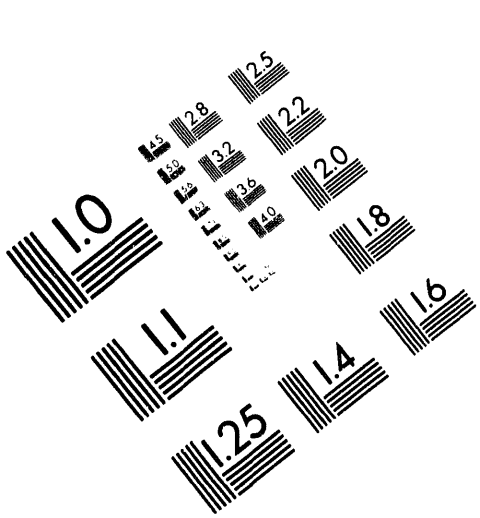

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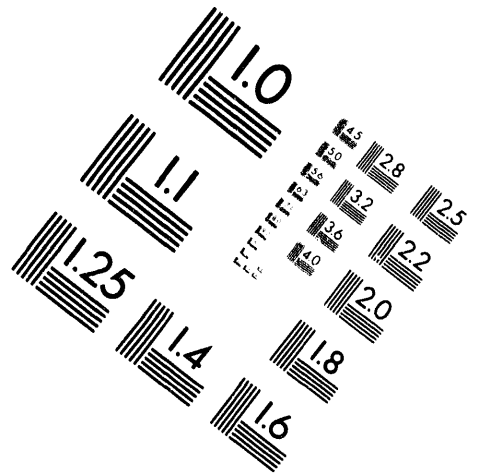

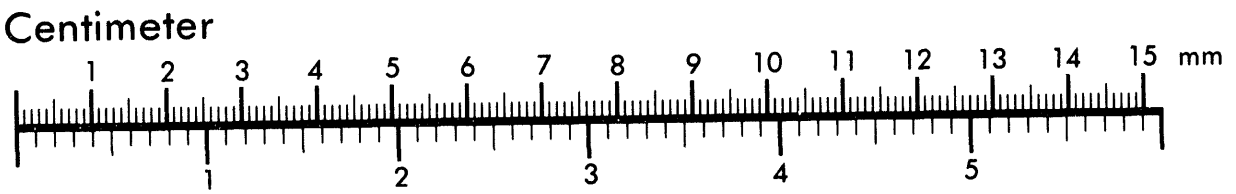

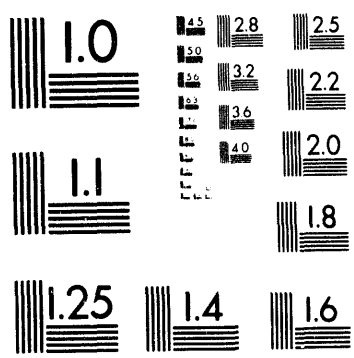

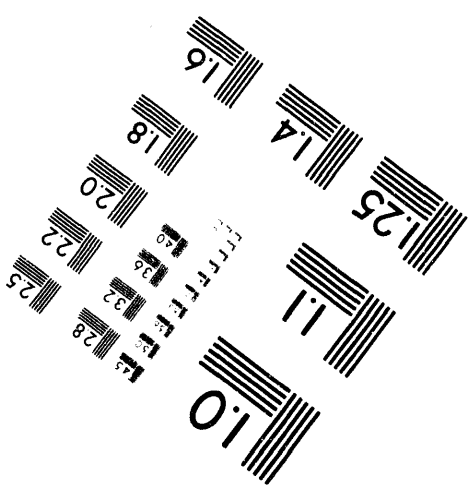

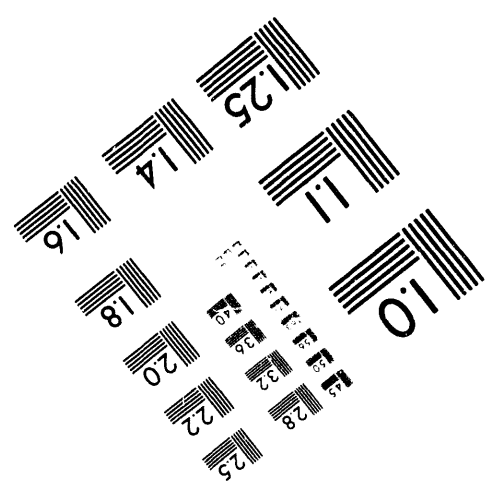

1 of 1

Report of the first international workshop on human chromosome 8 mapping

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The first international chromosome 8 workshop was held in Vancouver, Canada May 2-4, 1993. The conference was attended by 23 participants from Australia, Canada, Germany, the Netherlands, Sweden, the United Kingdom and the United States.

The workshop was supported by CGAT/CTAG (Canadian Genome Analysis & Technology Program/Programme Canadien de Technologie & D'Analyse du Génome) as well as by travel funds allocated by the National Institutes of Health and the Department of Energy of the United States and by agencies within the countries of overseas participants.

The goals of the workshop were to evaluate new locus assignments, review new data obtained for previously assigned loci, develop a consensus marker order for chromosome 8, assess and integrate physical mapping information, identify resources and foster collaboration.

New gene assignments

Four new gene assignments were reported at this workshop; CEBPD, CMT4, EBN2, and EXT1. In addition data suggesting that putative loci affecting tumor suppression and vertebrate segmentation are located on chromosome 8 were presented. The CEBPD locus, encoding the CCAAT/enhancer binding protein delta that is a member of the family of leucine zipper transcription factors, maps between PLAT and D8S165 (Cleutjens et al., 1993). Other loci are disease associated and are described below.

Physical mapping

Much of the physical mapping of markers on chromosome 8 has relied on the somatic cell hybrid panel of Wagner et al. (1991). This panel divides the chromosome into 10 regions, A-J. Localizations of four cloned genes on this panel were reported: SFTP2 and POLB in region C; CEBPD in region D, and IL7 in region F. Localization of SFTP2 was further refined to 8p21 by FISH. A number of polymorphic STR markers were also localized on this panel, including 12 markers from the Weissenbach et al. (1992) genetic linkage map (Fig. 1).

A new panel of hybrid cells that divides the large I interval into nine subintervals was reported. Using this

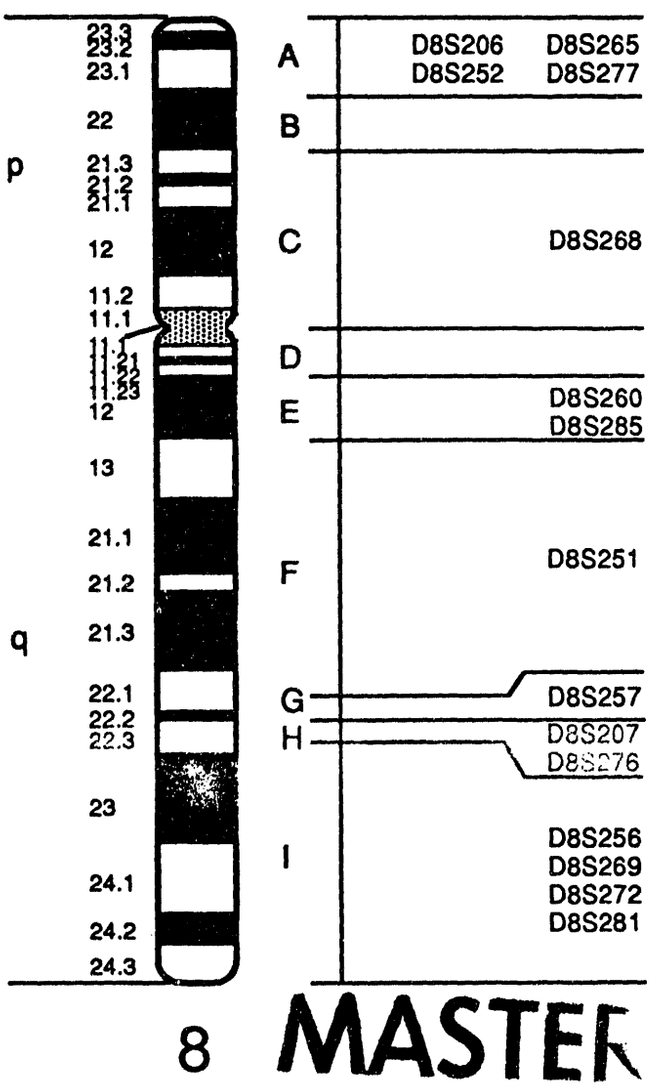

Fig. 1. Polymorphic markers mapped in somatic cell hybrids

new panel two STR markers, D8S269 and D8S281, that could not be ordered by genetic linkage analysis were placed into separate subintervals. This physical ordering places D8S281 proximal to D8S269. These markers flank the Langer-Giedion syndrome chromosome region (LGCR). Nine loci spanning the LGCR were ordered, using a combination of YAC contig and deletion mapping,

as follows: cen-D8S42-D8S50-D8S98-D8S51-D8S67-D8S43-D8S114-D8S48-D8S47-qter. An estimate of the minimum size of this region, based on the size of the YAC contigs and pulsed-field gel electrophoresis is 5 Mb.

Four groups reported radiation hybrid panels, two of which have been extensively characterized by STS content mapping. A consensus order, based on markers used by both groups, was derived (Fig. 2). The radiation hybrid data allows the placement of D8S268, which could not be uniquely positioned on the consensus genetic map, between D8S135 and D8S165. There is good agreement between the radiation hybrid map consensus order and that determined by genetic linkage analysis.

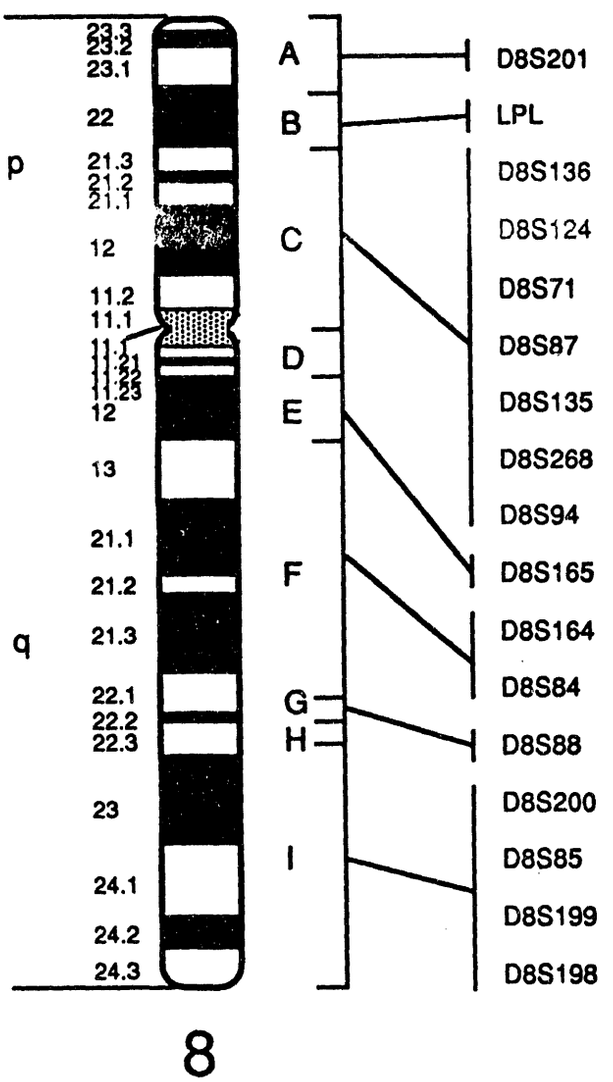

Fig. 2. Consensus marker order based on radiation hybrid maps of Oshima et al and Wagner et al (see abstracts). Markers are arranged in a continuous linear order from 8pter to 8qter.

Disease gene loci

A number of disease gene loci have been assigned to chromosome 8. These include three new loci first reported at this workshop. Information on these loci, presented at the meeting, can be found in the abstracts accompanying this report. Further data can be found in OMIM and GDB. The consensus locations of all the disease gene loci discussed at the workshop was determined (Fig. 3).

Benign familial neonatal convulsions (epilepsy, benign neonatal, EBN2)

Linkage analysis in one large Hispanic family has mapped the EBN2 gene to 8q24.1 closely linked to MYC. There are no recombinants with MYC, D8S256 or D8S284. The highest lod score of 4.43 was obtained with both D8S256 and D8S284 at $\theta = 0$. This is the second EBN locus to be assigned, EBN1 maps to 20q (Leppert et al., 1989).

Charcot-Marie-Tooth (CMT4)

CMT4, a recessive disorder, has been mapped to the region 8q13-q21.1 in four large inbred Tunisian families. No recombinants were detected with D8S164 and D8S286. Recombination events in these families suggest that the markers D8S279 and D8S84 flank the CMT4 locus.

Multiple exostosis (EXT1)

The Langer-Giedion syndrome (LGS) includes exostoses as one of the clinical features. Multiple exostoses have been reported in patients with a chromosomal inversion or translocation involving 8q24 (Frydman et al., 1992; Ogle et al., 1991). This has suggested that familial, dominantly inherited, exostoses might be due to a single gene locus within the minimal Langer-Giedion chromosomal region (LGCR) that is deleted in the contiguous gene deletion syndrome, LGS. A maximum lod score of 8.11 for linkage of EXT1 to STR markers within 8q24 was found, with significant genetic heterogeneity for familial exostosis (Cook et al., 1993). The locus assigned to chromosome 8 is EXT1, while at least one other locus is unlinked to 8q24 (Le Merrer et al., 1992).

Branchio-oto-renal syndrome (BOR)

The gene for BOR has been localised to 8q11.2-q12 (Smith et al., 1992; Kumar et al., 1992) between the flanking markers PENK and D8S164 within a 15 cM interval. The location for BOR has been refined to between the Genethon markers D8S260 and D8S279, which span approximately 10 cM.

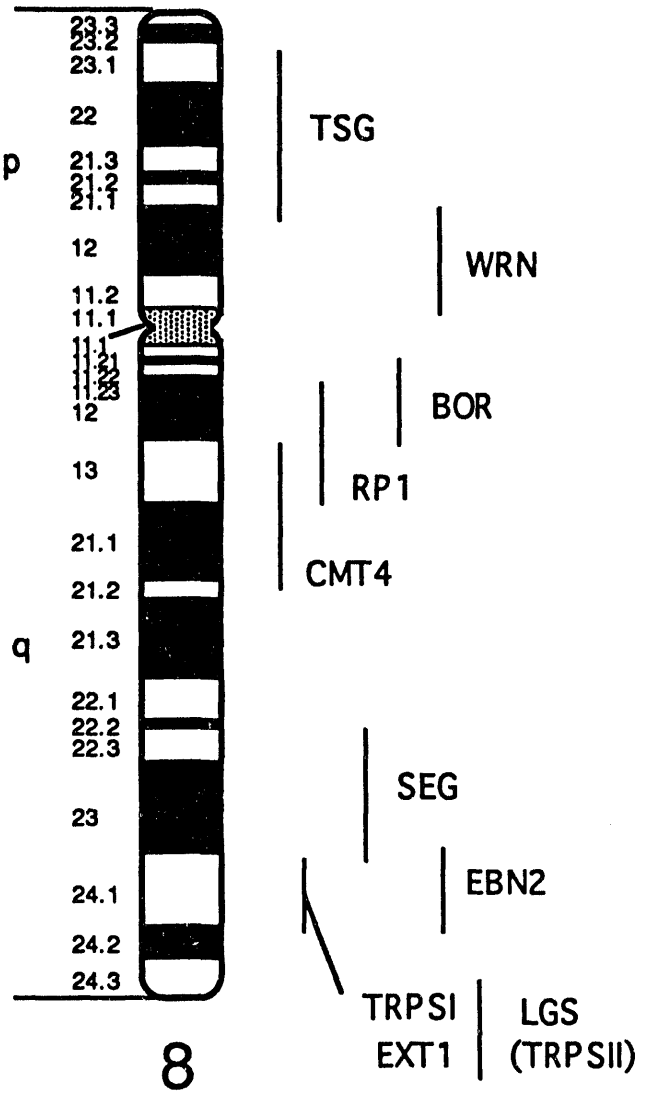

Fig. 3. Ideogram indicating the localization of disease loci discussed at the workshop. TSG and SEG indicate the putative tumor suppressor gene and segmentation gene respectively. All other symbols are described in text.

Langer-Giedion syndrome (LGS)

Langer-Giedion syndrome has been localized to 8q24.1 within the LGCR. There is evidence that Langer-Giedion syndrome (LGS or TRPSII) is a contiguous gene syndrome, combining the phenotypic features of trichorhino-phalangeal syndrome type I (TRPSI) and multiple cartilaginous exostoses (EXT1). Deletion and translocation breakpoint mapping in patients with TRPSI, TRPSII and EXT1 indicates that the TRPSI gene is located proximal to the EXT1 gene. The TRPSI gene is close to the markers D8S98 and D8S51 while EXT1 is close to D8S67.

Retinitis pigmentosa (RP1)

RP1 has been mapped to region 8q12-q13 in one large family (Blanton et al., 1991). Further analysis places RP1 between D8S165 and D8S260.

Werner syndrome (WRN)

Linkage to a number of markers in proximal 8p has been reported by two groups (Goto et al., 1992; Schellenberg et al., 1992) with D8S87 and ANK1 flanking WRN. A new closely linked STR, D8S339, was reported that has a maximum Θ of 0.006 (Thomas et al., 1993).

Putative loci located on chromosome 8

Tumor suppressor gene(s)

Three groups presented data on loss of heterozygosity of 8p loci in prostate cancer that suggest the existence of tumour suppressor gene(s). Roughly half of all prostate tumours examined showed losses of a broad region approximately centered at 8p21-8p22 and including the following loci: D8S133, D8S136, D8S137, D8S258, D8S261, D8S282, D8S298, LPL, NEFL, and SFTP2. Deletion mapping indicates that the distal locus, D8S265, and the proximal locus, D8S283, are retained. Losses within this region were also reported in colon and lung cancer, with evidence for at least two independent regions of loss in colon cancer.

Segmentation gene

A four generation family has been identified with a unique sequence of fusions of cervical vertebrae in association with vocal impairment. Karyotyping of metaphases from blood lymphocytes from affected family members has identified an inversion of the long arm of chromosome 8. The inversion involves 8q22.2-q23.3 and suggests that a locus involved in vertebrate segmentation lies at one of the breakpoints.

Consensus order map

The consensus genetic order of polymorphic markers was determined (Fig. 4). This consensus map is based on several published linkage maps (Steinbrueck et al., 1992; Tomfohrde et al., 1992; Weissenbach et al., 1992; Emi et al., 1993), several unpublished linkage maps presented at the workshop as abstracts and additional physical mapping data. All of these linkage maps are based on CEPH reference families.

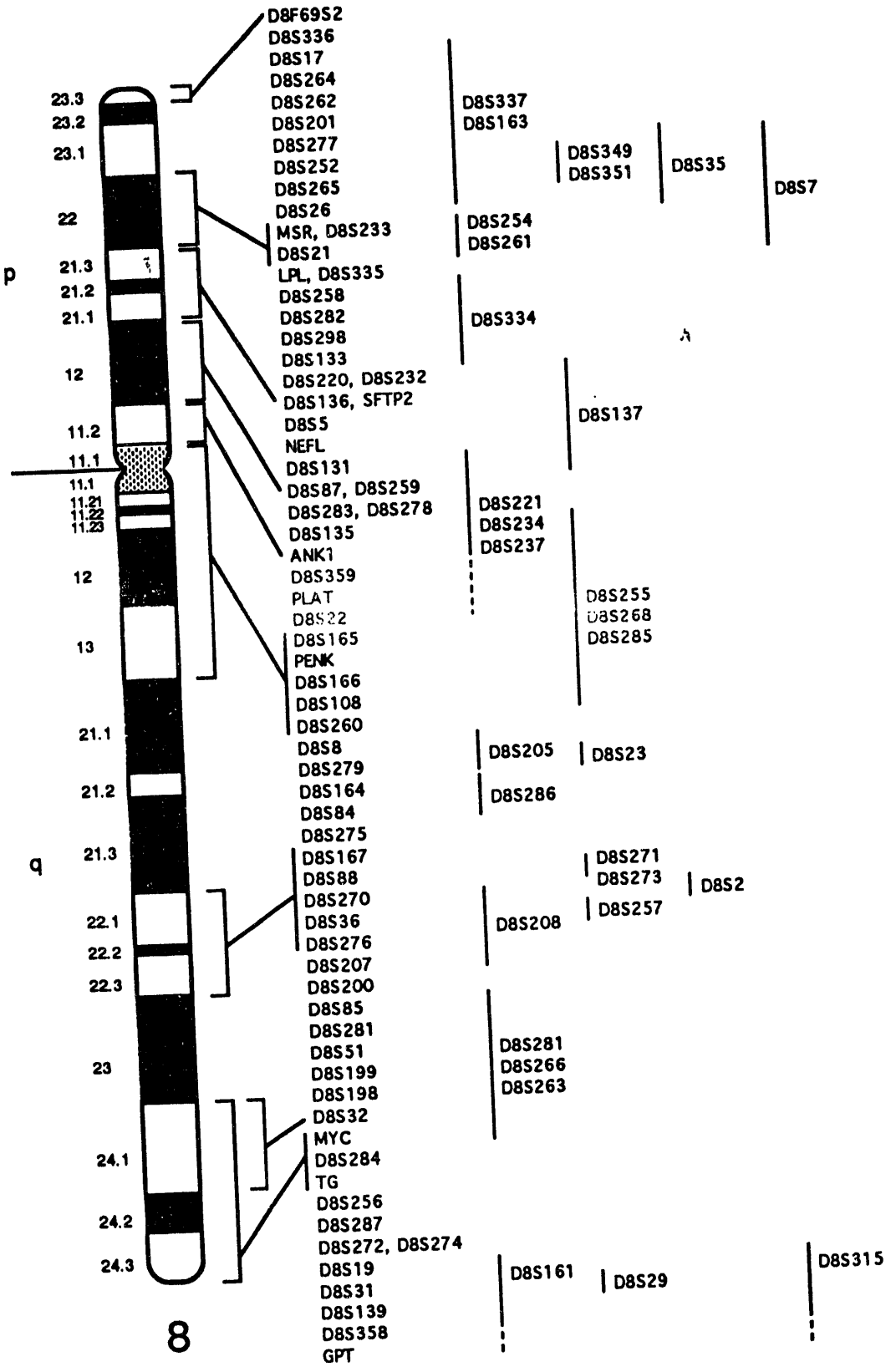

Fig. 4. Consensus order of polymorphic markers. Markers that are separated by commas show 0% recombination with respect to each other in one or more linkage maps.

TABLE I. Listing of polymorphic markers placed on consensus order map

Symbol	Probe name	Type	Enzyme	Location	Het	Refs[a]
ANK1	ANK1.PCR2.1/ANK1.PCR2.2	RS	PCR/NcoI	8p21.1-p11.2	0.49	1,2
D8F69S2	HTY275C24	UNKN	HindIII	8pter	0.37	1
D8S2	82B	UNKN	TaqI	8	0.38	1
D8S5	TL11	UNKN	HindIII	8p21.3-q11.1	0.39	1,2
D8S7	D8S7.PCR1.1/D8S7.PCR1.2	UNKN	PCR	8p23	0.30	2
D8S8	lambdaEMBL3.287	UNKN	TaqI	8q13-q21.1	0.55	1
D8S17	pYNM3	VNTR	PstI	8pter-p22	0.33	
D8S19	pHHH171	UNKN	MspI	8q	0.38	1
D8S21	pABLA-2	UNKN	RsaI	8	0.44	1
D8S22	CRI-V1225	UNKN	EcoRI	8p21.3-q11.1	0.69	1
D8S23	CRI-V822	UNKN	EcoRI	8q13-q22.1	0.44	1
D8S26	CRI-R191	RS	MspI	8p22-p21.3	0.53	1
D8S29	CRI-L1251	UNKN	MspI	8	0.24	
D8S31	CRI-L580	UNKN	TaqI	8	0.49	1
D8S32	CRI-L413	UNKN	PstI	8	0.52	1
D8S35	CRI-L40	UNKN	BglII	8	0.46	1
D8S36	CRI-C96	UNKN	BglII	8	0.70	1
D8S51	L48	UNKN	SacI	8q23.2-q24.11	0.40	
D8S84	Mfd8CA/Mfd8GT	DINUC	PCR	8q13-q21.2	0.63	1,2
D8S85	Mfd18CA/Mfd18GT	DINUC	PCR	8q23-qter	0.74	1,2
D8S87	Mfd39CA/Mfd39GT	DINUC	PCR	8p12	0.68	1,2
D8S88	Mfd45CA/Mfd45GT	DINUC	PCR	8q22.1-q22.3	0.84	1,2
D8S108	pBS8-96	UNKN	SacI	8cen-q13	0.44	
D8S131	Y19-1D	RS	BamHI	8p21	0.31	1,2
D8S133	D8S133CA/D8S133GT	DINUC	PCR	8p21.3-q11.1	0.78	1,2
D8S135	D8S135CT/D8S135GA	DINUC	PCR	8p21.3-q11.1	0.17	
D8S136	D8S136CA/D8S136GT	DINUC	PCR	8p	0.88	1
D8S137	D8S137CA/D8S137GT	DINUC	PCR	8p21.3-q11.1	0.68	1,2
D8S139	CEB6	VNTR	HaeIII	8q24.3	0.63	1
D8S161	Wis2L/Wis2R	DINUC	PCR	8q	0.79	
D8S163	pKSR2	RS	EcoRI	8pter-p22	0.49	4
D8S164	Mfd104CA/Mfd104GT	DINUC	PCR	8q13-q22.1	0.81	1,2
D8S165	Mfd117CA/Mfd117GT	DINUC	PCR	8q11.23-q12	0.53	1,2
D8S166	Mfd159CA/Mfd159GT	DINUC	PCR	8q11.23-q12	0.85	1,2
D8S167	Mfd185CA/Mfd185GT	DINUC	PCR	8q22.1-q22.3	0.87	1,2
D8S198	Mfd167	DINUC	PCR	8q23-qter	0.83	1,2
D8S199	Mfd177	DINUC	PCR	8q23-qter	0.83	1,2
D8S200	Mfd196	DINUC	PCR	8q23-qter	0.76	1,2
D8S201	Mfd199	DINUC	PCR	8pter-p22	0.92	1,2
D8S205	MS45-1/MS45-2	DINUC	PCR	8	0.78	
D8S207	MS142-1/MS142-2	DINUC	PCR	8	0.74	
D8S208	MS91-1/MS91-2	DINUC	PCR	8	0.75	
D8S220	cCI8-319	UNKN	TaqI	8p21.3-p21.2	0.44	4
D8S221	cCI8-326	UNKN	TaqI	8p21.2-p21.1	0.57	4
D8S232	cCI8-448	UNKN	MspI	8p21.3	0.28	4
D8S233	cCI8-487	RS	MspI	8p22-p21.3	0.49	4
D8S234	cCI8-494	UNKN	TaqI	8p11.23-p11.22	0.50	4
D8S237	cCI8-511	UNKN	PstI	8q11.21-q11.22	0.44	4
D8S252	D8S252CA/D8S252GT	DINUC	PCR	8pter-p22	0.27	
D8S254	Mfd210	DINUC	PCR	8	0.58	

TABLE I cont'd. Listing of polymorphic markers placed on consensus order map

Symbol	Probe name	Type	Enzyme	Location	Het	Refs[a]
D8S255	AFM023xc1a/AFM023xc1m	DINUC	PCR	8	0.74	3
D8S256	AFM073yb7a/AFM073yb7m	DINUC	PCR	8	0.84	3
D8S257	AFM077ya5a/AFM077ya5m	DINUC	PCR	8	0.73	3
D8S258	AFM107xb6a/AFM107xb6m	DINUC	PCR	8	0.71	3
D8S259	AFM107yb2a/AFM107yb2m	DINUC	PCR	8	0.59	3
D8S260	AFM114xe7a/AFM114xe7m	DINUC	PCR	8	0.83	3
D8S261	AFM123xg5a/AFM123xg5m	DINUC	PCR	8	0.78	3
D8S262	AFM127xh2a/AFM127xh2m	DINUC	PCR	8	0.72	3
D8S263	AFM141xa5a/AFM141xa5m	DINUC	PCR	8	0.76	3
D8S264	AFM143xd8a/AFM143xd8m	DINUC	PCR	8	0.84	3
D8S265	AFM144zb2a/AFM144zb2m	DINUC	PCR	8	0.79	3
D8S266	AFM151ye3a/AFM151ye3m	DINUC	PCR	8	0.53	3
D8S268	AFM156xa3a/AFM156xa3m	DINUC	PCR	8	0.61	3
D8S270	AFM165xh4a/AFM165xh4m	DINUC	PCR	8	0.80	3
D8S271	AFM165yb10a/AFM165yb10m	DINUC	PCR	8	0.78	3
D8S272	AFM175xb4a/AFM175xb4m	DINUC	PCR	8	0.82	3
D8S273	AFM179yf6a/AFM179yf6m	DINUC	PCR	8	0.81	3
D8S274	AFM182xa3a/AFM182xa3m	DINUC	PCR	8	0.78	3
D8S275	AFM185xe9a/AFM185xe9m	DINUC	PCR	8	0.76	3
D8S276	AFM192xc5a/AFM192xc5m	DINUC	PCR	8	0.66	3
D8S277	AFM198wd2a/AFM198wd2m	DINUC	PCR	8	0.74	3
D8S278	AFM200ye1a/AFM200ye1m	DINUC	PCR	8	0.66	3
D8S279	AFM203wc1a/AFM203wc1m	DINUC	PCR	8	0.88	3
D8S281	AFM205yh4a/AFM205yh4m	DINUC	PCR	8	0.65	3
D8S282	AFM234vf4a/AFM234vf4m	DINUC	PCR	8	0.73	3
D8S283	AFM238yh12a/AFM238yh12m	DINUC	PCR	8	0.80	3
D8S284	AFM248td9a/AFM248td9m	DINUC	PCR	8	0.84	3
D8S285	AFM255yb9a/AFM255yb9m	DINUC	PCR	8	0.79	3
D8S286	AFM268ve9a/AFM268ve9m	DINUC	PCR	8	0.82	3
D8S287	LCAW4	RS	HindIII	8q	0.50	1
D8S298	AFM234yh10a/AFM234yh10m	DINUC	PCR	8	0.70	3
D8S315	38A/38B	UNKN	PCR	8q22.3-q24.3		
D8S334	cCI8-190	RS	TaqI	8p21.3	0.44	4
D8S335	cCI8-245	RS	TaqI	8p22-p21.3	0.43	4
D8S336	cCI8-266	RS	TaqI	8p23.2-p23.1	0.55	4
D8S337	cCI8-388	RS	MspI	8p23.2-p23.1	0.50	4
D8S349	Mfd280	DINUC	PCR	8	0.84	
D8S351	Mfd295	DINUC	PCR	8	0.84	
D8S358	CEB42			8q24		
D8S359	cos199E8	DINUC	PCR	8p21.3-q11.1	0.39	
GPT						1
LPL	LPL-3'-CA/LPL-3'-GT	DINUC	PCR	8p22	0.84	1,2,4
MSR1	cMSR-32	UNKN	MspI	8p22	0.45	4
MYC	MYC.PCR3.1/MYC.PCR3.2	DINUC	PCR	8q24.12-q24.13	0.86	1
NEFL	NF68, pHNFL, NFL915PA	UNKN	TaqI	8p21	0.47	1,4
PENK	105/106	DINUC	PCR	8q11.23-q12	0.53	1,2
PLAT	PLAT.PCR2.1/PLAT.PCR2.2	DINUC	PCR	8p12-q11.2	0.78	1,2
SFTP2	311.1	RS	EcoRI	8p	0.38	
TG	IRI-ULB	UNKN	PvuII	8q24	0.50	1

[a]Refs 1: Steinbrueck et al., 1992; 2: Tomfohrde et al., 1992; 3: Weissenbach et al., 1992; 4: Emi et al., 1993

Certain loci are included in figure 4 where the local order is known but the exact position with respect to the consensus map is not certain. These loci are shown flanked by two markers on the consensus map indicated by the endpoints of the adjacent lines. In addition the cytogenetic localization of selected loci is given, indicated by brackets beside the ideogram.

These loci are listed in Table I with descriptive information, cytogenetic localization where known, maximal heterozygosity and reference to published linkage maps that include the locus (additional data is available in GDB).

Most of the loci, in the ordered map, are included in two or more published or unpublished linkage maps. Most of the remaining markers were placed on the consensus map by interpolation between maps where warranted. Thus, if markers A and C are in 2 or more maps but marker B is present in only one map, though flanked by A and C, then B was included in the consensus map.

Finally, the loci D8S51, D8S201 and D8S281 were placed on the consensus map by physical localization using somatic cell hybrid panels.

Resources

Somatic cell hybrid panels

In addition to the widely distributed panel of Wagner et al. (1991), 6 other hybrids now subdivide the 8q23-8qter interval of the chromosome. Four of the lines were derived from Langer-Giedion syndrome patients (Parrish et al., 1991) and the remaining 2 lines are from a hereditary renal cell carcinoma and define a single breakpoint (Drabkin et al., 1985).

Three radiation-reduced hybrid panels have been constructed using the donor line, GM10156B, which contains 1 to 2 copies of chromosome 8. A 10 krad dose of radiation was used for two panels (Wagner, Houston; Schellenberg, Seattle) and 7 krads for the third panel (Leach, San Antonio). A total of 90 human DNA containing lines have been isolated. A fourth panel (Spurr, ICRF) of 105 radiation-reduced hybrids was constructed using a 10 krad dose on the donor line C4a, which is a monochromosomal human hamster hybrid. DNA from all hybrid panels is available by contacting the individual investigators.

Libraries

A flow sorted chromosome 8 cosmid library, LA08NC01, (Wood et al., 1992) has been distributed to five laboratories. Information concerning distribution and availability of this library is available from Dr. Larry Deaven, Los Alamos National Laboratory.

YAC contigs

YAC contigs of approximately 4 Mb and 2 Mb have been assembled for 8q24.1 and 8q11, respectively. This information has been deposited in GDB and will be regularly updated as the contigs are expanded. Four additional YAC contigs have been described for human chromosome 8 (Bellanne-Chantelot et al., 1992) and regionally localized by FISH. These contigs are being screened for STS content and the information will be placed in GDB.

STS sites

Twenty-four new STRs were reported (Drayna, Menlo Park). All of these are CA repeats, mostly with heterozygosities over 50%. Two hundred and forty-one new clones have been isolated from the 8q24.1 microdissection library (Lüdecke et al., 1989). DNA sequence of the clones and suggested primer pairs are freely available (Lüdecke, Essen, FRG). Additional STSs and STRs for other regions of the chromosome were described by numerous investigators and their primer sequences have been deposited in GDB.

Acknowledgement

We would like to thank Michael Schertzer for his excellent work in support of the workshop.

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DISCLAIMER

Abstracts of the first international workshop on human chromosome 8 mapping

AUTOSOMAL RECESSIVE CHARCOT-MARIE-TOOTH DISEASE TYPE A: EVIDENCE OF GENETIC LINKAGE OF CMT4 TO CHROMOSOME 8q

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Autosomal recessive Charcot-Marie-Tooth disease (RCMT) is a severe neuropathy of childhood. Previous reports have suggested genetic heterogeneity, however, classification remains ambiguous. From a series of RCMT Tunisian families, a homogeneous set was selected according to clinical, electrophysiological and pathological criteria and designated RCMT type A (RCMTA). Characteristics of RCMTA are: (1) early age of onset, rapidly progressive distal weakness and atrophy of the limbs leading to an inability to walk in the late childhood or adolescence, (2) normal cerebrospinal fluid (3) slow nerve conduction velocity (NCV) (mean median motor NCV=30m/s) and (4) hypomyelination with basal laminal onion bulbs. Using linkage analysis, we studied four inbred Tunisian families with RCMTA. Significant evidence for linkage was found for several markers from chromosome 8q13-21.1 (D8S84, D8S164, D8S260, D8S275, D8S279 and D8S286). A peak two point lod score of $z=9.12$ at $\theta=0.00$ was obtained for D8S164. Analysis of key recombinants suggest the following order: Cen-D8S279-(RMCTA,D8S286,D8S164)-D8S84-D8S275-qter. The present chromosomal localization of one form of RCMT, gene symbol CMT4, will have implications in clarifying the nosology of this complex group of disorders.

HIGH FREQUENCY OF DELETIONS ON THE SHORT ARM OF CHROMOSOME 8 IN HUMAN PROSTATIC CARCINOMA

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A detailed deletion mapping of a series of human prostatic carcinomas, using restriction fragment length polymorphism (RFLP) analysis of all chromosomes, showed allelic losses on individual chromosomes at variable frequencies. Allelic losses occurred on chromosomes 8p, 10pq, 16q, and 18q in more than 30% of the cases. Loss of genetic information from one or two of the chromosomes 8, 10 or 16 were always present in tumors showing allelic losses, indicating that genes on these chromosomes have a central role in prostatic cancer. A more extensive study of these chromosomes was thus carried out and showed the highest frequency of allelic deletions to occur on the short arm of chromosome 8 (65%) (where the minimally deleted region was between the PLAT locus and pter). The long arm of chromosome 16 had allelic deletions in 56% of informative cases, with three different breakpoints. Chromosome 10 exhibited a complex deletion pattern, showing allelic losses from both the short (p) and the long (q) arms. Our data indicate that tumor suppressor genes involved in the oncogenesis of prostatic carcinoma may be localized between 8pter and the PLAT locus and that additional/alternative tumor suppressor genes are likely to be localized on chromosome 10 and on the long arm of chromosome 16.

REGIONAL ALLELIC LOSSES OF CHROMOSOME 8p IN HUMAN PROSTATE CANCER

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Allelic markers on chromosomes 8p were lost in over 50% of 18 prostate carcinomas studied by Bergerheim et al. We have confirmed and extended these data in a set of 64 paired tumor / normal DNA samples using PCR- and Southern blot-based polymorphisms on chromosome 8. Twenty-seven polymorphic markers in twenty-three genetic loci were surveyed, all but four of which were on the p arm. Thirty of 64 cases showed allelic losses of at least one locus. In twenty-nine of these (45%), multiple adjacent loci were lost, usually including the LPL gene (8p22) as well as D8S261, D8S258, D8S282, and D8S298, all located in region B defined in a somatic cell hybrid panel (Wagner et al., 1991). In most cases, one or more proximal markers in region C were retained. Markers located distally in region A were retained in 15 of these 29 tumors. These data preliminarily define a consensus deletion region localized to 8p22 and adjacent subbands and including markers in region B. Similar losses are observed in lung, hepatocellular and colon carcinomas (Emi et al., 1992). It is therefore hypothesized that a suppressor gene for these cancers is located within or in close proximity to chromosome band 8p22.

HUMAN SEGMENTATION SYNDROME

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Vertebral fusions are best explained by an error of segmentation during somitogenesis early in development. Vertebral fusions (Klippel-Feil anomaly) have been previously reported as predominantly sporadic cases. We report a unique familial case of vertebral fusions associated with vocal impairment and laryngeal abnormalities, where 73% are affected within four generations. A unique chromosome 8 inversion (q22.2, q23.3), a likely locus for the segmentation gene (SGM1), segregates with the affected phenotype.

The unique sequence of cervical, vertebral fusions provides a window to the mode and range of SGM1 gene expression. Parrallels between this mutant expression profile and segmentation gene expression profiles (albeit in *Drosophila*) suggest that segmentation may be governed by similar mechanisms, with significance in vertebrate body patterning.

FASTER GENETIC LINKAGE COMPUTATIONS

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Linkage analysis using maximum likelihood estimation is a powerful tool for locating genes. As available data sets have grown, the computation required has grown exponentially, and become a significant impediment. Others have previously shown that parallel computation is applicable to linkage analysis and can yield order of magnitude improvements in speed. We demonstrate that algorithmic modifications can also yield order of magnitude improvements, and sometimes much more.

Using the software package LINKAGE, we describe a variety of algorithmic improvements we have implemented, demonstrating how these techniques are applied, and their power. Experiments show that these improvements speed up the programs by an order of magnitude on problems of moderate and large size. All improvements were made only in the combinatorial part of the code, without resorting to parallel computers. These improvements synthesize biological principles with computer science techniques to effectively restructure the time-consuming computations in genetic linkage analysis.

A principle motive for improving the computation speed was a particular multipoint analysis of the chromosome 8 form of autosomal dominant retinitis pigmentosa (RP1). With our improved algorithms we were able to reach solutions in a week that would have taken almost a year, and therefore were impossible. Nevertheless, we recognize that no matter what algorithmic improvements we achieve, geneticists will want to solve larger and more difficult problems. Further research is underway to work on additional algorithmic improvements and to

investigate new parallel algorithmic approaches.

ISOLATION OF MICROSATELLITE MARKERS ON CHROMOSOME 8

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Starting from a chromosome 8-specific phage library, we have isolated clones containing microsatellite sequences by hybridization to oligonucleotide probes. We isolated 180 clones containing $(CA)_n$ sequences, 200 clones containing $(TTA)_n$ sequences, and 100 clones each containing $(TTTA)_n$, $(TCTA)_n$, and $(TTTC)_n$ sequences. We are now obtaining single copy DNA sequence flanking these repeats, designing primers for PCR amplification, and testing these sequences for polymorphic variation. For CA-containing clones, we have obtained flanking DNA sequences from 65 clones. Of these, 40 produce a PCR product under our standard conditions, and 24 of these are usefully polymorphic. These sequences have been shown to be present in human but not hamster DNA, and preliminary evidence suggests they are distributed throughout the length of the chromosome. These markers have proven themselves useful in linkage studies of genes on chromosome 8.

PRELIMINARY REPORT OF LOSS OF HETEROZYGOSITY AT THE MYC LOCUS DURING THE EVOLUTION OF A CASE OF CHRONIC MYELOID LEUKEMIA (CML) WITH TRISOMY 8 AT BLAST CRISIS

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CML inevitably evolves from a chronic disease into an acute phase (AP) leukaemia. This progression is usually marked by the acquisition of secondary non-random chromosomal abnormalities, the most common of which is trisomy 8.

The reason for the incidence of trisomy 8 in association with the onset of AP is not known. Elevated *c-myc* mRNA and protein expression has been demonstrated in some studies on patients in AP compared with chronic phase samples and there is one example of *c-myc* amplification associated with trisomy in AP CML.

We present the case of a patient with CML (with the karyotype in AP: 48,XY,+8,t(9;22),i(17q),+Ph) shown to have loss of heterozygosity (LOH) at a locus 3' to the *c-myc* gene when in AP. The LOH could indicate that abnormal cell divisions led to complete loss of one copy of chromosome 8 followed or preceded by duplications of the other copy of chromosome 8. It is also possible that the LOH is restricted to the particular region of chromosome 8 studied here. More information will be provided by a study of other polymorphic loci on chromosome 8.

There could be selection of allelic forms of genes on chromosome 8 or differentially expressed genes due to genomic imprinting. We are investigating the possibility that allelic forms exist in the general population by examining polymorphisms closely linked to candidate oncogenes on chromosome 8.

FINE MAPPING AND NARROWING OF THE GENETIC INTERVAL OF THE REGION OF BRANCHIO-OTO-RENAL SYNDROME ON CHROMOSOME 8q BY LINKAGE STUDIES

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Branchio-oto-renal syndrome (BOR) is an autosomal dominant disorder. The syndrome is characterized by ear abnormalities, cervical fistulae, hearing loss and renal abnormalities. The prevalence of BOR syndrome is approximately 1:40,000, and it has been reported to occur in about 2% of profoundly deaf children.

The clinical features of the BOR syndrome vary significantly from one family to another. Several families have been described with branchial anomalies, preauricular pits, and hearing loss with no renal dysplasia; branchio-oto-syndrome (BO). The phenotypic expression of the branchial arch, audiologic and renal development can be quite variable even within the same family. The complex clinical symptoms suggest the possibility of more than one gene involved in the development of BOR syndrome. The gene for autosomal dominant branchio-oto-renal syndrome has been mapped to chromosome 8q. The distance between the flanking markers D8S87 and PENK, reported earlier (Kumar et al., 1992), was approximately 15 cM. The BOR region has been refined further using CEPH and Genethon markers. The disease gene lies between the markers D8S260 and D8S279 which spans about 10 cM. The work in now underway to develop STS markers using microdissection library for isolation of YAC/cosmid clones from the BOR region.

A FRAMEWORK MAP OF THE EBN2 REGION ON CHROMOSOME 8q

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Benign familial neonatal convulsions (epilepsy benign neonatal, EBN) is a rare autosomal dominant disorder characterized by unprovoked seizures in the first weeks of life. A form of this disorder has been mapped to chromosome 20q. Our analysis of a large EBN pedigree has identified a new locus for EBN on chromosome 8q, and confirmed genetic heterogeneity for this disorder. We have compiled a genetic map of the EBN2 region. Data for microsatellite markers D8S164, D8S88, D8S85, D8S198 (Marshfield) and D8S284, D8S256, D8S274 (Genethon) were extracted from CEPH database 6.0 and combined with data from our newly developed microsatellite marker, D8S315. The framework map is presented below with recombination estimates and odds against local inversion shown. Multipoint linkage analysis indicated that the most likely position for the newly identified EBN2 locus is between D8S284 and D8S256, with a maximum LOD score of 4.43.

cen-D8S164-.070($1.97x10^{14}$)-D8S88-.156($4.37x10^{73}$)-D8S85-.085($6.82x10^{19}$)-D8S198-.199($2.29x10^{30}$)-D8S284-.057($8.14x10^{6}$)-D8S256-.076($1.24x10^{19}$)-D8S274-.141($7.79x10^{10}$)-D8S315-ter

PHYSICAL MAPPING OF THE LANGER-GIEDION SYNDROME CHROMOSOME REGION

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We have previously reported a deletion map of the Langer-Giedion syndrome chromosome region (LGCR, 8q24.1) based on 13 clones isolated from a 8q24.1 microdissection library. Since then, we have refined the deletion map by including additional markers and by constructing YAC contigs spanning the shortest region of deletion overlap in patients with Langer-Giedion syndrome. The results of the YAC contig mapping confirm the locus order within the LGCR.

This locus order spanning the critical region is cen - D8S42 - D8S55 - D8S98 - D8S51 - D8S67 - D8S43 - D8S114 - D8S48 - D8S47 - tel.

ANCHOR CLONES FOR THE CONSTRUCTION OF A 8q24.1 YAC CONTIG

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Five hundred and thirty-three clones from a human 8q24 microdissection library were analyzed by automatic DNA sequencing

337 different insert sequences were found. The insert size ranged from 52-376 bp (mean size, 170 bp). 291 of these sequences (86%) are free of repetitive DNA. With the help of a computer program, primer pairs for 260 of the 291 single copy clones (89%) could be designed. In a pilot study, 19 microclones which had been mapped to the Langer-Giedion syndrome chromosome region (LGCR, 8q24.1) were successfully translated into STSs. From the FISH analysis using the whole microdissection library as a probe we conclude that most of the clones are derived from the region between the LGCR and the MYC gene in 8q24.1. Assuming that the clones are evenly distributed throughout 8q24.1 they will be useful to connect the LGCR and MYC loci with a YAC contig.

RADIATION HYBRID MAPPING OF THE PROXIMAL REGION OF THE SHORT ARM OF HUMAN CHROMOSOME 8

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To construct a detailed physical map of chromosome 8, we have developed radiation hybrids using a hamster cell line carrying an intact human chromosome 8. The radiation hybrid panel consists of 2 sets of hybrid cell lines, each of which includes 34 Alu-positive cells. Over 60 known markers of human chromosome 8 have been genotyped. The most likely order of the markers of the central region of human chromosome 8 is as follows: D8S137 - D8S293E - D8S131 - D8S124 - D8S278 - D8S259/D8S71 - D8S283 - D8S87 - D8S105 - D8S135:PAa - D8S135:PBa - D8S255 - ANK - D8S268 - MS8-2 - D8S94 - D8S292E - D8S291E - D8S165. This panel should be useful in providing information on the order of markers within other regions of human chromosome 8.

LINKAGE AND PHYSICAL MAPPING OF THE CHROMOSOME 8 FORM OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA (RP1)

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We have mapped a locus for autosomal dominant retinitis pigmentosa (RP1) in an extended Kentucky family to the pericentric region of chromosome 8. To date we have tested 25 markers from chromosome 8 for linkage in the family; of these, 12 show statistically significant evidence of linkage to the RP1 locus. Nine of these markers form a continuous framework linkage map spanning the region containing the disease locus, with a cluster of markers less than 1 cM apart centered on the disease. Two markers, D8S108 and D8S165, show 0% recombination with RP1, with maximum lod scores of 3.6 and 9.9 respectively. We have isolated YAC clones containing these two markers and are constructing contiguous YAC maps covering these regions. In addition, all of the framework markers which show linkage to RP1 have been placed on an ordered set of radiation-reduced hybrids that span much of chromosome 8. The combined multipoint linkage data and physical mapping information place RP1 in the region 8q11-q21 and probably localize the disease within a 6 cM span.

The principal difficulty in linkage analysis in this family has been computational. The linkage pedigree contains 192 individuals (of whom 120 are typed for all markers) and two inbreeding loops. Because of the substantial number of untyped individuals and the loops, multipoint analysis can be prohibitively time consuming. By rewriting the computational algorithms in the LINKAGE package we have achieved a 40 fold increase in the speed of calculations. However, multipoint linkage analysis of RP1 is still difficult.

Finally, we have begun screening a retinal-specific cDNA library for candidate genes that map to this region of chromosome 8. These cDNAs will be applicable to other retinal diseases that map to 8 such as VMD1.

Supported by the George Gund and National RP Foundations.

CONSTRUCTION OF A GENETIC LINKAGE MAP OF CHROMOSOME 8 USING A PANEL OF RADIATION HYBRIDS

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We have constructed a panel of radiation hybrids for chromosome 8 using the somatic cell hybrid C4a as the parental cell line fused to the hamster cell line a23. The hybrid C4a contains human chromosome 8 as the only cytogenetically detectable human material. It was irradiated with 10 Krads of X-rays prior to fusion.

A total of 105 independent clones were isolated, frozen stocks and DNA were prepared from each clone. Initially all clones were screened for the presence of human DNA using specific Alu primers and PCR. A total of 80 clones, 82% of the total, were shown to contain human DNA. These clones were screened for the occurrence of chromosome 8 single copy genes and other loci using PCR amplification and oligonucleotide primers.

We have used primers specific for the genes PENK and CA2 and the anonymous D segment D8S88. Approximately 10-15% of the clones show the presence of a single copy sequence. Further mapping is currently being undertaken. It is anticipated that this resource of DNA will be of value in helping to integrate the genetic and physical maps of this chromosome. Microtitre plates of DNA will be available to the chromosome 8 workshop community following this meeting.

AN UPDATED GENETIC LINKAGE MAP FOR HUMAN CHROMOSOME 8

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We expect to complete construction of an "index map" for human chromosome 8 in 1993 that includes markers with minimum heterozygosity of 70% and intermarker spacing of no more than 15 cM. We recently published a comprehensive baseline map for this chromosome (Science, 258: 67, 1992) and have continued to add microsatellite markers (e.g. from the Genethon collection, Weissenbach et al., Nature 359: 794 1992) and further define index marker candidates. Our current map has a sex-average distance of 237 cM and was constructed using genotypes from the CEPH pedigree resource and the program package CRI-MAP (odds for order are 1000:1). Nineteen of the 20 markers that are uniquely localized are microsatellites with heterozygosities of at least 70%. A microsatellite marker is being developed to replace the single RFLP on the map (at the NEFL locus). There are currently 6 intervals with spacing greater than 15 cM, but the maximum interval length is only 23.3 cM. We have closed the 8p terminus of the map with a RFLP marker from a telomere clone (D8F69S2), confirmed its localization to 8pter by FISH mapping, and are continuing efforts to identify a microsatellite marker that would serve as the 8p endpoint for the map. For the long arm of the chromosome D8S161 currently defines the q terminus (D8S161 maps below D8S139 which has previously been mapped to 8q24.3 by *in situ* hybridization).

THE HUMAN C/EBPδ (CRP3/CELF) GENE: STRUCTURE AND CHROMOSOMAL LOCALIZATION

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In an attempt to identify C/EBP- like transcription factors expressed in the prostate, a cDNA homologous to the mouse C/EBPδ

(CRP3) and the rat CELF gene was isolated. A genomic clone containing the entire C/EBPδ gene was isolated using a cDNA fragment as a probe. The gene was characterized by restriction mapping and sequence analysis. By fluorescence in situ hybridization, using the biotinylated genomic clone as a probe, the C/EBPδ gene was assigned to the pericentromeric region of human chromosome 8, most probably to 8q11. This chromosomal location was confirmed by analysis of a panel of human x hamster somatic cell hybrid DNA samples with a C/EBPδ specific STS. As a result, the C/EBPδ gene could be positioned between the PLAT and D8S165 locus.

A CANDIDATE TUMOR SUPPRESSOR GENE INVOLVED IN PROSTATE CANCER IS LOCATED ON CHROMOSOME 8p

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DNA was isolated from twenty-five progressively growing prostate tumors obtained by transurethral resection. Sections with a high percentage of tumor cells (80% or more) were selected; control DNA was obtained from white blood cells or from normal prostate sections. Fourteen chromosome 8p microsatellite markers were used to examine allele loss. All twenty-five tumor DNAs were informative for at least one marker. In fifteen cases (60%) loss of one or more loci was observed. In contrast, in only four DNAs loss of heterozygosity of an 8q marker has been found. These findings extend the previous observation of Bergerheim et al. [Gene Chromosome Cancer 3, 215-220 (1991)], and show that chromosome 8p harbours one or more candidate tumor suppressor genes involved in prostate cancer. The smallest interstitial deletion detected in one tumor of the series is between the markers D8S265 and D8S283.

SOMATIC CELL HYBRID RESOURCES FOR HUMAN CHROMOSOME 8

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We have characterized three panels of human/hamster somatic cell hybrids containing fragments of human chromosome 8 that are useful for mapping of DNA probes to specific regions of the chromosome. The first panel consists of a collection of 11 cell lines derived for various purposes by other investigators which were identified as containing fragments of human chromosome 8 by screening with chromosome 8-specific probes (Wagner et al., Genomics 10:114-125, 1991). This panel divides the chromosome into 10 intervals of varying sizes. The second panel is composed of four cell lines carrying deletion derivatives of chromosome 8 from patients with Langer-Giedion syndrome and two cell lines containing the reciprocal products of a 3;8 translocation derived from patients with hereditary renal cell carcinoma. This panel divides one of the largest intervals defined by the first mapping panel (the "I" interval, covering 8q23 and 8q24) into 9 subintervals, improving the mapping resolution in this region. The third panel is composed of cell lines derived by gamma irradiation of a chromosome 8-only hybrid and fusion to a nonirradiated recipient hamster cell line to rescue fragments of chromosome 8. These radiation hybrids have been characterized by PCR analysis for the presence of nearly 50 sequence tagged sites distributed across all regions of chromosome 8. The majority of the STSs are also in the recently published genetic maps of chromosome 8, allowing the integration of the genetic and physical maps. Eleven unique radiation hybrids have been characterized which each have from 1 to 4 fragments of chromosome 8, providing many new mapping intervals to refine the physical localizations of DNA probes. The radiation hybrids are also useful as sources for developing region-specific probes for chromosome 8.

HUMAN CHROMOSOME 8 LINKAGE MAP BASED ON SHORT TANDEM REPEAT POLYMORPHISMS

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We have constructed a new linkage map of short tandem repeat polymorphisms (STRPs) for chromosome 8 which encompassed and extended earlier maps for this chromosome (Tomfohrde *et al.* Genomics 14:144-152, 1992; Steinbrueck *et al.* Science 258:67-86, 1992; Weissenbach *et al.* Nature 359:794-801, 1992). The map was based on 60 dinucleotide STRPs and 2 tetranucleotide STRPs and included, among others, all available Genethon and Marshfield markers. Markers were typed on 8 to 40 CEPH reference families. Total lengths of the female and male maps were 262 and 133 cM, respectively. At the chromosome ends, male recombination was about equal to female recombination, but throughout the interior of the chromosome, female recombination was in excess, particularly in the region near D8S260. The most distal STRP on 8p (D8S264) was apparently close to the telomere (Steinbrueck *et al.* 1992). On 8q, however, a gap may have remained between the most distal STRP (UT721) and the end of the linkage map, even though the map was extended by about 48 cM over the STRP map published by Tomfohrde *et al.* (1992). Order for 39 of the STRPs was supported with 1000:1 odds. Several pairs or sets of markers could not be ordered because of the absence of recombination between them. The largest gap between STRPs on the comprehensive sex-equal map was 16 cM.

The first chromosome 8 maps from the Cooperative Human Linkage Center (CHLC) have also been constructed. These maps and subsequent versions are publicly accessible through anonymous FTP (File Transfer Protocal) at ftp chlc.org (or ftp 131.249.3.27).

YAC AND COSMID CONTIG MAPS OF CHROMOSOME 8

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We are developing contig maps of two regions of chromosome 8 using YAC and cosmid clones. The first set of contigs surrounds the Langer-Giedion chromosomal region in 8q24.1. Sequence tagged sites mapped to this region were used to screen two human YAC clone libraries to initiate contigs. The contigs were expanded by chromosome walking in the YAC library using probes derived from the ends of the original YAC clones. Restriction maps of the YACs were constructed using the infrequently cutting enzymes BssHII, EagI, and SfiI. We now have four contigs covering a total of approximately 4 million base pairs in this region. Human sequences were obtained from the YAC DNA in several of the yeast clones by Alu-PCR and used to screen a chromosome 8-specific cosmid library. The cosmids obtained have been characterized by restriction fingerprinting and Southern blot hybridization. Approximately 600 kb of cosmid contigs have now been assembled. The second region for which we are constructing contigs is 8q11-q13, which contains the RP1 (retinitis pigmentosa) and BOR (branchio-oto-renal syndrome) genes. We have already isolated and partially characterized YACs surrounding the D8S165 and D8S108 loci. Our strategy to extend these contigs is to use STS content mapping in the YAC library. Twenty to thirty additional sequence tagged sites some of which have been previously mapped to this region, but most of which will be newly isolated and characterized from radiation hybrid containing this region of chromosome 8, will be used to screen YAC pools. Based on the coincident occurrence of STSs in YAC pools, the STSs will be ordered and a minimum number of YACs needed to cover the region will be identified and isolated from the pools. In addition, we have carried out a linkage analysis on 11 EXT families using 4 STS markers surrounding LGCR (8q24.11 - 8q24.13). Multipoint linkage analysis found significant evidence for heterogeneity, with linkage

EXT1 to these markers with 70% of the families.

DELETION MAPPING OF A TUMOR SUPPRESSOR GENE ON CHROMOSOME 8 IN COLORECTAL AND LUNG CARCINOMA

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Loss of heterozygosity (LOH) for 8p has been demonstrated for a variety of cancers including colorectal, lung, prostate, pancreatic and hepatocellular carcinoma, suggesting the presence of a tumor suppressor gene. We are attempting to localize this gene by deletion mapping. Southern analysis using RFLP markers showed LOH in 33% of 75 informative primary colorectal carcinomas with the following distribution.

Probe	Locus	Band	#Cases	#Inf(%)	#Loss(%)
pSW50	D8S7	8p23	77	11(14%)	5(45%)
pLPL	LPL	8p22	80	32(40%)	4(13%)
pSP-C	SFTP2	8p21-22	71	40(56%)	11(28%)
pHNF-L	NEFL	8p21	70	35(50%)	11(31%)
PolB	POLB	8p11-12	75	15(20%)	0(0%)

Deletion mapping showed 4 cases with distal loss only (pSW50), while 5 cases showed LOH as 8p21-22 without pSW50 loss; these data suggest there may be two distinct regions of LOH. These data were compared to allelotyping in 11 lung cancer cell lines using microsatellite repeat markers; 7 of 11 markers showed LOH with the following markers (#LOH/#informative): D8S201 (3/4), D8S252 (1/2), LPL5' (3/6), SFTP2 (4/6), D8S133 (4/8), D8S137 (3/4), D8S131 (0/4). Two lines with i(8q) showed loss of all markers. Deletion mapping established D8S131 as the proximal boundary. These studies demonstrate a significant degree of 8p allele loss in the tumor types, with 8p21-22 being a common region of LOH in both lung and colorectal cancer.

PHYSICAL LOCALIZATION OF STR-POSITIVE MARKERS FOR THE SHORT ARM OF CHROMOSOME 8

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Allele loss from the short arm of chromosome 8 has been reported in several human tumors, including gastrointestinal, prostate and lung. We are actively studying tumor samples to define the region of minimum overlap. To prepare a higher resolution map of 8p, we are physically localizing cosmid clones containing short tandem repeats (STRs) from the LA08NC01 library. New cosmid clones are identified by filter hybridization with known genes and polyCA/GT. Localization of the clones is performed by fluorescence in situ hybridization (FISH), and determined as fractional length from the p terminus (FLpter). We report markers that have been mapped by genetic linkage, Table 1(a), and three new markers, Table 1(b).

Table 1.

Locus	Clone	Band	Flpter	St. Dev.
a.				
LPL	17B7	8p22	0.14	0.02
D8S133	24E10	8p12-21	0.15	0.01
D8S136	140D4	8p12-21	0.14	0.02
D8S137	171B10	8p12-21	0.19	0.02
D8S131	132C2	8p21	0.19	0.02
b.				
SFTP2	104G2 12H8(contig)	8p21	0.15	0.02
New	104F4	-	0.13	0.02
D8S359	199E8	-	0.25	-

A STRATEGY FOR CORRELATING GENETIC LINKAGE AND PHYSICAL MAPS FOR CHROMOSOME 8

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Detailed genetic linkage maps, now being developed with highly informative index markers, will enable disease gene mapping. Gene isolation requires the linkage map to be correlated with physical maps. We are developing a series of anchor points based on rare cutter restriction sites. We have developed CA repeat markers at 5 loci on 8p containing NotI (GCGGCCGC) sites.

Locus	Cosmid	#NotI sites	cM from 8pter
D8S252	140B11	3	17.8
D8S133	24E10, 73D1	3	43.9
D8S136	140D4	2	45.6
D8S137	171B10	1	55.7
D8S135	129E11	1	70.8

The cosmid library, LA08NC01, is now being screened to identify GGCGCGCC, the sequence for the new AscI restriction enzyme. Both subcloning of Sau3AI/AscI fragments and colony hybridization with a degenerate 12mer containing the AscI recognition site are being used for screening. We have isolated 41 independent cosmids with AscI sites, 24 containing CA repeats. Unique sequence subclones will provide probes for long range restriction mapping while YACs may be identified using the STS procedure.

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