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An Integrated Map of Human Chromosome 13 Allowing Regional Localization of Genetic Markers

Key Words

Chromosome 13
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Abstract

37 CA repeats, 5 STSs, 9 ESTs, and 4 genes were mapped to 19 different intervals of chromosome 13 determined by the cytogenetic breakpoints of 19 different cell lines with interstitial deletions or translocations involving various parts of chromosome 13. A framework genetic linkage map was constructed from 25 of these microsatellite markers, to which 26 markers from other genetic maps were added. Thus, an integrated map of chromosome 13 resulted. Since the microsatellite markers included in this study derive from different genetic maps, an approximate regional localization can now be assigned in principle to any genetic marker on chromosome 13.

Introduction

Chromosome 13 contains 3.6% of the human haploid genome, i.e. 114 Mb DNA. It has a genetic length of 130 cM [1]. According to the genome database (GDB version 5.4, November 1994), 56 genes have been mapped to this acrocentric chromosome. Among these are the genes responsible for retinoblastoma, RB1 [2]; Wilson's disease, ATP7B [3, 4]; chronic B cell leukaemia, BCLL [5-7]; Duchenne-like muscular dystrophy, DLMD [8]; Hirschsprung's disease, HSCR2 [9]; a nonsyndromic form of recessively inherited childhood deafness, NSRD1 [10]; and a locus for

familial breast cancer, BCRA2 [11]. In addition, 68 ESTs and 755 other anonymous DNA segments have been assigned to chromosome 13.

Relatively few of these loci have been regionally assigned. Six deletion hybrid maps have been presented, ordering a total of 171 markers in maximally 11 different intervals [12-16]. Genotypes have been generated for a total of 120 microsatellite and 59 restriction fragment length polymorphism (RFLP) markers. Linkage maps have been built from these data, but none of these genetic maps provide detailed cytogenetic locations for the microsatellite loci [17-25].

In the present study, a first integrated map of chromosome 13 is presented, based upon the breakpoints of nineteen cell lines with interstitial deletions or translocations involving various parts of chromosome 13. Fifteen of the cell lines have been provisionally described in the HGM11 report [26]. Three cell lines, ICA, ICC, and ICD were generated by irradiating monochromosomal hybrid GF7 [27] with a low dosage of X rays [28]. The other cell lines have been derived from individuals with a rearranged chromosome 13 by fusion of cells from that individual with a rodent cell line and selection of a hybrid clone containing the der(13), but not the complete homologue of chromosome 13. In contrast to the three cell lines mentioned earlier, these rodent cell lines always contain other human chromosomes in addition to the der(13).

37 CA repeats, 5 STSs, 9 expressed sequence tags (ESTs), and 4 genes were mapped to 19 different intervals determined by the cytogenetic breakpoints in the cell lines. A framework genetic linkage map was constructed from 25 of these microsatellite markers, to which 26 markers from other genetic maps were added. Thus, an integrated map of chromosome 13 resulted, allowing an approximate regional localization of any genetic marker on chromosome 13.

Materials and Methods

Cell Lines

The hybrid cell lines used were GF7 containing human chromosome 13 only [27], ICA containing 13pter-13q14.1 [28], ICC containing 13pter-13q14.1 [Buys, unpubl. data], ICD containing 13pter-13q14.3 [28], WC-H38B3B6 containing 13pter-q13::13q21.1-qter [29], WC-H12D12 containing 13pter-q14::13q22-qter [29], NM-87-26XT containing 13q12-q14::13q22-qter [30], PKII-90-P5b (= PK88-25) containing 13pter-q12::13q21.2-qter [30], BARF7 containing 13pter-q22::13q34-qter [12], KSF39 containing 13pter-q14.1 [12], PPF22 containing 13pter-q14::13q31-qter [12], KBF11 containing 13pter-

q12::13q14-qter [12], E8 containing 13q14.1-qter [17], D1 containing 13pter-q14.1 [17], RHF 407 containing 13q14.3-13qter [6], RHF 2324 containing 13pter-13q14.3 [6], GS89a containing 13pter-q14::13q32-qter [16], X13-C9 containing 13pter-q13, CF25 containing 13q12-13qter [31], and CF27 containing 13pter-q14.1::13q22-qter [32]. The cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. After treatment of the cells with SDS and proteinase K, DNA was isolated by phenol extraction according to standard procedures.

Polymerase Chain Reactions

Reactions were carried out on a thermocycler with 96-well microtiter plates in 20- μ l vol of 'Supertaq reaction buffer' (Sphearo Q HT Biotechnology, Leiden, The Netherlands) with final concentrations of 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM Mg²⁺; 0.1% Triton X-100; 0.01% gelatin; 0.2 mM dATP, dGTP, and dTTP; 0.02 mM dCTP; 0.025 μ l [³²P]dCTP; 0.125 u Taq polymerase (Supertaq, Sphearo Q HT Biotechnology, Leiden, The Netherlands); 100 ng of each primer, and 100–300 ng template DNA. Thermal cycling was carried out for 30 cycles, consisting of denaturation at 93 °C for 45 s (first step 3 min), annealing at 5–10 °C below T_d for 1 min, and extension at 72 °C for 1 min, followed by a final extension step for 3 min. Products were size-separated on 4–6% polyacrylamide gels and analyzed after exposure to an X-ray-sensitive film.

Map Construction

The genetic map was constructed using the expert system MultiMap [24], based on CRI-MAP [33]. It was run on a DEC5000/25 workstation under Ultrix 4.3, with a CLISP interpreter. Sex differences in recombination were calculated using the program sexdif_d, written and made publicly available by J.E. Blaschak and A. Chakravarti.

For the genetic map we used the most informative loci from table 1. In addition, the following markers were included: D13S115, D13S122, D13S125 [20]; D13S138, D13S141 [21]; D13S154, D13S159, D13S172, D13S173, D13S176, D13S219 [18]; D13S232, D13S260, D13S263, D13S267, D13S274, D13S283, D13S285, D13S289, D13S291 [22]; D13Z1 [34]; COL4A1 [36]; D13S107, D13S234, D13S235 [23, 37]; sLDA-1 [38]. Forty CEPH families were used in the genetic analysis: 1331, 1332, 1347, 1362, 1413, 1416, 884, 102, 13291, 13292, 13293, 13294, 1333, 1334, 1340, 1341, 1344, 1345, 1346, 1349, 1350, 1375, 1377, 1408, 1418, 1420, 1421, 1423, 1424, 66, 12, 23, 21, 2, 17, 37, 35, 28, 45, 104.

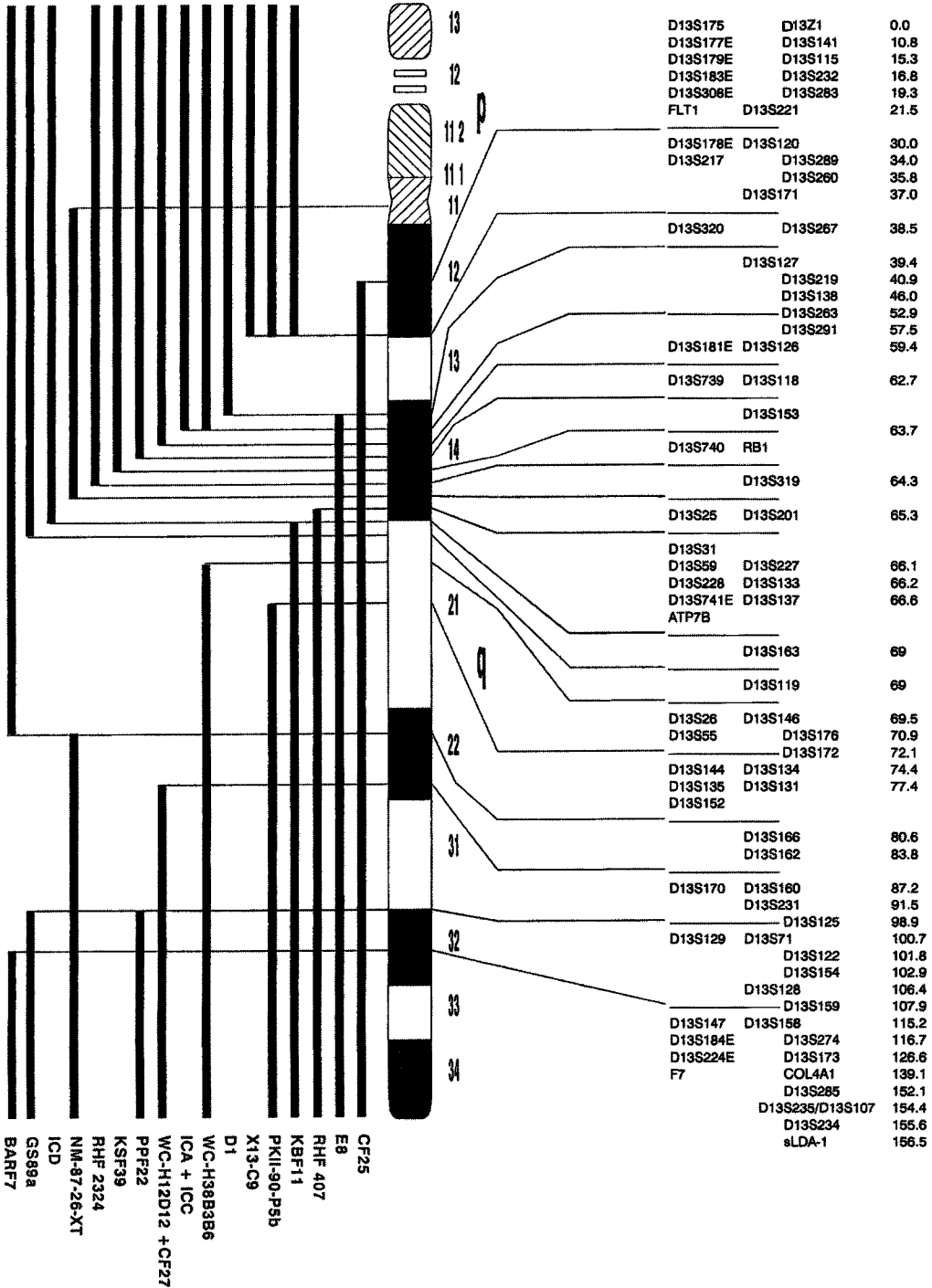
Table 1. Chromosome 13 loci used as STS in the deletion hybrid map

Locus	Amplified sequence	Reference No.	Locus	Amplified sequence	Reference No.
ATP7B	STS	3	D13S163	(CA) _n *	18
D13S25	STS	45	D13S166	(CA) _n *	18
D13S26	STS	16	D13S170	(CA) _n	18
D13S31	STS	41	D13S171	(CA) _n *	18
D13S55	STS	16	D13S175	(CA) _n	18
D13S59	STS	41	D13S177E	EST	48
D13S71	(CA) _n *	46	D13S178E	EST	48
D13S118	(CA) _n *	20	D13S179E	EST	48
D13S119	(CA) _n *	20	D13S181E	EST	48
D13S120	(CA) _n *	20	D13S183E	EST	48
D13S126	(CA) _n *	20	D13S184E	EST	48
D13S127	(CA) _n *	20	D13S224E	EST	48
D13S128	(CA) _n *	21	D13S201	(CA) _n *	39
D13S129	(CA) _n	21	D13S217	(CA) _n	18
D13S131	(CA) _n *	21	D13S221	(CA) _n *	18
D13S133	(CA) _n *	21	D13S227	(CA) _n *	45
D13S134	(CA) _n *	21	D13S228	(CA) _n	45
D13S137	(CA) _n *	21	D13S231	(CA) _n *	49
D13S135	(CA) _n	21	D13S308E	(CAT) _n	50
D13S144	(CA) _n	20	D13S319	(CA) _n *	16
D13S146	(CA) _n *	47	D13S320	(CA) _n	16
D13S147	(CA) _n	47	D13S739	(CA) _n	this study
D13S152	(CA) _n	18	D13S740	(CA) _n	this study
D13S153	(CA) _n *	18	D13S741E	EST	this study
D13S158	(CA) _n *	18	FLT1	(CA) _n	51
D13S160	(CA) _n *	18	F7	VNTR	52
D13S162	(CA) _n *	18	RB1	RFLP*	53

Asterisks indicate the most informative loci used for the genetic map. VNTR = Variable number of tandem repeat.

By a previously described approach [17, 37], two new markers were developed, D13S739, amplified by primers mgg11X: 5'-TGCTTCCTATGGCTGCCAG-3', and mgg11Z: 5'-CTGTCCGTGGAAGTTATGAG-3', and D13S740, amplified by primers mgg9X: 5'-GGGATAACAAAGAATGGAGG-3', and mgg9Z: 5'-GCTTAACTGTCTCTGATTCG-3'. D13S741E, amplified by primers mgg16X: 5'-CAAGTGCTACATACGGACA-3', and mgg16Y: 5'-GCTGACTCATATGGCCTTAG-3', is part of cDNA clone LGA3, identified when screening a liver cDNA library [40] with YAC 10H9.

Fig. 1. An integrated map of chromosome 13. The bars on the left schematically represent the breakpoints of the der(13) of the cell lines. Of the three columns of loci, the first two were physically mapped on the deletion hybrid panel to 1 of the 19 intervals, the last two were genetically ordered. The numerals represent the sex-average cumulative genetic distance from the centromeric region to the telomeric region in Kosambi cM. The KSF39 breakpoint is a genetic anchor for RB1, as the der(13) of this cell line has a break interrupting the RB1 gene [11]. The RB1 haplotype used in the genetic map [23] falls in intervals 7 and 8. D13S153 is also an RB1 intragenic marker.



Results and Discussion

Definition of Intervals

The markers used to construct the EURO-GEM map of chromosome 13 [23] were tested by radioactive polymerase chain reaction for their possible presence in one or more of the 19 hybrid cell lines. Thus, 13 intervals could be distinguished. To increase the resolution of the map, a further number of markers were tested, including those described previously [17]. All markers were tested at least in duplicate. Included were 37 CA repeats from different genetic maps 5 STS markers, generated from probes that detect RFLP, 4 genes and 9 EST markers (see table 1). Out of 30 potential intervals that can be inferred from the chromosome 13 breakpoints in the 19 cell lines, these 54 loci define 19 different intervals (see fig. 1, first and second row of loci). For all cell lines the cytogenetic breakpoints of chromosome 13 are known (for references see Materials and Methods). All breakpoints and deletions described, except the distal deletion of cell line KBF11 and the proximal breakpoint of cell line NM-87-26-XT, have been used in constructing the intervals. Some breakpoints, however, were indistinguishable from each other. When the intervals were portrayed alongside chromosome 13, no ambiguities appeared, neither with respect to groups of loci per interval, nor with respect to the locations of a few probes that have been well-assigned previously, including D13Z1 at the centromeric region [34], RB1 at 13q14.2-q14.3 [35], D13S31 at the junction of bands 13q14.3-21.1 [41], D13S71 at proximal 13q32 [42], D13S158 at 13q33 [43], and COL4A1 [36], D13S107, D13S234, and D13S235 at 13q34 [23, 37].

The 19 intervals divide 98 Mb of the long arm of chromosome 13, but are not evenly distributed, as 8 out of 19 intervals identified are in band 13q14.

The Genetic Map

A genetic linkage map was constructed along the deletion hybrid breakpoint map. Using the MultiMap program [24], an initial linkage map was constructed with the most informative microsatellite markers ordered by the breakpoints of the cell lines. This initial map consisted of 25 ordered loci (second row in fig. 1). It was subsequently used as a framework map for the incorporation of a further 26 highly informative markers taken from different genetic maps. Genotypes were generated as described [23], downloaded from public databases, or obtained from the authors. All orders determined genetically are supported by at least 1,000:1 odds. Markers that increased any interval with more than 10% of its original genetic length were not incorporated. The final result was a map consisting of 50 distinct loci, mostly ordered genetically. In interval 11 the order D13S227-D13S133-D13S137 has been determined, however, by mapping to a YAC contig (Kooy, unpubl. data, confirming results obtained previously with a different YAC contig [44]). The genetic map spans 156.5 cM (fig. 1). It is the first chromosome 13 map to approach the genetic endpoint of the long arm by including sLDA-1, a marker isolated from a telomeric YAC [38]. The average female to male recombination ratio is 0.169. An excess male over female ratio is observed in the (peri-)centromeric region, the telomeric region, and in the intervals between the markers D13S171-D13S118, D13S146-D13S176, and D13S154-D13S128.

By comparing markers from genetic maps of human chromosome 13, with our integrated map (fig. 1), and genetic marker can now in principle be assigned to a specific cytogenetic region. A direct application of the map can be found, e.g. in the selection of YACs to characterize by fluorescence in situ hybridization abnormalities of chromosome

13, preliminarily identified by conventional chromosome analysis. YACs to be used for a more detailed characterization should contain either an STS which is on the map or one which is not on the map but flanked by two others that are included in the map.

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