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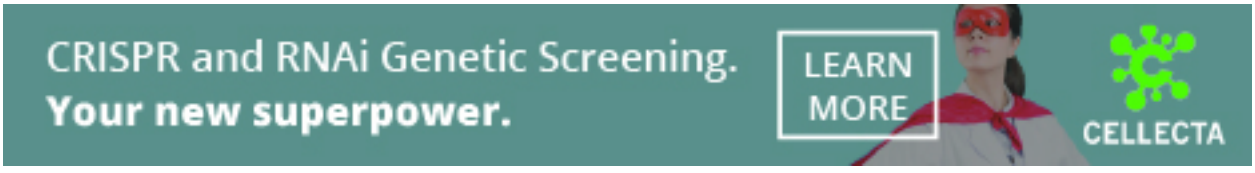

RESEARCH

A Radiation Hybrid Map of 40 Loci for the Distal Long Arm of Human Chromosome 8

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We generated a panel of 97 radiation hybrids from the cell line GM10156B, which contains only human chromosome 8 in a Chinese hamster ovary cell line background. Statistical analysis of the cosegregation of markers in the 97 radiation hybrids was used to construct a physical map delineating the order and intermarker distance of 40 8q24 loci. Twenty-one loci were ordered with maximum likelihood ratios greater than 1000:1. A high level of consistancy was seen between our RH map and the published genetic map, suggesting that our panel will be a valuable resource for the rapid mapping of markers derived from human chromosome 8.

The construction of a high resolution map for the human chromosome 8q24 region is of particular biological interest as it contains several disease gene loci, including a locus for benign familial neonatal convulsions (BFNC) (Lewis et al. 1993) and loci for Langer–Giedion syndrome (LGS) (Parrish et al. 1991). BFNC is characterized by tonic–clonic seizures with onset in the first week of life. Spontaneous remission of the seizures occurs by 6 months of age. Aside from the seizures themselves, there is no other neurological involvement; growth and development are normal (Miles and Holmes 1990). Transmitted in an autosomal dominant fashion, BFNC is a genetically heterogeneous disorder with loci on both chromosome 20 (EBN1) (Leppert et al. 1989) and chromosome 8 (EBN2) (Lewis et al. 1993). Tight linkage to chromosome 8q24 markers D8S284, MYC, and D8S256 has been demonstrated for EBN2 (Lewis et al. 1993).

LGS has been localized to 8q24.1, and evidence suggests that LGS is a contiguous gene syndrome combining the phenotypic features of two autosomal dominant disorders, multiple exostoses (EXT), and tricho-rhino-phalangeal syndrome, type I (TRPSI) (Parrish et al. 1991). EXT is characterized by the growth of multiple benign cartilage-capped tumors on the long bones (Raskind et al. 1995). Recent loss of heterozygosity studies in sporadic and EXT-affected chondrosarcomas suggests that EXT functions as a tumor suppressor (Hecht et al. 1995; Raskind et al. 1995). Features of TRPSI include sparse scalp hair, bushy eyebrows, bulbous nose, long philtrum, short stature, and cone-shaped epiphysis (Ludecke et al. 1995). Deletion, translocation, and linkage mapping have localized an EXT gene (EXT1) to ~1.5–2.0 Mb centromeric to the 8q24 marker D8S199 (Cook et al. 1993; Ludecke et al. 1995). Data indicate that the TRPSI gene (TRPS1) maps centromeric to EXT1, with a maximal distance between the two genes of 200 kb (Ludecke et al. 1995).

Positional cloning of disease gene loci assigned to 8q24 can by assisted by a high resolution map of this genomic area. In an effort to improve the resolution of the 8q24 map, we have undertaken radiation hybrid (RH) mapping. This method complements the existing mapping strategies of meiotic mapping (Gyapay et al. 1994) and somatic cell hybrid (SCH) panels (Wagner et al. 1991; Parrish et al. 1994) but at a higher resolution and with particular emphasis on defining the order and intermarker distance of loci in regions of interest.

RH mapping is a powerful complement to these existing maps as it integrates both physical and genetic mapping data and achieves a high level of resolution. With this technique, a high dose of radiation is used to lethally fragment the chromosomes in a donor cell line, followed by recovery of fragments in a recipient cell line. The resulting hybrid clones are then typed for the re-

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tention of chromosomal markers of interest. Monomorphic, as well as polymorphic, loci can be utilized as markers in RH mapping, offering a major advantage over meiotic mapping. These two techniques are analogous, as the frequency of breakage, or recombination, between loci is directly related to the distance between the loci. Analysis of the concordance/discordance of marker retention in independent RH clones generates a statistical map of the relative order of marker loci and interlocus distance (Boehnke et al. 1991; Cox et al. 1991; Lunetta and Boehnke 1994).

In our study RHs were generated using the SCH GM10156B, which contains only human chromosome 8 in a Chinese hamster ovary (CHO) cell line background (Jones et al. 1981), as the donor cell line. Chromosomal fragments were rescued by fusion to the hypoxanthine phosphoribosyl transferase (HPRT)- and adenine phosphoribosyl transferase (APRT)- deficient hamster cell line, CHO–ATS–49tg. A panel of 97 radiation hybrid clones was used to delineate the order and intermarker distance of 40 8q24 loci, including 37 anonymous loci and three genes. Our RH map of 8q24 improves the resolution in the vicinity of the EBN2, EXT1, and TRPS1 loci and should assist in the positional cloning of disease genes.

RESULTS

Radiation Hybrid Panel

Ninety-seven RHs were scored for the presence or absence of 40 human-specific chromosome 8q24 markers. Fifty-one (52.5%) of the hybrids were positive for at least one 8q24 marker and a different 10 hybrids are also known to retain centromeric (8q11.1) or 8p23 fragments (Bookstein et al. 1994). A total of 61 of the 97 RHs (62.8%) are thus known to contain human chromosome 8 DNA.

To assess the number of chromosome 8-derived fragments retained in a RH, metaphase spreads were prepared from 10 randomly chosen RH cell lines. Ten spreads from each of the randomly chosen RH cell lines were analyzed by FISH using biotinylated total human DNA as a probe. One to six integrated human chromosome fragments were observed in the hybrid cell lines tested.

The retention frequencies of 40 8q24 markers ranged from 10.3% to 21.6% with an average retention frequency of 15.8% in the panel of 97 RHs (Table 1). The centromeric marker D8S1212 (8q11.1) had a retention frequency of 30.9% in the RH panel. The most centromeric marker from 8q24, D8S304, had a retention frequency of 15.5%. There were two sets of markers that had complete concordance: D8S272/D8S502/D8S274 and D8S537/D8S554. In an additional four sets (D8S346/D8S345, D8S347/KW257, D8S557/D8S558, and D8S199/D8S323), the markers were concordant for all but one cell line. A high confidence of order could only be established using one marker of each of these sets. The second markers of each set had tight linkage with the first marker (lod > 12), but order was unable to be determined.

Multipoint Analysis of RH Data

The RHMINBRK program was used to order the loci according to the minimum number of obligate chromosome breaks required to explain the data. The orders requiring the fewest breaks were accepted as candidate orders for maximum likelihood analysis. Because the marker D8S1212 (8q11.1) had a retention frequency that was approximately double (30.9%) the average retention of 8q24 markers, maximum likelihoods were analyzed under both the equal and centromeric retention models. Both models gave the same best locus order, and no significant difference was found in the fit to the data for either model ($\chi^2 = 0.016$, df = 1, $P < .9$, likelihood ratio test). The loci order suggested by minimum breaks criteria matched with the best order generated by maximum likelihood analysis. This order was further supported by data from the two-point analysis.

The resulting framework map is shown in Figure 1. The map contains 21 loci ordered with ⩾1000:1 odds using maximum likelihood ratios and spans a distance of 558.6 cR_{5000}. The comprehensive RH map is shown in Figure 2. The order of the 19 remaining markers is consistent with the framework map. A comparison of a genetic map (Gyapay et al. 1994) of the region is also illustrated in Figure 2. The order of the 17 markers common to the two maps is in close agreement. The genetic map spans 30 cM between D8S527 and D8S274, whereas the same region is 333.1 cR_{5000} on the RH map. Thus, 1 cR_{5000} = 0.09 cM or 1 cM = 11 cR_{5000} in this region.

Table 1. PCR information for 8q markers

Marker name	Annealing temp. (°C)	Mg^{2+} Conc.	Product size (bp)[a]	Retention frequency (%)	Reference
D8S320	60	3.0	386–426	18.8	Riley et al. (1993)
D8S300	60	2.5	496–	17.5	K. Ward[b]
D8S343	55	1.5	134–	17.5	UMDG (1995)
D8S341	55	1.5	355–	20.6	K. Ward[b]
D8S428	55	2.0	191	21.6	K. Ward[b]
D8S51	50	2.5	127–	13	Ludecke et al. (1991)
D8S527	50	2.5	272–284	14.6	Gyapay et al. (1994)
D8S323	60	2.5	192–200	18.6	Lu et al. (1993)
D8S199	45	1.5	226–	17.9	Tomfohrde et al. (1992)
D8S198	55	2.0	165–	20.8	Tomfohrde et al. (1992)
D8S514	50	2.0	209–227	15.8	Gyapay et al. (1994)
D8S508	45	2.0	214–232	13.4	Gyapay et al. (1994)
D8S342	60	3.0	360–412	15.5	Lu et al. (1993)
D8S450	60	2.0	261	14.4	K. Ward[b]
D8S348	60	2.5	408–	15.5	K. Ward[b]
KW435	60	2.0	310–327	16.5	K. Ward[b]
D8S266	45	1.5	153–165	16.8	Gyapay et al. (1994)
MYC	52	1.5	87–125	16.5	Polymeropoulos et al. (1992)
D8S347	60	2.0	322–382	17.7	K. Ward[b]
KW257	55	3.0	500–	18.6	K. Ward[b]
D8S321	60	3.0	228–	16.5	K. Ward[b]
D8S263	45	2.0	275–289	13.4	Gyapay et al. (1994)
D8S284	50	2.5	243–273	13.4	Gyapay et al. (1994)
ACDY8	55	1.5	280	10.3	T.B. Lewis[b]
D8S557	50	1.5	233–251	14.4	Gyapay et al. (1994)
D8S558	60	2.0	160–180	13.4	Gyapay et al. (1994)
D8S529	52	2.0	244–262	14.4	Gyapay et al. (1994)
TG	65	2.0	392	14.4	Theune et al. (1991)
D8S256	50	2.0	210–232	12.6	Gyapay et al. (1994)
D8S523	55	2.0	243–257	13.4	Gyapay et al. (1994)
D8S537	60	3.0	146–176	15.5	Gyapay et al. (1994)
D8S554	60	3.0	161–177	15.5	Gyapay et al. (1994)
D8S534	50	1.5	176–210	16.5	Gyapay et al. (1994)
D8S274	55	2.0	108–118	16.5	Gyapay et al. (1994)
D8S272	48	2.0	192–239	16.5	Gyapay et al. (1994)
D8S502	50	2.5	211–221	16.5	Gyapay et al. (1994)
D8S345	60	2.0	276–296	13.4	Lu et al. (1993)
D8S346	60	2.5	232–252	14.4	Riley et al. (1994)
D8S315	60	2.0	368–384	15.5	Lu et al. (1993)
D8S304	60	2.0	550–	15.5	K. Ward[b]

Primer sequences can be retrieved from the Genome Data Base.
[a]Size ranges are indicated if known. Where the precise size range has not been established, a known reference allele size and a dash indicating polymorphism is shown.
[b]Primer sequences first described in this publication.

DISCUSSION

A panel of 97 RHs was constructed from a chromosome 8-only SCH line and used to order 8q24 markers. Despite the absence of selection for chromosome 8 sequences, a majority of the RHs have been shown to retain human DNA. FISH analysis indicated one to six fragments of human DNA in 10 RH cell lines analyzed. The complete RH panel was screened by PCR for the presence or absence of 40 8q24 markers. The 61 hybrids known to contain chromosome 8 fragments

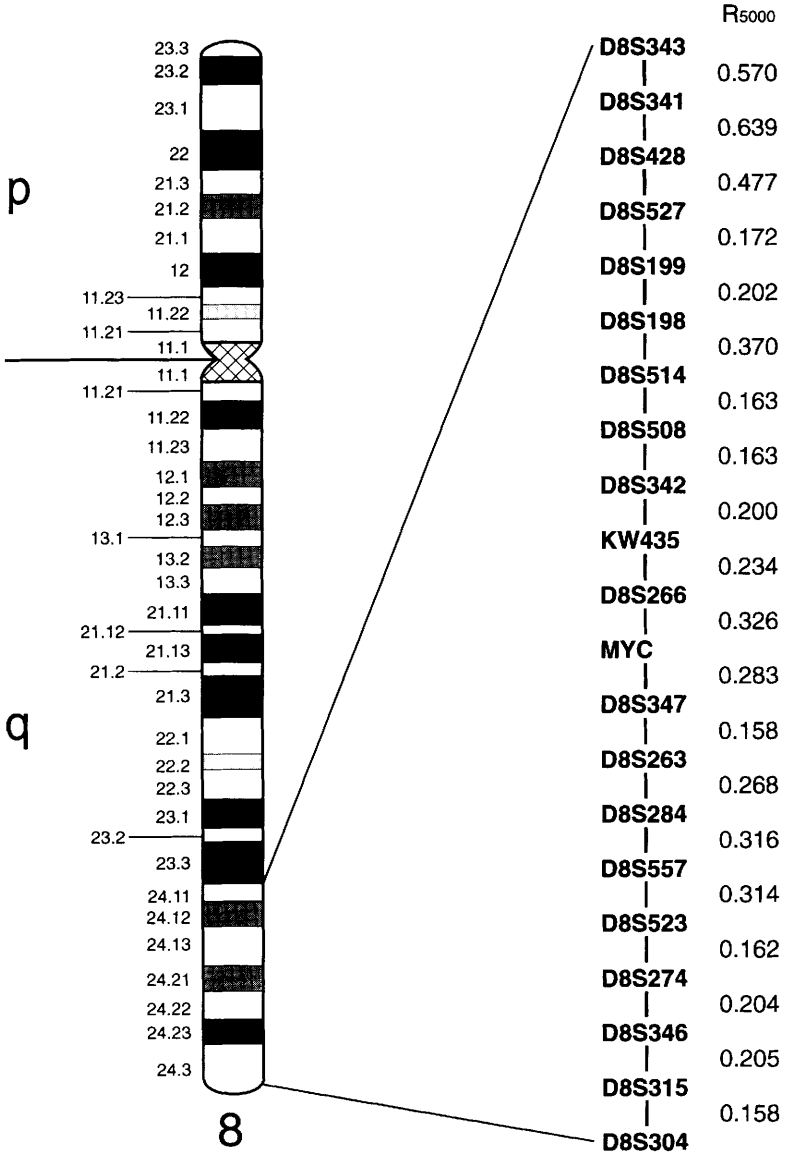

Figure 1 Framework map of 8q24. Twenty-one markers were ordered with >1000:1 maximum likelihood ratio. R_{5000} distances are shown at *right*. 1 cR_{5000} corresponds to ~90 kb.

clearly represented independent clones, as none of these hybrids have matching retention patterns of markers. Retention frequencies of the 8q24 markers ranged from 10.3% to 21.6%.

The retention data were analyzed using the multipoint method of maximum likelihoods. Data from an 8q11.1 marker suggested that fragments near the centromere may be retained at a higher frequency; thus, multipoint analysis was performed under different marker retention models. Essentially identical results were obtained under models allowing for equal retention probabilities for all markers or for higher retention probabilities toward the centromeric endpoint of the map. No gradient of retention was observed toward the telomere. This pattern of similar retention frequencies along a chromosome arm, with an increase in retention for markers in close proximity to the centromere, has been observed in other sets of RHs (Lawrence et al. 1991; Gorski et al. 1992; Francke et al. 1994).

Twenty-one loci in band 8q24 were ordered with high support (≥1000:1 maximum likelihood ratios), providing a framework map of the region. Five loci on the framework map contained sets of markers that had no obligate chromosome breaks and could not be ordered by RH analysis. An additional 16 loci, representing 19 markers, could be ordered with respect to the framework map.

Comparison of the cR_{5000} distances to actual physical distances is difficult because precise physical measurements among 8q24 markers are not known, but estimates can be made from genetic mapping data. As rates of recombination vary across the genome, estimates should be calculated separately for each chromosomal region. The RH map spans 333.1 cR_{5000} between markers D8S527 and D8S274; the same region on the genetic map is 30 cM (Gyapay et al. 1994). Using the assumption that 1 cM equals ~1000 kb, then within this interval, 1 cR_{5000} corresponds to ~90 kb. This RH panel was created using a dose of 5000 rads, resulting in larger fragments as compared to using a higher dose of radiation to induce chromosome breaks. Taking the low radiation dose into account, the estimate of cR-to-kb conversion is consistent with reports for other RH panels (Frazer et al. 1992; Gorski et al. 1992; Abel et al. 1993; Richard et al. 1993, O'Connell et al. 1994).

The RH map order generated by maximum likelihood analysis is in good agreement with the order proposed by the genetic linkage map (Gyapay et al. 1994). The RH map was able to resolve several sets of markers that had no observed re-

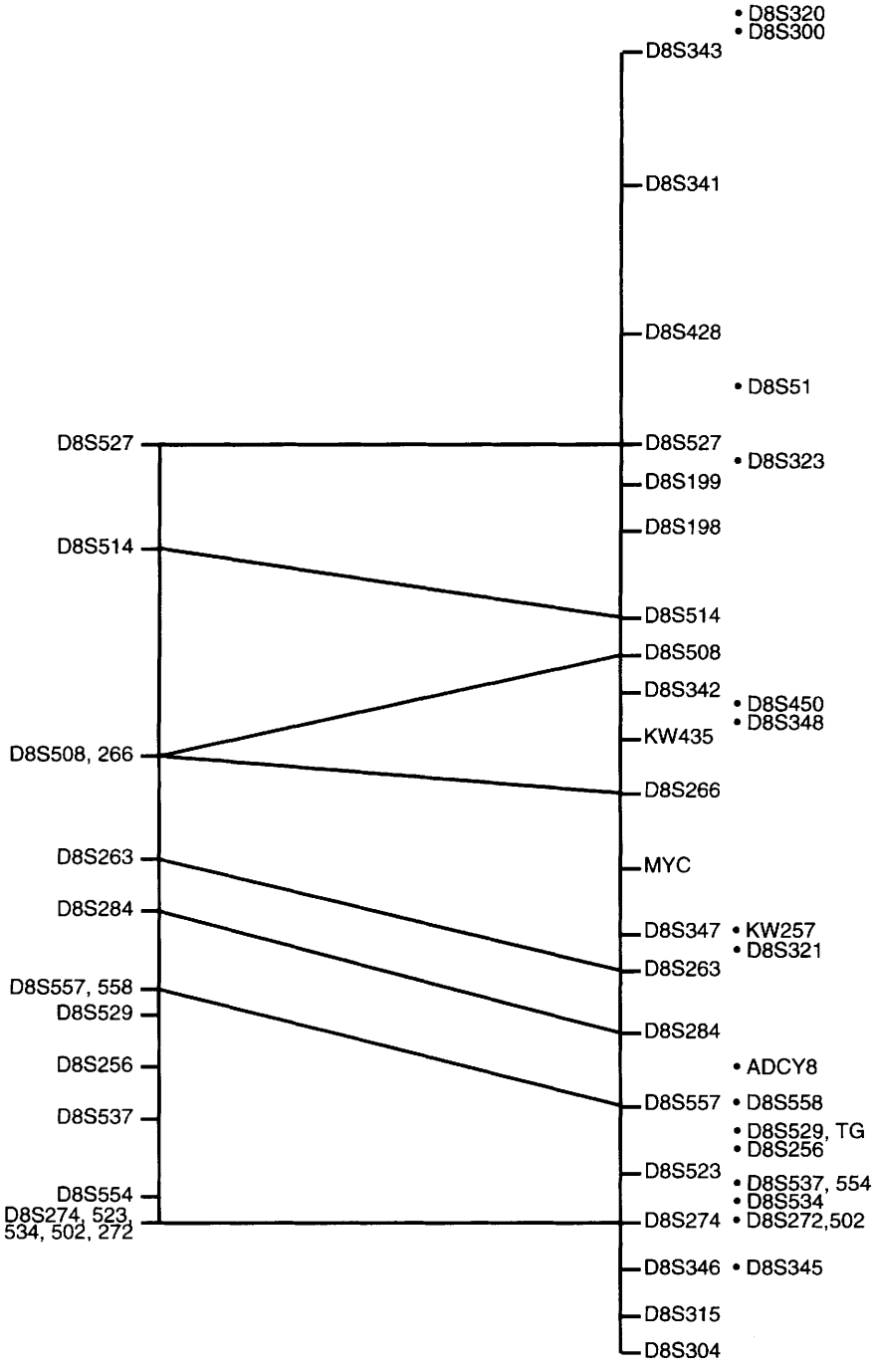

Figure 2 Comparision of the genetic map and the comprehensive RH map. The genetic map is shown at *left*, and the comprehensive RH map at *right*. Markers placed to the side could not be ordered with a 1000:1 maximum likelihood ratio. Data from Gyapay et al. (1994).

combination in the Genethon analysis of 8 CEPH (Centre d'Etudes du Polymorphisme Humain) pedigrees. The marker set D8S508/D8S266 is indicated as a single locus by linkage analysis, yet in the RH panel the markers are separated by 59.7 cR_{5000}. Eight chromosome breaks were observed between D8S508 and D8S266, and four additional markers were mapped into this interval. The physical distance between the marker pair is estimated as 5.3 Mb based on the RH panel. A marker cluster on the genetic map consisting of D8S274/D8S502/D8S272/D8S534/D8S523 was able to be partially resolved on the RH map. Three of the markers (D8S274/D8S502/D8S272) continued to map as a single unit on the RH map. Marker D8S534 had two chromosome breaks with this marker group, placing it 7.5 cR_{5000}, or ~680 kb, proximal to D8S274/D8S502/D8S272. Marker D8S523 was the only marker whose RH localization was in disagreement with the genetic map. RH mapping ordered the region as D8S274/D8S502/D8S272–D8S534–D8S554/D8S537–**D8S523**–D8S256, whereas genetic mapping predicted the order D8S274/D8S502/D8S272/D8S534/**D8S523**–D8S554–D8S537–D8S256. The RH placement was ascertained by a cell line indicating a chromosome break between the marker set of D8S274/D8S502/D8S272/D8S534/D8S554/D8S537 and D8S523. Additional cell lines indicated a grouping of D8S523 with D8S554 and D8S537. The support for the RH map localization of D8S523 between D8S554/D8S537 and D8S256 was 1.1×10^4 and 4.1×10^7, respectively.

The creation of a high resolution map of 8q24 is a powerful resource in the efforts to positionally clone disease genes assigned to this region. A gene responsible for benign familial neonatal convulsions, EBN2, was originally mapped by linkage analysis to the

8q24 interval defined by markers D8S272 and D8S198, a distance that was reported to be 30 cM (Lewis et al. 1993). Originally, few markers had been well ordered for band 8q24. Only three polymorphic markers were reported for the EBN2 critical interval: D8S256, D8S284, and MYC (Lewis et al. 1993). Using the RH panel, 20 additional markers have now been mapped to this area. Eighteen of the newly mapped markers are polymorphic and useful in redefining the EBN2 critical region. Based on the RH map and the estimate 1 cR_{5000} equaling ~90 kb, the original EBN2 interval was calculated to be 27 Mb. Linkage analysis using the original pedigree has narrowed the critical region to D8S272–D8S342, an interval of 226 cR_{5000}, or ~20 Mb. This figure is close to the genetic estimate of 18 cM separating D8S272 and D8S508. D8S508 is a marker slightly proximal to D8S342, as the latter is not included on the genetic map (Gyapay et al. 1994). The high concentration of polymorphic markers that have been ordered using the RH panel will assist future efforts to further narrow the EBN2 critical region by linkage analysis in additional families.

Another disorder mapping to 8q24 is LGS. LGS is considered a contiguous gene syndrome of chromosome 8q24 that combines the phenotypic features of two autosomal dominant disorders, EXT and TRPSI. Linkage studies have mapped the EXT locus ~1.5 cM centromeric to the marker D8S199 (Cook et al. 1993). Breakpoint analysis have concurred with the linkage studies, placing the EXT1 locus 1.5–2.0 Mb centromeric to D8S199 (Ludecke et al. 1995) . This previous mapping data suggest the EXT1 locus is between markers D8S199/D8S323 and D8S527, a distance of 17.2 cR_{5000}. Mapping of the chromosomal breakpoints in EXT, TRPS1, and LGS patients had indicated the maximum distance between the EXT1 and the TRPS1 loci to be $\leq$2000 kb, with TRPS1 mapping centromeric to EXT1 (Ludecke et al. 1995). The TRPSI critical region is defined on the RH map as the interval D8S199/D8S323–D8S527–D8S51, a span of 49.0 cR_{5000}.

RH mapping is a powerful technique, particularly when combined with other mapping strategies. The use of a RH panel produces a higher resolution map than meiotic mapping or somatic cell hybrid panel localization. RH mapping is a bridge spanning the large-scale genetic mapping and fine-scale contig building. The high level of consistency between our RH map and the genetic map suggests that this panel will be a valuable resource for the rapid mapping of markers derived from human chromosome 8.

METHODS

Cell Lines and Culture Conditions

GM10156B is a human–hamster hybrid cell line that retains an intact human chromosome 8 as its only human component. One copy of human chromosome 8 is retained in 12% of the cells; 88% of the cells contain two copies of human chromosome 8. The GM10156B cell line (obtained from Coriell Cell Repository, Cambden, NJ) was cultured in KAO medium (Jones et al. 1981) with 10% fetal bovine serum, 500 U of penicillin/ml, 0.5 mg/ml of streptomycin, 60 mM hypoxanthine, and 10 mg/ml of proline. This cell line served as the donor for the radiation hybrid fusion. The recipient cell line was an APRT and HPRT -deficient cell line, CHO–ATS–49tg (gift of Jerry Adair, Science Park, Smithville, TX). The cell line was cultured in a 1:1 (vol/vol) mixture of Ham's F12 medium and Dulbecco's modified Eagle medium (F/DV), with the addition of 10% fetal bovine serum, 500 U/ml of penicillin, and 0.5 mg/ml of streptomycin.

Generation of Radiation Hybrid Cell Lines

GM10156B donor cells (2×10^7) were trypsinized, washed, and placed in 10 ml of serum-free KAO medium. The cells were placed on ice and exposed to 5000 rads of γ-irradiation using a ^{137}Cs source at a rate of 115.1 rads/min. The cells were immediately fused with 50% polyethylene glycol 1500 (Boehringer Mannheim Biochemical, Indianapolis, IN) to the same number of nonirradiated CHO–ATS–49tg cells. The fused population was plated at a density of 1×10^6 cells per 75 cm^2 flask with complete F/DV selective medium containing hypoxanthine, aminopterin, and thymidine (HAT) (Littlefield 1964). After 3 weeks, 97 HAT-resistant hybrids were isolated, expanded, and DNA was extracted. No clones were observed in flasks containing CHO–ATS–49tg cells placed in complete HAT medium or in flasks containing GM10156B-irradiated cells placed in nonselective medium.

Fluorescence In Situ Hybridization of Cell Lines

To detect the number of chromosome 8-derived fragments retained in a RH, fixed metaphase spreads of several hybrids were analyzed by fluorescence in situ hybridization (FISH) (Pinkel et al. 1986). One hundred nanograms of sonicated human placental DNA was labeled by nick translation in the presence of biotin-14–dATP (GIBCO–BRL) and used as the probe. After hybridization of the probe to the metaphase spreads, human DNA in the hybrids was detected with fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories). Propidium iodide was used to counterstain the hamster DNA. FISH results were photographed with a Zeiss Axioskop photomicroscope.

Marker Screening of the Radiation Hybrids

For each of the 97 RHs, the presence or absence of each of 40 different markers from 8q24 was determined by PCR.

For each primer set, Mg^{2+} concentration and annealing temperature were optimized using either an Omnigene (Hybaid) or a PHC-3 (Techne) thermocycler (Table 1). PCR consisted of 100 ng of template DNA, 10 ng of each primer, 0.2 mM dNTPs, and 1.2 units of *Taq* polymerase (Perkin Elmer) in a 20-μl volume. PCRs were denatured for 5 min at 95°C followed by 30 cycles with each cycle comprised of 94°C for 1 min, annealing temperature for 1 min, and 72°C for 1 min. The final cycle was followed by a 10-min extension at 72°C. PCR products were separated by electrophoresis on a 2% agarose gel (FMC BioProducts) in 1 × TAE buffer and visualized with ethidium bromide staining. Data were scored as present, absent, or ambiguous; questionable results were retyped at least once. Only 0.3% of the data was scored as ambiguous.

Multipoint Mapping of RH Data

The order and intermarker distance of chromosome 8q24 markers were determined using the multipoint maximum likelihood method of statistical analysis (Boehnke et al. 1991). This method depends on two assumptions: (1) γ-ray breakage occurring randomly along a chromosome, and (2) independent retention of the resulting fragments in the RHs. In the N-locus case, the likelihood of the RH data is a function of the N – 1 breakage probabilities between adjacent loci and one or more fragment retention probabilities. The program RHMAP v2.01 (Boehnke et al. 1991), consisting of the routines RH2PT, RHMINBRK, and RHMAXLIK, was utilized for the statistical analysis.

The RH2PT routine was used to estimate retention frequencies, linkage groups, intermarker lod scores, and intermarker distances. Next, multipoint analysis under the minimum breaks criterion identified the map orders with the fewest number of obligate breaks. The branch and bound strategy of minimum breaks guarantees that the best locus order is identified, but with a large number of loci this strategy is computational intensive. Because of the large data set, multilocus order probabilities for the 40 markers were first estimated using the simulated annealing option. Next, the most probable orders identified were tested using the branch and bound option with overlapping sets of 10 markers. The orders with the smallest number of breaks were further verified by comparison with the lod scores and distances obtained by RH2PT. These orders were then accepted as candidate orders for RHMAXLIK. The data were analyzed by RHMAXLIK in overlapping groups of 10 markers and again as the total set of 40 markers. Locus orders were compared by their maximum likelihoods under both the equal and centromeric retention models, with the order with the largest maximum likelihood being the one best supported by the data.

The map with the highest maximum likelihood that contained all of the loci was considered to be the comprehensive map. The framework map was constructed by adding loci one at a time and analyzing the overall map support using RHMAXLIK. If all of the loci under consideration were jointly ordered with a 1000:1 support for order, then the map was considered as a framework. In all, 21 markers were jointly ordered with >1000:1 support. This framework map is shown in Figure 1. Interloci lod scores ranged from 6.29 to 12.97 on the framework map.

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