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An Integrated Physical Map of 210 Markers Assigned to the Short Arm of Human Chromosome 11

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Using a panel of patient cell lines with chromosomal breakpoints, we constructed a physical map for the short arm of human chromosome 11. We focused on 11p15, a chromosome band harboring at least 25 known genes and associated with the Beckwith–Wiedemann syndrome, several childhood tumors, and genomic imprinting. This underlines the need for a physical map for this region. We divided the short arm of chromosome 11 into 18 breakpoint regions, and a large series of new and previously described genes and markers was mapped within these intervals using fluorescence *in situ* hybridization. Cosmid fingerprint analysis showed that 19 of these markers were included in cosmid contigs. A detailed 10-Mb pulsed-field physical map of the region 11p15.3–pter was constructed. These three different approaches enabled the high-resolution mapping of 210 markers, including 22 known genes.

INTRODUCTION

Physical maps of human chromosomes will be of great help in the isolation and characterization of genes, including those that are disease related. Significant attention has been focused on the short arm of chromosome 11 because of its role in genetic disorders such as Wilms tumor and aniridia, both of which map to 11p13. Chromosome region 11p15 plays a role in various human neoplasia since specific loss of alleles from this region has frequently been demonstrated for Wilms tumor, rhabdomyosarcoma, adrenocortical carcinoma, hepatoblastoma, bladder carcinoma, and breast cancer (Seizinger *et al.*, 1991). This same region is associated with an overgrowth malformation syndrome, the Beckwith–Wiedemann syndrome (BWS), as demonstrated by linkage analysis and chromosomal rearrangements (Koufos *et al.*, 1985; Ping *et al.*, 1989; Waziri *et al.*, 1983; Mannens *et al.*, 1994). BWS children have a highly increased risk for the childhood tumors mentioned above. A number of the genes mapped to 11p15 are imprinted (Junien, 1992). A detailed 11p map will be of great help in identifying genes involved in BWS and tumor formation.

In this study we combined data obtained with metaphase and interphase fluorescence *in situ* hybridization (FISH), pulsed-field gel electrophoresis (PFGE) techniques, and cosmid contig construction, resulting in an accurate map of 210 11p DNA markers. The use of reference markers enabled us to integrate this new map with previously published maps.

MATERIALS AND METHODS

Patient cell lines. Lymphoblastoid cell lines from patients with the following chromosomal abnormalities were used: patient B01, 46,−11,+der(11)(11pter−11q23::11p13−11pter); patient B02, 46,−11,+der(11)(11pter−11p15.1::11p15.5−11qter) (described as patient 1 and patient 2 in Waziri *et al.*, 1983); patient B04, inv(11)(p15.4q22.3); patient B05, t(4;11)(p15.2;p15.3); patient B06, t(11;22)(p15.5;q12); patient B07, t(9;11)(p11.2;p15.5); patient B08, t(11;16)(p15;q12) (described as WH5.1, B10.1, B901, B(9;11)1.1, and 1217 in Mannens *et al.*, 1994); patient B12, t(11;12)(p15.5;q24.11); patient B15, 46,−14, +der(14),t(11;14)(p15.3;q32.3) (this study); patient A01, t(9;11)(9pter−9q31::11p14−pter;11qter−11p13::9q31−9qter) (described as A9.1 in Mannens *et al.*, 1991); patient A03, del(11)(p12p14) (described as YB in Gilgenkrantz *et al.*, 1982); patient A05, der(11)ins(1411)(q23;p12−14) (described as LEV7 in Lavedan *et al.*, 1989); patient A08, t(4;11)(q22;p13) (described as H.V. in Gessler *et al.*, 1989); patient A07, t(4;11)(q28;p14) (this study).

Markers. The 68 cosmid and phage probes used for *in situ* hybridization in this study were ZnFP83 (D11S776), ZnFP94 (D11S777),

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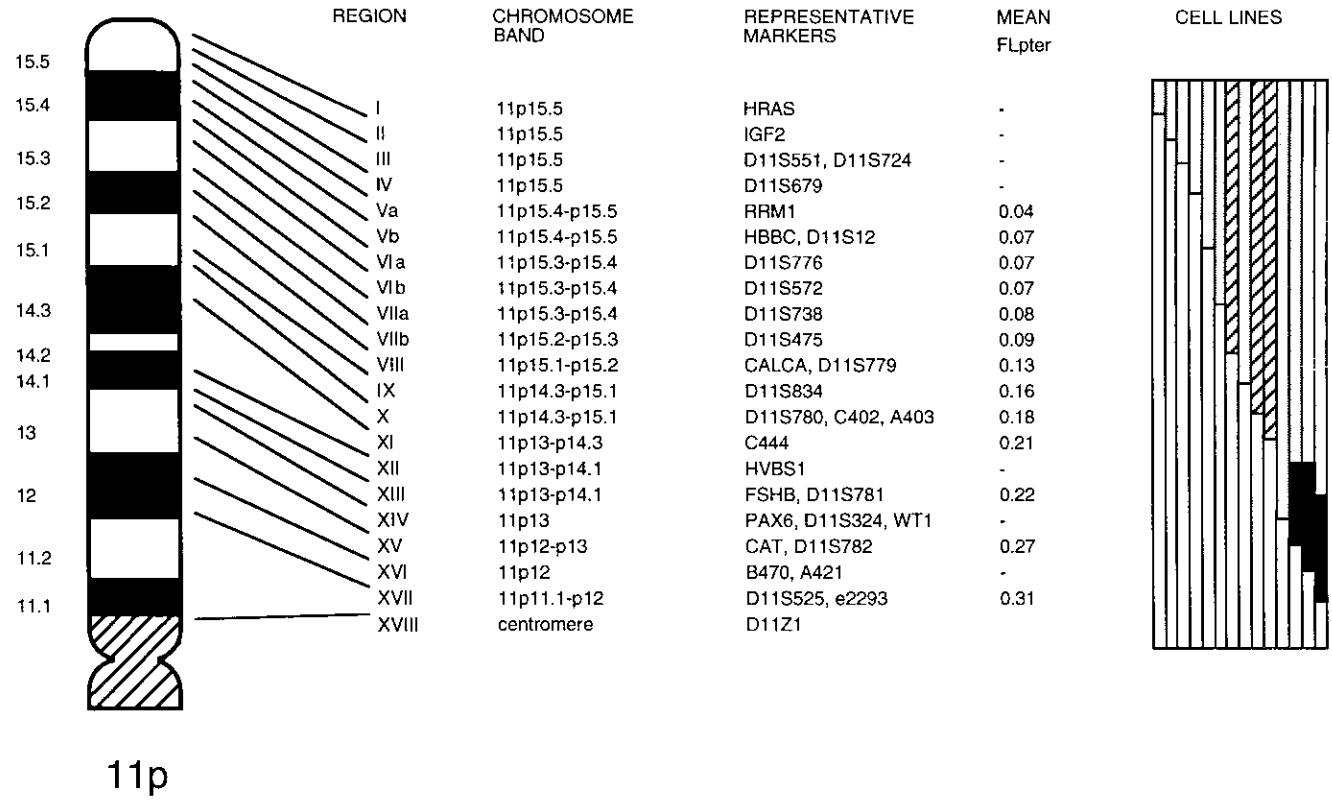


FIG. 1. Location of the 18 regions on the short arm of chromosome 11. The cell lines used to define the regions are indicated. From left to right, cell lines B12, B08, B06, B07, B04, B05, B15, A07, B02, B01, A08, A01, A03, and A05 are given. Gray, translocated or inverted segments; hatched, duplicated segments; black, deleted segments. The subdivision of regions V, VI, and VII is explained under Discussion. Representative markers for each region, determined with FISH, PFGE, or Southern analysis, are shown. Although not identical, the proximal breakpoints of cell lines A01 and A03 are virtually the same, since there are no markers between these breakpoints. The mean FLpter values for markers within various regions are as indicated. A dash indicates not measured.

ZnFP04 (D11S778), ZnFP40 (D11S779), ZnFP65 (D11S834), ZnFP17 (D11S781), and ZnFP13 (D11S782) (Hoovers *et al.*, 1992b); L07 (D11S494), L22 (D11S498), L29 (D11S501), L163 (D11S517), and L201 (D11S525) (Wadey *et al.*, 1990); cos536/537 (HRAS), C397, C428, C402, A403, C444, C445, C464, B470, and A421 (Harrison-Lavoie *et al.*, 1989); ZNF140 (clone 238); cosINS/IGF2 (IGF2/INS/TH) (De Pagter-Holthuizen *et al.*, 1987); e2328 (D11S12) (this study); cosHG50 (HBBC); cCl11-10 (D11S431), cCl11-38 (D11S441), cCl11-237 (D11S454), cCl11-253 (D11S459), cCl11-280 (D11S466), cCl11-289 (D11S470), cCl11-310 (D11S475), cCl11-314 (D11S477), cCl11-330 (D11S483), cCl11-385 (D11S551), and cCl11-440 (D11S572) (Tokino *et al.*, 1991); cCl11-565 (D11S601), cCl11-395 (D11S648), cCl11-421 (D11S657), cCl11-469 (D11S679), cCl11-479 (D11S682), cCl11-489 (D11S690), cCl11-516 (D11S704), cCl11-555 (D11S724), cCl11-546 (D11S719), cCl11-583 (D11S738), and cCl11-598 (D11S742) (Tanigami *et al.*, 1992; Hori *et al.*, 1992); cCl11p15-25 and cCl11p15-46 (Takita *et al.*, 1992); pLC11A (D11Z1) (Waye *et al.*, 1987); coshCT2 (CALCA) (Steenbergh *et al.*, 1984); cosSG7.1 (CALCB) (Steenbergh *et al.*, 1986); lambdaTG3 (CALCP) (Höppener *et al.*, 1988); FAT5 (PAX6), B2.1 (WT1), (Fantes *et al.*, 1992); p60 (D11S324) (Compton *et al.*, 1988); C1.1 (D11S87) (Lewis *et al.*, 1988); c19A2 (CARS) (Cruzen *et al.*, 1993); e3413 (D11S16) (Heding *et al.*, 1992); e3148, e1355, e2624, e3083, e2218, e3198, e2293, and ZnFP104 (this study).

The DNA probes from chromosome region 11p15 used for the pulsed-field mapping were pCD1.1 (CTSD) (Faust *et al.*, 1985); pB28.1 (DRD4) (Van Tol *et al.*, 1991); pADJ762 (D11S12) (Barker *et al.*, 1984); H19S1 (D11S813E) (Brannan *et al.*, 1990); pHd3.2 (HBBC) (Little *et al.*, 1980); pEJ6.6 (HRAS) (Shih and Weinberg, 1982); phins311 (IGF2) (Xiang *et al.*, 1987); MLP (Xu *et al.*, 1992); SMUC41 (MUC2) (Gum *et al.*, 1989); JER57 and JER58 (Van Cong *et al.*, 1990); Probe 2-1 (Brookes *et al.*, 1989); PRI(A) (RNH) (Lee *et al.*, 1988); pE2.8 (RRM1) (Byrne and Smith, 1990); nonrepetitive subclones were used from cosmids cCl11-330 (D11S483), cCl11-p15-49, cCl11p15-25, cCl11p15-46, cCl11-385 (D11S551), cCl11-555 (D11S724), e3760, cCl11-469 (D11S679), cCl11-395 (D11S648), L163 (D11S517), ZnFP104, cCl11-253 (D11S459), cCl11-289 (D11S470), cCl11-479 (D11S682), cCl11-546 (D11S719), ZnFP83 (D11S776), cCl11-421 (D11S657), cCl11-10 (D11S431), cCl11-280 (D11S466), and cCl11-440 (D11S572).

The clones of the cosmid contigs are listed in Table 2.

Experimental procedures. Probe labeling and *in situ* hybridizations on metaphase chromosomes were performed as described previously (Hoovers *et al.*, 1993). For two-color *in situ* hybridization, biotinated and dioxiginated probes were hybridized simultaneously to the target DNA and detected by different fluorochromes, FITC (green) and TRITC (red), according to Wiegant *et al.* (1991). Map positions of the probes were expressed as the fractional length of the whole chromosome relative to pter (FLpter), with a 95% confidence interval (Lichter *et al.*, 1990). *In situ* hybridizations and probe ordering on interphase chromosomes were performed according to Trask *et al.* (1991).

Southern hybridization and dosimetric analysis were carried out as previously described (Mannens *et al.*, 1991).

PFGE, including DNA preparation, digestion, electrophoresis, blotting, hybridization, and washing, was performed as described previously (Hoovers *et al.*, 1993).

As part of an ongoing research program to construct an overlapping cosmid clone map for the short arm of human chromosome 11, all cosmids used in the present study were fingerprinted to identify potential overlaps with other cosmids in the database for the con-

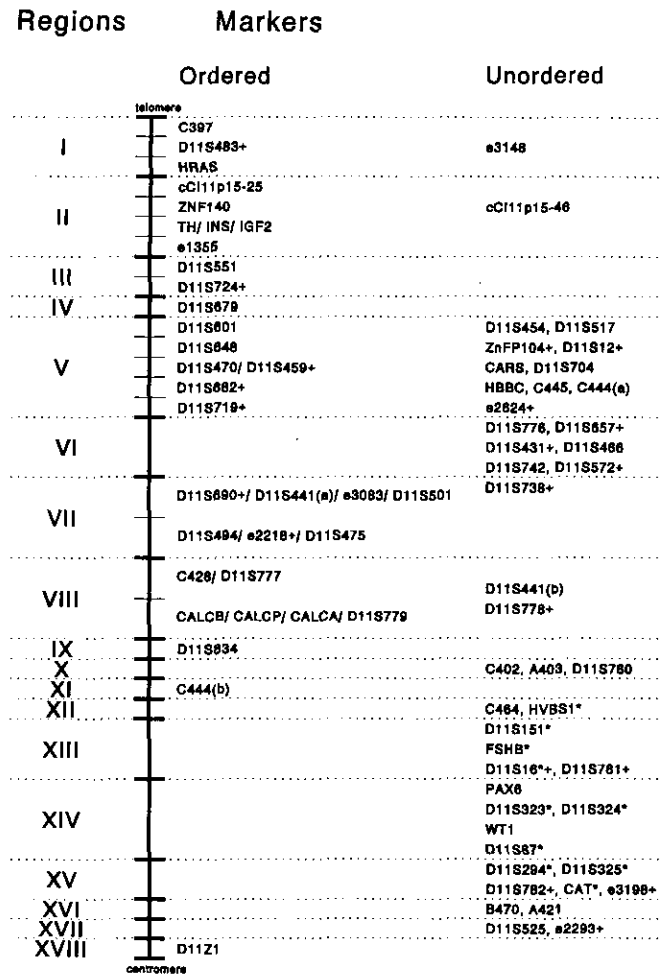


FIG. 2. FISH mapping of markers to the breakpoint regions along the short arm of chromosome 11. Most of the markers from regions I, II, III, and V were ordered using (multicolor) FISH on interphase chromosomes. Markers from regions VII and VIII were ordered using FLpter measurements (Table 1). Markers C444 and D11S441 show signals on two different positions on 11p, C444 in regions V and XI, D11S441 in regions VII and VIII. Probes marked with an asterisk are mapped relative to deletion breakpoints with Southern blot hybridization. The mapping of D11S16, D11S324, and D11S87 is also confirmed with FISH. Probes marked with a plus indicate cosmids integrated in cosmid contigs (Table 2).

struction of cosmid contigs. Cosmid fingerprints are analyzed by a computerized matching procedure, and contig size and fractional overlap between cosmids are calculated. Fingerprinting and construction of contigs at the Imperial College were performed as described by Heding *et al.* (1992).

RESULTS

FISH Mapping

Regional localization of a large series of 11p cosmid and phage clones was carried out with (multicolor) FISH techniques. First, we localized 68 markers by hybridization to metaphase chromosomes and determined the relative position of the marker (FLpter) on the chromosome. A panel of patient cell lines with breakpoints in chromosome 11p enabled us to divide 11p into 18 different chromosome regions (Fig. 1). Second, we precisely established the localization of the 68 markers to these 18 chromosome regions (Fig. 2).

For chromosome regions I to V, 16 markers were further ordered using multicolor FISH (Fig. 3) on interphase chromosomes. Region I, the most distal one, contains four markers. The order of three markers within this region has been determined, with C397 the most distal marker on this map. Also, for regions II to V, the order is presented in Fig. 2.

FLpter values with a 95% confidence interval were used to order the markers within regions VII and VIII (Table 1). Within region VII, D11S690, D11S441 (a), e3083, and D11S501 are ordered distal to D11S494, e2218, and D11S475. No further ordering could be established within this region. Within region VIII, C428 could be ordered distal to D11S441 (b), D11S778, CALCB, CALCP, CALCA, and D11S779. Marker D11S777 is distal to CALCB, CALCP, CALCA, and D11S779.

Two markers previously mapped to 11p (Tanigami *et al.,* 1992; Wadey *et al.,* 1990) were assigned to the q arm. D11S498 was localized to 11q24.2–q25, and D11S477 was localized to 11q23.3–q24.1 (data not shown).

Southern Analysis

The 11p13–11p14 reference markers HVBS1, D11S151, FSHB, D11S16, D11S323, D11S324, D11S87, D11S294, D11S325, and CAT were mapped by Southern blot analysis and dosimetric measurement relative to deletion breakpoints in cell lines A01, A03, and A05 (Mannens *et al.,* 1991) (see Fig. 1). The data presented in Fig. 2 show that HVBS1 is located in region XII, D11S151 and FSHB are located in region XIII, D11S323 is located in region XIV, and the markers D11S294, D11S325, and CAT are located in region XV. The mapping of D11S16 to region XIII and D11S324 and D11S87 to region XIV is confirmed with FISH.

Cosmid Contig Construction

All cosmids used in the present study were fingerprinted to identify potential overlaps with other cosmids in a database for the construction of cosmid contigs. This database contains 7438 fingerprinted cosmids from chromosome 11, with 3401 cosmids (46%)

FIG. 3. *In situ* hybridization of D11S781 combined with the centromeric probe D11Z1 on metaphase chromosomes of patient A03. D11S781 is deleted on the del(11) chromosome (**A**). D11S517 on metaphase chromosomes of patient B06. The signal of D11S517 is visible on both the normal chromosome 11 and the der(11) chromosome. D11S517 is therefore located proximal to the breakpoint (**B**); D11S679 (green) as a reference marker and the more proximal probes D11S601 (green) and D11S648 (red) on interphase chromosomes. D11S648 is mapped proximal to D11S601 (**C**).

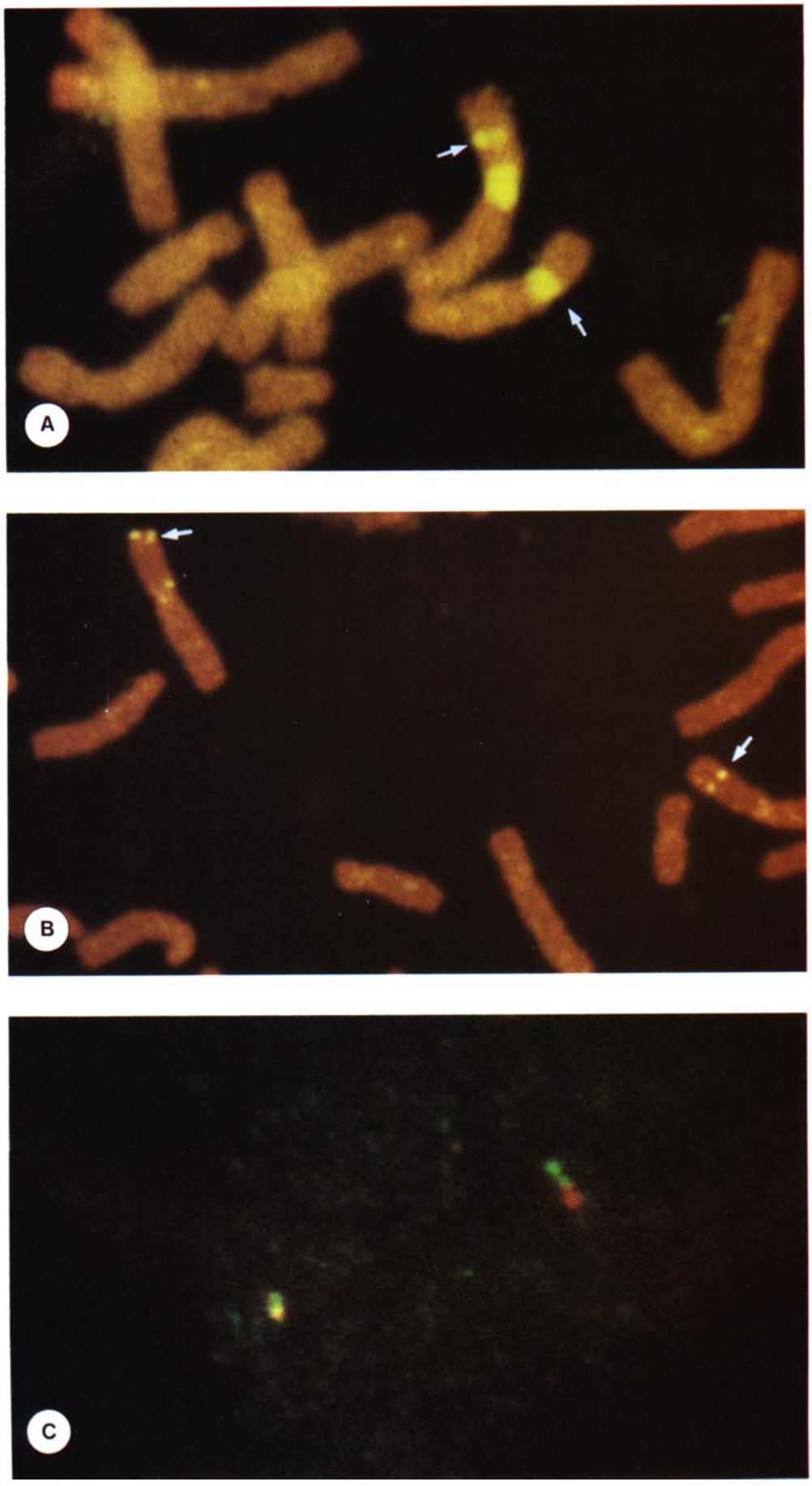

A
B
C

TABLE 1

Markers from Regions VII and VIII Ordered Using FLpter Measurements

Marker	Locus	FLpter (95% CI)
	Region VII	
cCI11-489	D11S690	0.056–0.076
cCI11-38-a	D11S441	0.058–0.079
e3083		0.062–0.087
L29	D11S501	0.063–0.086
L07	D11S494	0.092–0.116
e2218		0.095–0.114
cCI11-310	D11S475	0.096–0.119
	Region VIII	
C428		0.089–0.113
ZnFP94	D11S777	0.100–0.118
cCI11-38-b	D11S441	0.114–0.140
ZnFP04	D11S778	0.113–0.144
cosSG7.1	CALCB	0.122–0.143
lambdaTG3	CALCP	0.121–0.147
coshCT2	CALCA	0.131–0.148
ZnFP40	D11S779	0.132–0.151

Note. The order of two markers is established only when their 95% confidence intervals do not overlap.

within contigs (949 contigs). Within the database the contigs have an average size of 56 kb, and the cosmids within these contigs have an average overlap of 56%. Nineteen contigs could be constructed after fingerprint analysis of the cosmids that we mapped with FISH. These contigs contain 2 to 21 cosmids; the calculated sizes range from 36 to 153 kb (Table 2).

PFGE Mapping

The reference lymphoblastoid cell line AMSTR2 was used as a reference cell line for the pulsed-field mapping of 35 11p15 markers. Of these markers, 23 were also assigned to 11p15 by FISH (this paper). The rare-cutting restriction enzymes *Mlu*I, *Not*I, *Asc*I, *Sal*I, *Cla*I, *Bss*HII, and *Nru*I were used in an attempt to localize and link these 35 markers. A summary of the fragment sizes obtained with the 35 probes is listed in Table 3.

Most of the listed fragments are partials. Up to three of the largest bands are given in Table 3. The pulsed-field map thus generated is presented in Fig. 4.

Table 3 and Fig. 5 show that markers D11S483, cCl11p15-49, DRD4, HRAS1, RNH, and cCl11p15-25 recognize the same 650-kb *Mlu*I partial fragment. These six markers could be linked with MLP, MUC2, and probe 2-1 on the same *Cla*I fragment. MLP, MUC2,

TABLE 2

Mapping of 19 Cosmid Contigs to the Breakpoint Regions

Locus	Region	Size	Cosmids with their relative orientation
D11S483	I	88	D11S483–cCIp23–e3763–e2026–e1567–e216–u827–e2170–e2376–e4269 (e856, E1384, e2042, e4278, u2410)
D11S724	III	153	u820–e4228–q34–q32–q8–e3528–e3760–e2992–e3735–e1927–E1352–D11S724 (E494, c1024, e1579, e1673, e2107, e2813, e4965, q22, q36)
D11S459	V	81	e790–c1258–D11S459–cCIp18
ZnFP104	V	102	e570–ZnFP104–e1012–e2011 (E534, e776, E1186)
D11S682	V	36	e23591–D11S682
e2624	V	62	e2624–e2461–e2627
D11S12	V	81	e29441–e2313–e2328–e2819–e4178–e4101 (e3203, e31392, e3770)
D11S719	V	70	e1729–D11S719–u94 (e3250, m76)
D11S657	VI	85	e3351–e3903–D11S657–u36
D11S572	VI	117	e2611–e3577–e1021–e2041–e2053–D11S572–c959 (c2310)
D11S431	VI	55	q17–u943–D11S431
D11S738	VII	63	D11S738–e3395–e3311
e2218	VII	120	c3598–e831–e2951–e2218–e1120–m5364 (e836, e1478, e1682, e1686, e2704, e2823, e3325, e3394, e3533, e4288, e4334)
D11S690	VII	127	e4149–4883–D11S690–c748–e3151–e854–e2386–e1732 (e253, e1422, e1844, e2263, e31321, e3411, e4521)
D11S778	VIII	54	e3773–e1778–e3094–D11S778 (e1781)
D11S16	XIII	98	q119–e3413–e3745–D11S781–e4377–u2668–e1113 (e4378)
e3198	XV	44	e2598–e3198–e3690 (e3687)
D11S782	XV	72	D11S782–e2837–e1481–u160
e2293	XVII	94	e3746–e4268–e3362–e4839–e2674–e3405 (e904, e2293, e3478, e3948)

Note. Underlined cosmids are mapped in the chromosome regions by FISH and/or PFGE. The sizes of the contigs are calculated sizes in kb. The relative order of the canonical cosmids of all contigs is given. The buried cosmids, not informative for the contig, are in parentheses. Markers cCI11p15-18 and cCI11p15-23 are abbreviated as cCIp18 and cCIp23. The D11S16 cosmid contig also contains D11S781 as described before (Heding *et al.*, 1992).

TABLE 3

Summary of Restriction Fragment Sizes

	Enzyme						
Probe	*Mlu*I	*Not*I	*Asc*I	*Sal*I	*Cla*I	*Bss*HII	*Nru*I
D11S483	120	s.f.		300	<50		
	650			350	880		
cCI11p15–49	530	s.f.		300	760		
	650			350	880		
DRD4	530				760		
	650				880		
HRAS1	530	s.f.		220	760	s.f.	s.f.
	650			350	880		
RNH	530	s.f.					
	650						
cCI11p15–25	530						
	650						
MLP	220			280	760	400	
	460			380	880	480	
MUC2	220	350		280	760	400	720
	460	600		380	880	480	
probe 2–1	220			280	760	400	
	460			380	880	480	
JER58	220	350		100		400	
(MUC5)	460	600		380		480	
JER57	240	250		100		400	
(MUC5)	460	600				480	
ZNF140	220	250	730	180	s.f.	400	
	460	600				480	
CTSD	680	320	730	280	140	230	470
	740			390		390	620
cCI11p15–46	680	320	730	140	s.f.	230	
	740					390	
H19	680	300	730	s.f.	300	130	470
	740	550			430		620
IGF2	680	300	730	s.f.	120	s.f.	410
	740	550			430		1000
D11S551	500	110	490	s.f.	340	320	410
	680	300	820			480	1000
		460					
D11S724	500	110					
	680	300					
		460					
e3760	500	110				320	
	680	300				480	
		460					
D11S679	500	160		s.f.	s.f.	100	590
	680	350				480	1000
		460					
D11S648	200	50		280	200	50	590
D11S517	200						1000

TABLE 3—*Continued*

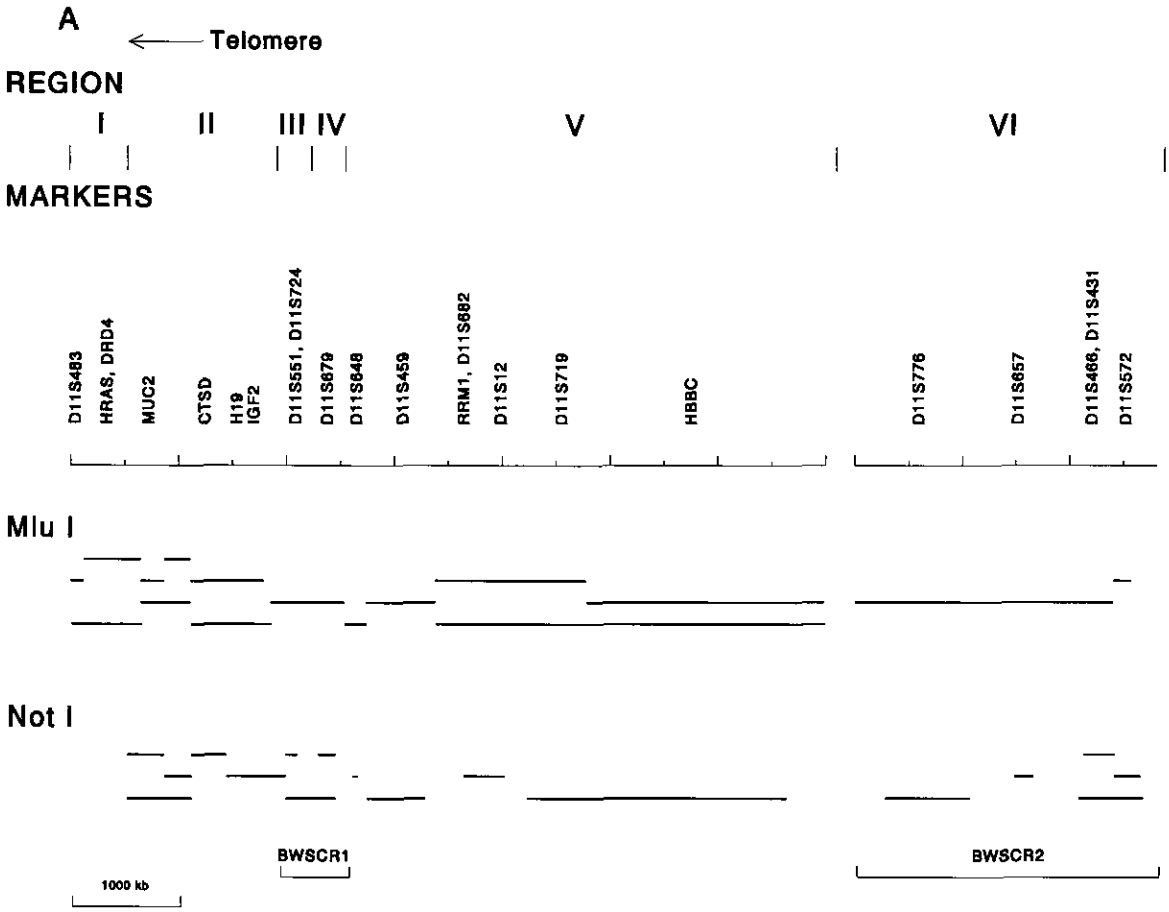
Probe	Enzyme						
	*Mlu*I	*Not*I	*Asc*I	*Sal*I	*Cla*I	*Bss*HII	*Nru*I
D11S470	650	390		420		170	
		540					
D11S459	650	390	410	420		170	
		540					
ZnFP104	650	390		420		170	
		540					
RRM1	1400	380		350			
	3600			440			
D11S682	1400	380				340	
	3600						
D11S12	1400	380		350		340	
	3600			440			
D11S719	1400	2400				1300	
	3600						
HBBC	2200	2400		310		1300	
	3600			620			
D11S776	2400	790		960		440	
						580	
D11S657	2400	70	420	340			900
		180		460			
D11S466	2400	290	390	570		180	430
		600		680			
D11S431		290		570		180	
		600		680			
D11S572	170	240		300	400	220	200
		600			490		

Note. D11S483 is the most telomeric marker, D11S572 the most centromeric one. Sizes are in kb. Boxes indicate the pulsed-field fragments recognized by more than one probe. s.f., small fragment (<50 kb), not informative.

and probe 2-1 produced identical hybridization patterns, and their relative order remains unclear. In addition, they recognize the same *Mlu*I and *Bss*HII partials as ZNF140, JER57, and JER58. The markers ZNF140, CTSD, cC11p15-46, H19, and IGF2 are located on a 730-kb *Asc*I fragment. The markers D11S551, D11S724, e3760, and D11S679 hybridize to identical *Mlu*I, *Not*I, and *Bss*HII partials. These markers are located on the same 1000-kb *Nru*I fragment as IGF2 and D11S648. Markers D11S517 and D11S648 are localized on a 200-kb *Mlu*I fragment. This region is covered with a 300-kb YAC from D11S517 (L. Kalikin, manuscript in preparation). This YAC also contains D11S679, D11S648, and D11S470. The markers D11S470, D11S459, and ZnFP104 recognize the same 650-kb *Mlu*I, 390- and 540-kb *Not*I, 420-kb *Sal*I, and 170-kb *Bss*HII fragments and could not be ordered.

From partial digestions we know that the proximal adjacent *Mlu*I fragment is a very large fragment of approximately 3600 kb. RRM1, D11S682, D11S12, D11S719, and HBBC are located on this large *Mlu*I fragment. In addition, it was found that D11S719 and HBBC also recognize the same 2400-kb *Not*I and 1300-kb *Bss*HII fragments.

FIG. 4. (**A**) Pulsed-field map for regions I to VI (because of the high marker density, not all markers of the telomeric part are shown). The relative orientation of the markers is given along a distance scale. The distance between HBBC and D11S776 is not known, since no overlapping fragments between these two markers could be found. Markers that are unordered to each other are placed on one line. The *Mlu*I and *Not*I fragments are shown. Regions I to V are contiguously linked from D11S483 to HBBC. In addition, the pulsed-field data for region VI are shown. The positions of BWS cluster regions 1 (BWSCR1) and 2 (BWSCR2) (Mannens *et al.*, 1994) are shown. (**B**) Pulsed-field map of the most distal 3000 kb (regions I to V) in detail. Markers for which the relative orientation is unknown are placed on one line. The *Mlu*I, *Nru*I, and *Cla*I fragments are shown.


A
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MARKERS
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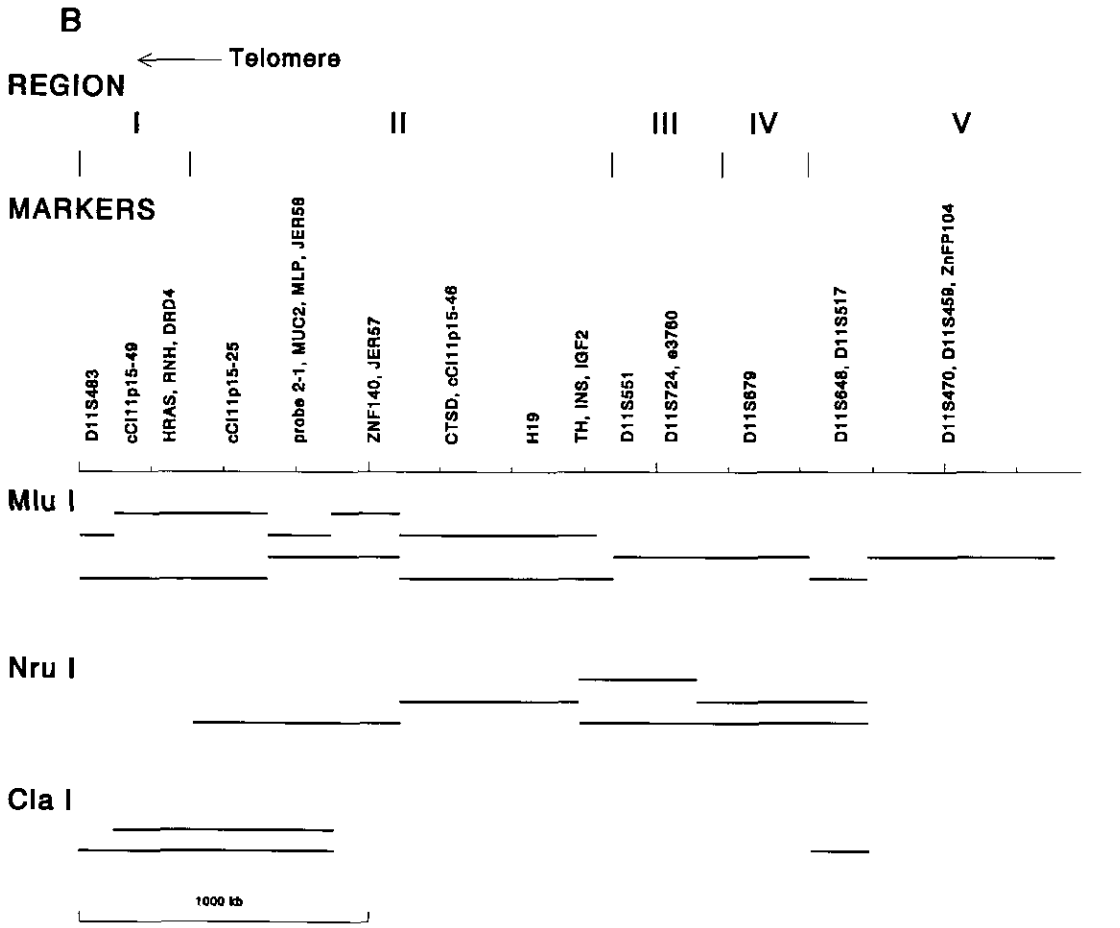
BWSCR2
1000 kb


B
Telomere
REGION
I
II
III
IV
V
MARKERS
D11S483
cCI11p15-49
HRAS, RNH, DRD4
cCI11p15-25
probe 2-1, MUC2, MLP, JER58
ZNF140, JER57
CTSD, cCI11p15-46
H19
TH, INS, IGF2
D11S551
D11S724, e3760
D11S679
D11S848, D11S517
D11S470, D11S459, ZnFP104
Mlu I
Nru I
Cla I
1000 kb

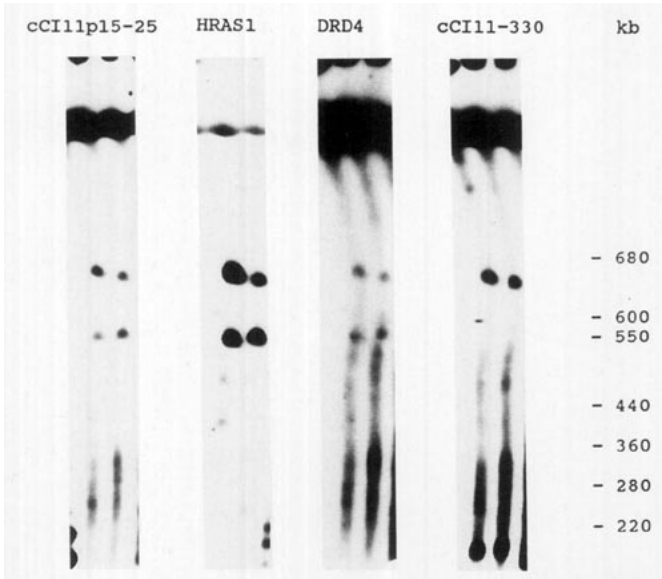


FIG. 5. Subsequent hybridization of cCl11p15–25, HRAS1, DRD4, and cCl11–330 (D11S483) to a blot with two lanes of *Mlu*I-digested DNA. HRAS1, DRD4, and cCl11p15–25 recognize bands of 530 and 650 kb. Marker cCl11–330 recognizes bands of 120 and 650 kb and maps distal to the previous three markers.

In the proximal region of this pulsed-field map, we were able to localize D11S466, D11S431, and D11S572 to the same 600-kb *Not*I partial fragment. D11S776, D11S657, and D11S466 recognize a 2400-kb *Mlu*I fragment.

DISCUSSION

In this study we describe the localization of 210 markers on the short arm of chromosome 11, with special emphasis on 11p15. Markers were positioned by FISH using fractional length measurement and mapped relative to a panel of chromosomal breakpoints, defining 18 regions. Cosmid contigs were identified for 19 mapped cosmids. A detailed PFGE map of the region 11p15.3–pter is constructed.

The combined use of FISH and PFGE mapping confers many powerful advantages over single-approach methods. The PFGE, contig, and FISH data presented in this paper show an internally consistent picture.

Markers from four regions were ordered with FISH on interphase chromosomes (Fig. 2). This ordering was in full agreement with the findings of the pulsed-field mapping and vice versa. FLpter values from many markers were determined. We found that the use of FLpter values for ordering markers within regions is limited, since the 95% confidence intervals of the determined FLpter values of most markers overlap with those of other markers.

A 10-Mb pulsed-field map was constructed using 35 markers of chromosome bands 11p15.5–11p15.3. This is a substantial portion of 11p, since the total length of 11p is calculated to be 54 Mb (Junien and van Heyningen, 1991). The most distal marker we could locate on the pulsed-field map is D11S483. However, with multicolor FISH we demonstrated that C397 is even more distal.

The most distal known genes on chromosome 11p15 are HRAS, RNH, and DRD4, which map on the same 530-kb *Mlu*I fragment. Markers cCl11p15-49 and cCl11p15-25 also map on this *Mlu*I fragment. The FISH analysis showed that HRAS maps in region I, distal to cCl11p15-25 (region II). Schneider *et al.* (1992) mapped RNH within 90 kb from HRAS, which is in agreement with our findings. Based on linkage analysis, DRD4 was found to map close and most likely proximal to HRAS (Gelernter *et al.*, 1992; Petronis *et al.*, 1993). Richard *et al.* (1993), however, mapped DRD4 distal to HRAS, using radiation hybrids. We now map DRD4 within 530 kb from HRAS. The order of the four probes could not be established with PFGE mapping.

The IGF2 and H19 genes are within 300 kb on the same *Not*I fragment. Zemel *et al.* (1992) mapped IGF2 and H19 to the same 200-kb *Bss*HII fragment. With the reference EBV lymphoblastoid cell line that we used, this finding could not be confirmed. This region was shown to be hypomethylated in various tumors and EBV cell lines (Mannens *et al.*, 1994), explaining this discrepancy. Our data revealed that H19 is located distal to IGF2. H19 recognizes the same *Nru*I fragment as CTSD. Our mapping of H19 proximal to CTSD is in agreement with the radiation hybrid map of Richard *et al.* (1993). IGF2 is located on the same *Nru*I fragment as D11S551, D11S724, e3760, and D11S679. These latter probes detect the same *Mlu*I, *Not*I, and *Bss*HII partials and define the distal BWS breakpoint cluster region (Mannens *et al.*, 1994).

The constructed pulsed-field map covers a 10-Mb region of 11p15.5–11p15.3, with FISH defined as regions I to VI, and harbors loci involved in BWS and tumorigenesis. As demonstrated on patients with balanced translocations, two different regions are involved in BWS (see Fig. 4A and Hoovers *et al.*, 1992a; Mannens *et al.*, 1994). The distal BWS region is near IGF2. The proximal BWS region is defined with FISH near region VI. This latter BWS region is not contiguously covered by the pulsed-field map presented in this study. Additional markers in this region are necessary to close this gap.

Several regions implicated in the etiology of tumorigenesis have been covered by the pulsed-field map. These include rhabdomyosarcoma, distal to D11S12, hepatoblastoma, distal to HBBC, and Wilms tumor, distal to D11S12. A possible second Wilms tumor locus at 11p15.3 proximal to HBBC may not be fully covered by this map (Junien, 1992).

Positive R-bands, particularly the telomeric bands, are in general gene-rich (Junien and van Heyningen, 1991; Saccone *et al.*, 1992). The gene density in the most distal 2000 kb of 11p15.5 is much higher than that in the remaining part of 11p (Fig. 4B). At least three mucin genes are located in that region, an intesti-

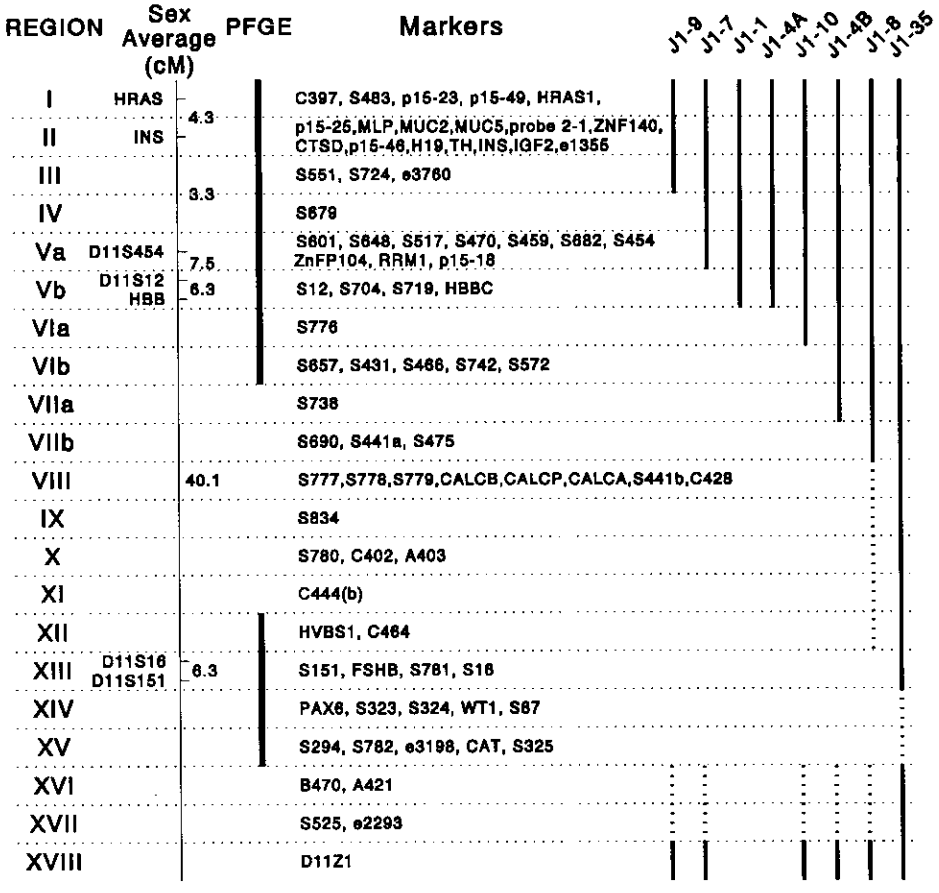


FIG. 6. Integration of various maps for the short arm of chromosome 11. On the left, the 18 breakpoint regions (this study) and the integration of a linkage map of 11p (Kramer *et al.*, 1992) to this map. Genetic distances between the reference linkage markers are given in cM. Regions I to VI are covered with PFGE (this study), as well as regions XII to XV (Compton *et al.*, 1988; Gessler and Bruns, 1989). The markers from this study that could be integrated are shown in the middle. On the right, the contents of eight J1-hybrids (Glaser *et al.*, 1989; Tanigami *et al.*, 1992; Puech *et al.*, 1992). Boldface vertical lines indicate the portion of chromosome 11p that each hybrid cell line contains. Dotted vertical lines indicate that the border of the breakpoint is not known.

nal mucin gene (MUC2), a tracheobronchial mucin gene (MUC5), and the intestinal mucin-like protein (MLP). The ribonuclease inhibitor gene (RNH), an inhibitor of ribonucleolytic and angiogenetic activities involved in tumor progression, is assigned close to HRAS. The gene for the dopamine D4 receptor is located in this same region. This receptor has a high affinity for clozapine, an effective drug for the treatment of schizophrenia. Cathepsin D, a lysosomal endoprotease, is also located in the most distal 2000 kb of the short arm of chromosome 11. Genetic disorders such as long-QT syndrome, Usher syndrome type 1C, and T-cell leukemia, are also localized to 11p15. Long-QT is mapped close to HRAS on 11p15.5 (Keating *et al.*, 1991). The locus for Usher syndrome type 1C is assigned to 11p14–11p15.3 (Smith *et al.*, 1992). Chromosome bands 11p15 and 11p13 are consistent sites of chromosome rearrangement in T-cell leukemia (Boehm *et al.*, 1991; Royer-Pokora *et al.*, 1991).

Construction of cosmid contigs are of great value for the characterization of disease-associated chromosome regions. In this study we mapped 19 constructed contigs along the short arm of chromosome 11. A number of these are close to BWS breakpoints. Contigs of markers D11S724, D11S657, D11S431, and D11S572 are located between BWS breakpoints. Efforts to expand these contigs to cross these and other BWS breakpoints are now in progress. The Imperial College database of fingerprinted cosmids contains about 37% of chromosome 11 in cosmid contigs. The high coverage makes it likely that most new cosmids fall within contigs of the database. Three of the nineteen mapped contigs of this study also contain a second previously described cosmid. The D11S483 contig also contains cCl11p15-23, the D11S459 contig also contains cCl11p15-18, and the D11S16 contig also contains D11S781.

Maps of the short arm of chromosome 11 were constructed using linkage analysis (Weissenbach *et al.*, 1992; Kramer *et al.*, 1992), PFGE (Compton *et al.*, 1988; Gessler and Bruns, 1989), and hybrid breakpoints such as the J1 hybrid series (Glaser *et al.*, 1989; Tanigami *et al.*, 1992; Puech *et al.*, 1992; Call *et al.*, 1992; Richard *et al.*, 1993). Integration of the combined data will greatly facilitate the completion of the physical map

for 11p. For our map we mainly used cosmid probes because of their easy use in *in situ* hybridizations and their suitability for contig construction. To ensure the integration with existing maps, we included previously described and commonly used markers for the FISH and PFGE mapping. For a number of these markers, sequenced-tagged sites are described (Miwa *et al.*, 1993; Richard *et al.*, 1993). Figure 6 shows the integration of several 11p maps. Seven of eleven 11p markers used for the genetic 11p map published by Kramer *et al.* (1992) could be physically mapped to the breakpoint regions. Our data combined with literature data reveal that pulsed-field maps are now available for 12 of the chromosome 11p regions described in this study. Mapping data of eight J1 hybrid cell lines could be integrated into our map. Using these latter data (Glaser *et al.*, 1989; Tanigami *et al.*, 1992; Puech *et al.*, 1992), three additional regions could be defined. Region V contains 17 markers. Thirteen of these markers were previously mapped relative to the J1-7 hybrid breakpoint and can be subdivided to define subregions Va and Vb. Region VI can be subdivided by the J1-10 hybrid breakpoint and region VII by the J1-4B hybrid breakpoint. This shows that integration of J1-breakpoints can give a refinement of our breakpoint map. Additional J1 breakpoints will be studied so that all published data can be incorporated into our map.

This study describes the construction of a detailed cloned DNA map for the short arm of human chromosome 11, with emphasis on 11p15. The positions of known genes, reference markers, and breakpoints have been integrated into it. We will use this map for the construction of YAC contigs and cosmid contigs. At present, we have isolated new YACs for H19, IGF2, D11S551, D11S724, D11S517, D11S12, HBBC, D11S776, D11S431, D11S466, and D11S501 (Kalikin *et al.*, manuscript in preparation), and we will continue to expand the map using chromosome 11p cosmid and YAC libraries.

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