–11qter (Evans & Lewis, 1989). A 1·4 kb *Eco*RI fragment from 8D11

which hybridized to an $(AC)_{10}$ oligomer was subcloned and sequenced. Unique sequence primers flanking the $(CA)_N$ repeat were designed as follows: GT strand: 5′-ACAGACCCACCA-GGACTAT-3′; CA strand: 5′-CCCGGATGTCTGCAAGGTGG-3′. This primer set amplified a product of around 160 bp under the following conditions: genomic DNA (~ 100 ng) was amplified in a 10 μl volume containing 0·5 μM each primer, 10 mM Tris pH 8·8, 50 mM KCl, 1·5 mM $MgCl_2$, 0·1% Triton, 100 μM each of dATP, dGTP and dTTP, 0·6 μM dCTP, 0·045 μCi [^{32}P]dCTP, 20% glycerol and 0·25 unit Taq polymerase. Reactions were heated for 1·5 min at 95 °C, then subjected to 30 cycles of 93 °C 0·5 min, 55 °C 1 min, 72 °C 1 min. Products were electrophoresed through an 8% acrylamide sequencing gel for 2·5 h.

Family studies

Segregation of the 8D11 CA repeat polymorphism with four other 11q22–23 markers was analysed in 38 families (segregating for tuberous sclerosis, Burley *et al.* 1992). DRD2 contains a CA repeat and was scored using a PCR-based assay, and D11S144, D11S29 and PBGD, which detect RFLPs, were scored on Southern blots, as described in Gillett *et al.* (1993). Two point lod scores were calculated using the LIPED programme.

Translocation hybrid lines

Seven somatic cell hybrid lines containing translocations involving human chromosome 11q were used to map D11S614. A3EW3B (Geurts van Kessel *et al.* 1985) retains the der 11 from the Ewing's sarcoma associated t(11;22); A3RS12B and A3RS17B (Sacchi *et al.* 1986) retain the reciprocal, leukaemia-associated t(4;11) chromosomes; P3.27A (Tokino *et al.* 1991) retains the der 11 from the constitutional t(11;22); M11X (Wadey *et al.* 1990) retains the der 11 from a t(11;X); MCH110.1c4 (Bader *et al.* 1989) retains the der X from a t(11;X). D11S614 was assayed using DNA from the hybrids, as described above, except that the reaction was carried out in a total volume of 100 μl using non-radioactive dNTPs. The product was run out on a 2% agarose gel and visualized with ethidium bromide. The presence or absence of fourteen additional 11q22–24 markers was scored by PCR or Southern blotting as described in Gillett *et al.* (1993).

RESULTS AND DISCUSSION

Cosmid 8D11 (D11S614) forms part of a 48 kb cosmid contig isolated from a library made from a somatic cell hybrid containing human 11q13–11qter. A dinucleotide repeat sequence was detected in this cosmid by screening with an $(AC)_{10}$ oligomer. Unique primers, designed from sequence flanking the repeat, amplified products ranging in size from 160 to 180 bp from human DNA. Segregation analysis of the amplified products in 35 families revealed ten different alleles which followed a co-dominant mode of inheritance. The observed heterozygosity at this locus in a population of 37 unrelated Caucasians was 85%, with allele frequency as follows: A1 (160 bp), 0·16; A2, 0·04; A3, 0·04; A4, 0·07; A5, 0·25; A6, 0·17; A7, 0·12; A8, 0·08; A9, 0·03; A10 (180 bp), 0·04. Co-segregation of this polymorphic marker with four markers from 11q22–23 (DRD2, D11S144, D11S29 and PBGD) was analysed in 35 families: two-point lodscores, given in Table 1, indicate linkage to all four markers.

Table 1. *Pairwise lodscores for D11S614 v. four markers in 11q22–23 (38 families)*

	Lod score (Z) at recombination fraction						
	0	0·01	0·05	0·1	0·2	0·3	0·4
D11S614 *v.*							
DRD2							
Males	$-\infty$	7·2	9·4	9·3	7·2	4·3	1·6
Females	$-\infty$	−8·9	0·35	3·2	4·2	3·1	1·4
D11S144							
Males	5·3	5·2	4·7	4·0	2·7	1·4	0·4
Females	5·3	5·2	4·8	4·2	2·9	1·6	0·5
D11S29							
Males	2·2	2·1	1·9	1·6	1·1	0·6	0·2
Females	2·8	2·7	2·4	2·1	1·4	0·8	0·3
PBGD							
Males	5·8	5·7	5·2	4·5	3·2	1·8	0·6
Females	8·3	8·2	7·4	6·5	4·7	2·8	1·1

Table 2. *Scoring of D11S614 and other markers in hybrid lines containing translocations in 11q*

Chromosome...		MCH 556.1 11	A3EW 3B t(11;22) Ewing's	A3RS 12B t(4;11) leuk	P3.27A t(11;22) constit	M11X (t(11;22)	A3RS 17B t(4;11) leuk	MCH 110.1c4 t(X;11)	Translocation
D11S614		+	+	−	−	−	+	+	
Markers									
DRD2	q22	+	+	+	+	+	−	−	
NCAM		+	+	+	+	+	−	−	
D11S144		+	+	+	+	+	−	−	
D11S351		+	+	+	+	+	−	−	
									t(11;22) constitutional
D11S29	q23.1–23.2	+	+	+	−	−	−	−	
APOC3		+	+	+	−	−	−	−	
CD3D		+	+	+	−	−	−	−	
D11S490		+	+	+	−	−	−	−	
									t(4;11) leukaemia
PBGD	q23.3	+	+	−	−	−	+	+	
D11S147		+	+	−	−	−	+	+	
THY1		+	+	−	−	−	+	+	
ETS1		+	+	−	−	−	+	+	
D11S420		+	+	−	−	−	+	+	
									t(11;22) Ewing's sarcoma
D11S543	q24	nt	−	−	nt	−	+	nt	

nt, not tested.

To localize D11S614 physically, the same PCR primers were used to score for the presence of D11S614 in DNAs from a series of somatic cell hybrids containing human chromosomes with translocations in distal 11q. Table 2 shows the results along with the scoring of 14 additional markers from 11q22–23. D11S614 maps to 11q23.3, in the interval flanked by the t(4;11) leukaemia breakpoint and the t(11;22) Ewing's sarcoma breakpoint, and which also contains

PBGD, THY1, D11S147, D11S420 and ETS. This localization was supported by FISH analysis in which the 8D11 cosmid gave a positive signal on the der22 from the cell line GM6229, which has the constitutional translocation chromosome t(11;22) (q23;q11.2) (data not shown). The physical mapping of D11S614 is entirely consistent with the genetic data, which indicate tight linkage to PBGD, CD3D and D11S29 (see above and Kramer *et al.* 1992). Regional physical localization of highly informative markers such as D11S614 will enable integration of the genetic and the physical maps of 11q23, and facilitate detailed mapping of genes in the region.

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