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Isolation of polymorphic DNA segments from human chromosome 21

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ABSTRACT

A somatic cell hybrid line containing only human chromosome 21 on a mouse background has been used as the source of DNA for construction of a recombinant phage library. Individual phages containing human inserts have been identified. Repeat-free human DNA subclones have been prepared and used to screen for restriction fragment length polymorphisms to provide genetic markers on chromosome 21. Nine independently isolated clones used as probes identified a total of 11 new RFLPs. Four of the DNA probes recovered from the library have been mapped unequivocally to chromosome 21 using a panel of somatic cell hybrid lines. A fifth probe detected an RFLP on chromosome 21 as well as sequences on other chromosomes. This set of RFLPs may now form the basis for construction of a genetic linkage map of human chromosome 21.

INTRODUCTION

Genetic linkage analysis, as an approach to mapping human disease loci, has become much more powerful with the availability of vast numbers of new genetic linkage markers generated by recombinant DNA techniques (1,2). These DNA markers, commonly termed restriction fragment length polymorphisms (RFLPs), represent heritable variations in the base sequence of human genomic DNA detected by Southern blot analysis. Polymorphic DNA markers, already far more numerous than the classical expressed polymorphisms, have been used successfully to map the Huntington's disease gene to chromosome 4 and to regionally localize the Duchenne muscular dystrophy locus and other disease genes on the X chromosome (3-5). Ultimately, it should be possible to construct complete linkage maps of all human chromosomes and thereby permit the systematic mapping of disease genes in family studies.

A chromosome for which it would be especially useful to have a large set of polymorphic markers is human chromosome 21. The role of trisomy 21 in causing Down Syndrome is well established (6). Furthermore, it represents a region of the genome in which there is currently a lack of genetic markers, making it impossible to test effectively for the presence of disease genes by linkage analysis. Finally, the small size of this chromosome increases the

probability that genes mapped to it could be isolated using a chromosome-specific cloning strategy (7).

We report here the isolation and characterization of polymorphic DNA markers from human chromosome 21. We have constructed recombinant libraries using genomic DNA from a human-mouse hybrid cell containing only human chromosome 21. Recombinant phage containing human inserts were identified and repeat-free subclones have been obtained in a number of cases. The extent to which each of these subclones detects RFLPs and is useful as a genetic marker has been evaluated. This work has resulted in the discovery of 9 new DNA markers, and represents our first step in generating a linkage map for human chromosome 21.

MATERIALS AND METHODS

Cell Lines

WA17 is a subclone of the mouse-human hybrid cell line WAVR4d-F9-4a which contains chromosome 21 as its only human chromosome (8,9). It was originally isolated by Dr Regina Dutkowski and kindly provided to us by Dr Gretchen Darlington. Cell fusion, isolation, and characterization of the human-mouse somatic cell hybrids comprising the mapping panel has been described (10,11). Cell hybrids were examined for human chromosome-specific enzyme markers (11,12), chromosomes (13), and hybridization to the chromosome 21 probes on the same cell passage.

DNA Isolation and recombinant library construction

Cell line DNAs used for polymorphism screening were prepared as described from lymphoblastoid cell lines obtained from unrelated individuals (14,15). DNA used for mapping studies was isolated from human, mouse, and hybrid cells as described (16). High molecular weight WA17 DNA was prepared as previously described (15), partially digested with EcoRI (Boehringer Mannheim) and size fractionated by agarose gel electrophoresis (18 hrs, 8 V/cm). DNA fragments of 10-22 kb were electroeluted and ligated to EcoRI digested Charon 4A phage arms previously treated with calf intestinal phosphatase (Boehringer Mannheim). A total of 75,000 recombinant bacteriophage were recovered by in vitro packaging of the ligated DNA (17). DNA from phage clones with human inserts (identified as described under "Results" was digested with EcoRI, fractionated on agarose gels, and transferred to nitrocellulose (18). Repeat-free DNA fragments identified by lack of hybridization to ^{32}P-labeled human DNA were isolated from agarose gels by binding the DNA to glass fiber filters (GF/C, Whatman) in the presence of 8M NaCl)(19). DNA was eluted in 0.1X TE (1 mM Tris-HCl, 0.1

mM EDTA, pH 8.0) and ligated to EcoRI-digested, dephosphorylated pBR328 vector. Recombinant plasmids were recovered by transformation of E. coli LE392.

DNA transfer and hybridization

Endonuclease digestions were performed as suggested by the supplier (EcoRI- Boehringer Mannheim, all others-New England Biolabs). Detection of RFLPs by Southern transfer and hybridization as previously described (3).

RESULTS

Isolation of phage containing chromosome 21 DNA

The species-specificity of interspersed repetitive sequences in mammalian genomes allows distinction between recombinant DNA molecules containing DNA inserts of human origin and those containing rodent DNA (18). We have therefore approached the isolation of DNA segments from human chromosome 21 by cloning DNA from WA17, a human-mouse hybrid cell line containing chromosome 21 as its only human component. In our phage library constructed from WA17 genomic DNA, each recombinant phage should contain a single insert of 10-20 kbp and any human DNA insert should derive from chromosome 21. To identify chromosome 21 clones, we prepared two nitrocellulose replicas from gridded arrays of phage plaques and hybridized these to human and mouse genomic DNA labelled with ^{32}P (20). Phage containing human DNA inserts were easily identified by their significant intensity of hybridization to human DNA relative to mouse DNA detected after a high stringency wash. This method has previously been shown to detect greater than 95% of human clones in libraries containing 12-20 kb inserts (18).

Overall, 43 phage clones from this initial screen were chosen for more detailed analysis. The previous use of a grid format in the screening permitted us to propagate these clones as pure stocks after only a single round of plaque purification without the need for additional hybridizations to human DNA. DNA prepared from each clone was digested to completion with EcoRI and the resulting fragments were resolved by agarose gel electrophoresis. The DNA from these gels was then transferred onto nitrocellulose, and each filter was hybridized to ^{32}P labelled human genomic DNA to determine which EcoRI fragments contained repetitive sequences. The results of this analysis are summarized in Table 1.

The phage clones contained a total of 580 kbp of human DNA corresponding to approximately 1 % of chromosome 21. The insert size averaged 13.5kbp/phage clone with a range of 1 to 7 EcoRI fragments per clone. The mean size of the

TABLE 1-PHAGE CLONES WITH HUMAN 21 INSERTS

CLONE NUMBER	ECORI FRAGMENTS (KB)							PLASMID SUBCLONES	HUMAN GENE MAPPING WORKSHOP SYMBOL
	A	B	C	D	E	F	G		
218	14.0	2.8	1.2						
219	6.3	3.9							
220	7.8	1.9*	1.5						
223	8.7	3.5	2.8	2.1*	1.9				
224	12.5	3.6							
225	9.2	2.6							
226	8.4	4.6							
227	8.0	3.3	2.8	1.2*					
228	6.0	3.4	1.5*					pPW228C	D21S1
229	12.0	6.9	1.9	0.9					
230	4.1	2.9	2.2*	1.2*				pPW230D	D21S2
231	6.1	2.7	2.1*	1.3*	1.2*	0.8*	0.7*	pPW231C pPW231D pPW231E pPW231F pPW231G	D21S3
232	3.7	3.6	1.4	1.1					
233	4.3	3.4	2.4	1.8	1.6	1.5*		pPW233F	D21S4
235	4.1	3.8	2.7	1.3*				pPW235D	D21S5
236	6.4	6.3	1.9*	1.1*				pPW236B	D21S11
237	6.9	2.7	1.9	1.4*				pPW237D	D21S6
238	5.0	3.7	3.5						
239	7.5	4.2	3.1	0.9					
240	6.9*	4.3	1.9						
242	3.1	2.7*	2.5	1.1*	0.9*			pPW242B pPW242D	D21S7
243	12.5								
244	5.4	2.8	2.2	1.0*				pPW244D	D21S23
245	5.0	4.0	2.9	2.0*				pPW245D	D21S8
247	8.9	5.4							
248	6.5	3.9*	3.1	0.9*				pPW248D	D21S9
249	10.1	2.5							
251	9.5	3.4							
255	6.4	1.8	1.0						
256	6.1	2.7	2.2						
258	4.7	4.4	3.2	2.8	1.1*			pPW258E	D21S10
259	10.0								
260	11.0	1.9							
262	9.2	5.5							
263	7.2	2.6	1.8	1.3*					
264	10.0	5.0							
265	7.3	3.0	2.8	1.1*				pPW265D	
266	5.1	3.6	3.4						
267	4.4	3.1	2.8*	2.0*	1.5			pPW267C pPW267D	D21S12
270	4.8	3.8	3.5	3.4	1.0				
271	7.8	3.2							
276	8.4	2.0	1.6						
278	5.0	3.9	3.6	3.5					

* indicates a fragment apparently free of repeat sequences

EcoRI fragments was 4.0 kbp, although fragments smaller than 500 base pairs would have been overlooked in this analysis. In all, 29 putative repeat-free EcoRI fragments representing 19 different phage clones were identified by their failure to hybridize significantly with the human total genomic DNA probe. For ease of subsequent subcloning, the clones were analyzed only with EcoRI, but it is likely that single-copy segments could have been identified in additional phage clones had other restriction enzymes also been employed. Twenty-one of these repeat-free segments were chosen for subcloning into the EcoRI site of pBR328 as indicated in Table 1.

Screening for DNA polymorphisms

To check the human origin of each single-copy fragment, we performed Southern blot experiments to detect specific hybridization to human genomic DNA and to WA17 DNA as compared to DNA from the cell line A9, the mouse parent of the somatic cell hybrid. The subclones were then tested for their ability to detect human DNA polymorphisms in three successive screens. Individual subclones derived from 12 different recombinant phage (all listed in Table 2) were used as probes against DNA digests from 6 unrelated individuals. Six restriction enzymes (EcoRI, HindIII, BamHI, MspI, TaqI, EcoRV) were used at this stage. Seven of the probes (pPW231F, pPW233F, pPW235D, pPW242B, pPW245D, pPW248D, pPW267C) were subsequently hybridized to BglII, HinfI, KpnI, PstI, PvuII, SalI and XmnI digests of genomic DNA from 4 unrelated individuals. In a final screen, 8 probes (pPW231F, pPW233F, pPW235D, pPW237D, pPW242B, pPW244D, pPW248D, pPW267C) were hybridized to SacI, BclI and RsaI digests of DNA from 4 unrelated individuals.

The results of the search for RFLPs are displayed in Table 2. Eighteen potential polymorphic restriction enzyme sites were identified as individual variations in the pattern of restriction fragments observed. Two of the subclones, pPW228C and pPW236B each detected putative RFLPs with two different enzymes, while one probe, pPW235D detected two separate putative RFLPs with the enzyme TaqI and single variants with MspI and BglII. None of the probes tested failed to detect a variant fragment in at least one of the 16 restriction enzyme digests. By summing the total number of fragments detected by each probe, we estimate that approximately 348 restriction enzyme sites comprising 1860 base pairs of DNA were monitored in each individual analyzed. We estimate therefore that in polymorphism screening panels of 4-6 individuals, approximately one cleavage site in 19 or 1 base in 100 is a potential site of frequent variation. This is similar to estimates for DNA probes from other human chromosomes (21,22).

TABLE 2--POLYMORPHIC DNA MARKERS FROM HUMAN CHROMOSOME 21

DNA PROBE	ENZYME	ALLELE (FRAGMENT SIZE/FREQUENCY) #1	#2	#3	CONSTANT FRAGMENTS	NUMBER PERSONS TYPED	PIC*	LOG REL. MENDELIAN LIKELIHOOD*
pPW228C	BAMHI	7.1/.21	6.3/.79			24	.29	5.3
	MSPI	7.6/.38	5.0/.62			26	.36	6.2
pPW231F	TAQI	4.5/.10	4.0/.90			20	.16	6.8
pPW233F	PSTI	8.7/.87	7.8/.13			20	.19	8.1
pPW235D	TAQI#1	8.0/.18	4.9/.69	4.0/.13	8.0+	15	.24	7.0
pPW236B	TAQI	5.5/.31	4.0/.69			32	.32	8.8
	ECORI	2.9/.25	1.9/.75		0.9	36	.31	7.1
pPW242B	RSAI	2.1/.37	1.8/.63			20	.35	9.2
pPW244D	ECORI	1.0/.36	0.9/.64		4.5	21	.35	9.3
pPW245D	HINDIII	3.2/.45	2.7/.55			33	.37	13.6
pPW267C	TAQI	15.0/.09	6.4/.91			20	.14	5.4

* Polymorphism information content (2);Log relative Mendelian likelihood (23) Allele frequencies were determined by gene counting for all codominant systems. Fragment sizes are in kilobases.

+ Probe pPW235D detected a constant band at 8.0 kb that obscures allele 1. In this case allele frequencies were calculated from phenotypic data assuming Hardy-Weinberg equilibrium using Bernstein's correction formula (27). Probe pPW235D also detects variable fragments of 18.0 kb, 13.1 kb and 11.0 kb but the underlying RFLP(s) has not yet been fully characterized. Additional variants detected but not characterized were with pPW235D (BglII, MspI), pPW237D (BamHI), pPW242B (PvuII), pPW248D (PvuII) and pPW258E (BamHI).

Characterization of putative RFLPs

An expanded panel of unrelated individuals was used to assess the frequencies of the variants in the general North American population. Allele frequencies observed are displayed for 11 of the putative RFLPs in Table 2 which also presents the calculated polymorphism information content (PIC) for each RFLP (1). This parameter represents the likelihood that progeny of an arbitrary mating will be informative for linkage analysis at the marker locus. The range of PIC values represented by the individual RFLPs was from 0.14 to 0.37. The informativeness of the other 7 potential RFLPs has not yet been fully investigated.

Initial identification of all putative RFLPs was performed by comparing unrelated individuals. Such analyses can be misleading if the differences observed are due to incomplete digestion of the genomic DNA or other factors unrelated to individual genotype. For the 11 RFLPs in Table 2, we have determined that the variation displays Mendelian inheritance and therefore represents a useful marker locus. Typical results of this analysis are displayed in Figures 1, and 2.

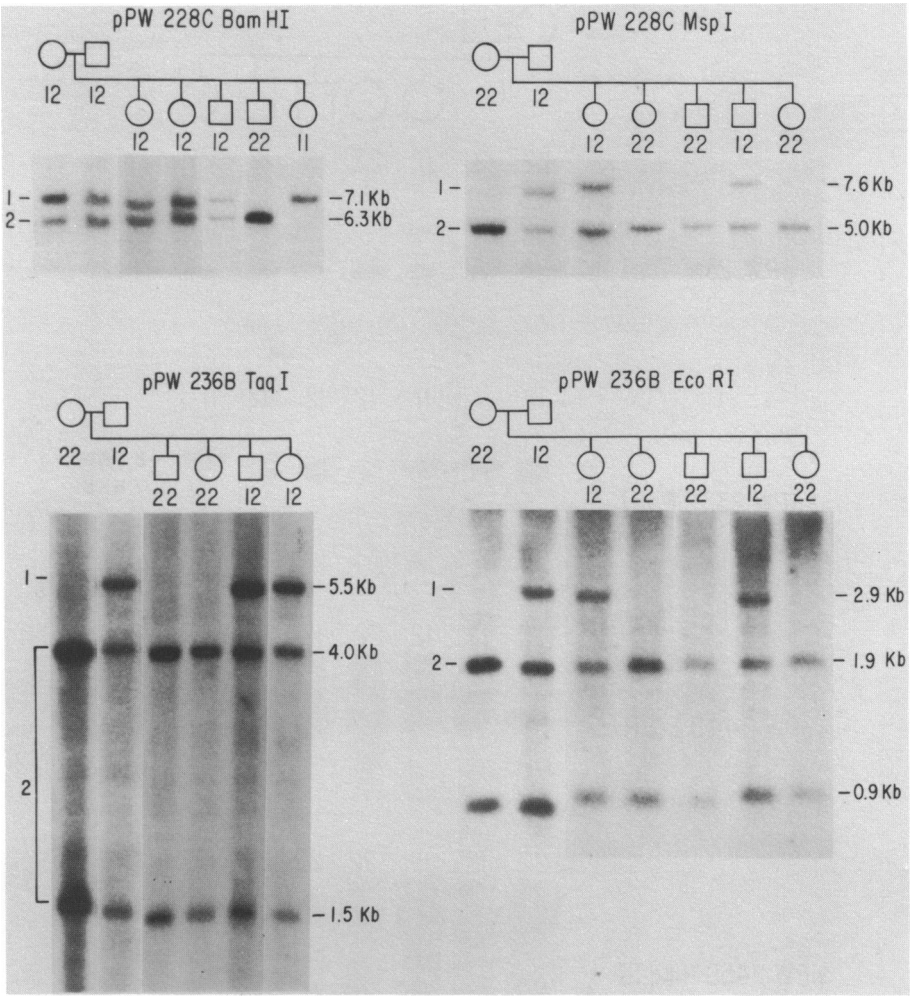

Figure 1. RFLPs detected by probes pPW228C and pPW236B
Five microgram aliquots of genomic DNA prepared from lymphoblast lines of family members were digested with the indicated enzyme, fractionated by agarose gel electrophoresis, transferred to a nylon membrane and hybridized to the indicated probe. Alleles are numbered in order of decreasing size. Slight discrepancies in the apparent sizes of bands result from the comparison of lanes run on different gels.

Figure 1 shows the pattern of fragments observed when either pPW228C or pPW236B was hybridized to Southern blots containing DNA from a small nuclear family. The family tested was identical in all cases, with the exception of pPW236B with TaqI. In a BamHI digest, both parents appear to be heterozygous,

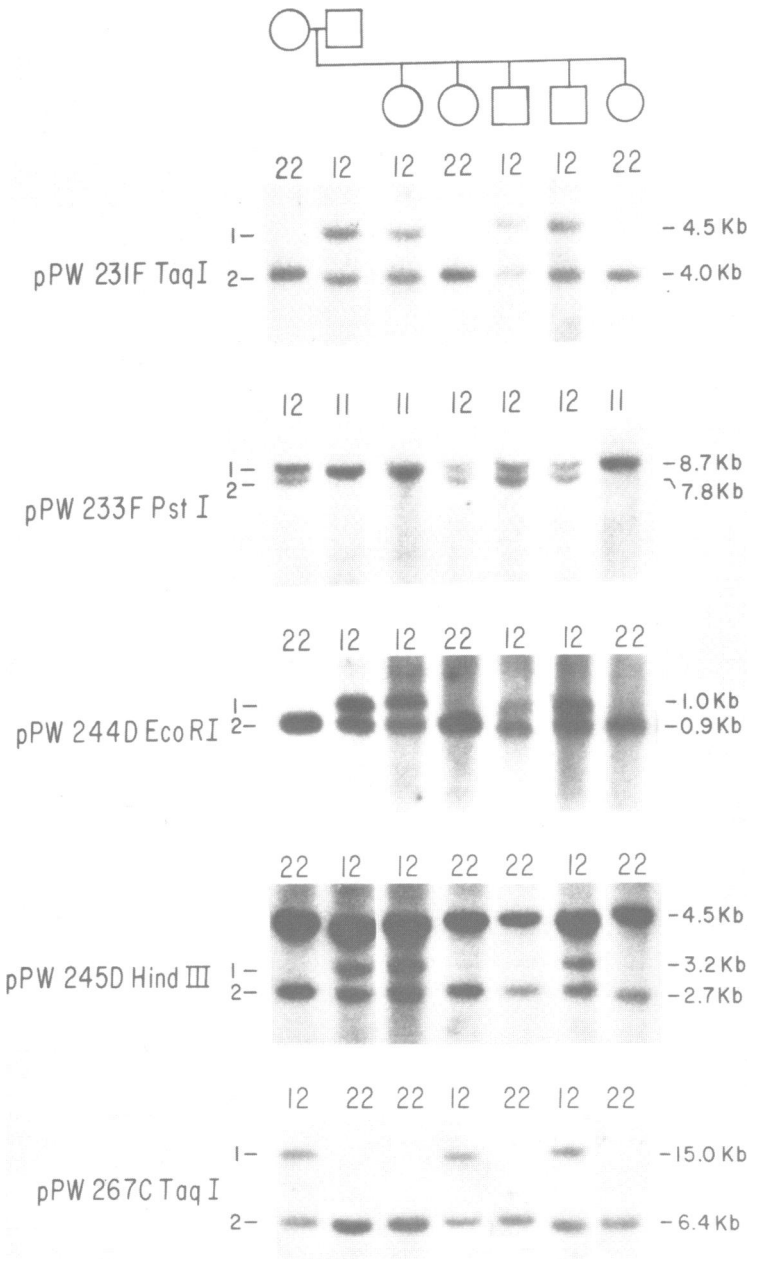

Figure 2. RFLPs detected in a nuclear family by five chromosome 21 probes Polymorphisms were detected using the indicated probe and enzyme as in Figure 1.

displaying both 7.1 kb and 6.3 kb fragments when tested with pPW228C. Three children are also heterozygotes, one child is homozygous for the 7.1 kb fragment, and one child is homozygous for the 6.3 kb fragment. In the same family, only the father is heterozygous for the 7.6 kb and 5.0 kb fragments detected by pPW228C in an MspI digest, while the mother is homozygous for the 5.0 kb fragment. As expected, the children were either heterozygotes or homozygotes for the 5.0 kb fragment. These data are consistent with the presence in the father of one chromosome 21 carrying the 7.1 kb BamHI and 5.0 kb MspI fragments with the second chromosome 21 carrying the 6.3 kb BamHI and 7.6 kb MspI fragments.

The father was also heterozygous for the 1.9 and 2.9 kb fragments detected in an EcoRI digest with pPW236B. The mother was homozygous for the 1.9 kb fragment. Again, all children were either identical to the mother, or heterozygous like the father. As expected, no children were apparent homozygotes for the 2.9 kb fragment. Furthermore, the pattern of inheritance observed for the 2.9 kb fragment is consistent with its presence on the same chromosome in the father as the 7.6 kb MspI fragment detected by pPW228C. A constant fragment was observed in all individuals at 0.9 kb indicating the presence in the genome of a second sequence related to pPW236B.

A Mendelian pattern of inheritance was also observed for the pPW236B TaqI polymorphism but this could not be compared to the previous sites since it was tested in a different nuclear family. Unlike the EcoRI digest, no constant fragment was observed with pPW236B in the TaqI digests. The variable 1.9 kb EcoRI fragment and the cross-hybridizing 0.9 kb EcoRI fragment might therefore reside in close proximity and derive from the same 4.0 kb TaqI fragment.

The same family used for pPW228C with BamHI and MspI and with pPW236B with EcoRI was also used to trace the inheritance of the polymorphisms detected by pPW231F, pPW233F, pPW244D, pPW245D and pPW267C as shown in Figure 2. In all cases, only one parent was heterozygous for the given polymorphism and the data were consistent with Mendelian inheritance.

For pPW233F and pPW267C, the mother was heterozygous for both polymorphisms while the father was homozygous. The pattern of inheritance of the alleles in the children indicated the presence of at least one recombination event between these two sites; the most parsimonious explanation is that the crossover occurred in the gamete giving rise to the eldest son. When pPW231F, pPW244D and pPW245D were used as probes, the father was heterozygous for each polymorphic site while the mother was a homozygote. No obligate recombination events were observed between the pPW231F TaqI and the

pPW244D EcoRI polymorphic sites, but both of these must have recombined with the polymorphic pPW245D HindIII site, again with the eldest son most likely being the only recombinant. From the data in Figure 1 discussed above, the pPW245D HindIII polymorphic site is segregating together with the pPW228C MspI and BamHI polymorphisms and the pPW236B EcoRI polymorphism with no obligate recombinants. Clearly this linkage data does not approach statistical significance, and many more families must be typed to achieve accurate estimates for the genetic distances separating these markers. The fact that recombination events have been observed does, however, indicate that these loci are not all clustered in a small region of chromosome 21.

Data from the families in figures 1 and 2 and from several additional sibships were used to calculate the relative likelihood of Mendelian inheritance versus random distribution of the different sized fragments for the 11 RFLPs as suggested by Barker et al. (23). The logarithm of the relative Mendelian likelihood which is a measure of the extent to which the data supports the hypothesis of Mendelian inheritance is shown for each RFLP in Table 3. In all cases, the odds are overwhelmingly ($>10^5$ to 1) in favour of Mendelian segregation. For each of these RFLPs, the frequency of homozygotes and heterozygotes observed in the general population does not deviate significantly from Hardy-Weinberg equilibrium ($P>0.10$ by chi-square analysis). The 7 other variants detected in our analysis must be considered unproven RFLPs until Mendelian inheritance and agreement with Hardy-Weinberg equilibrium have been investigated.

Mapping of cloned segments to chromosome 21

We presumed, based on the presence of chromosome 21 as the only human chromosome in the WA17 cell line, that all single-copy fragments we had subcloned derived from this chromosome. In order to check this assumption we chose 5 subclones at random to be mapped in the human genome using a panel of human-mouse somatic cell hybrid lines containing various human chromosomes (14). The clones were hybridized to Southern blots containing cell hybrid DNA digested with several different restriction enzymes. Each line was scored for the presence or absence of restriction fragments present in human DNA but absent from mouse DNA. The results of this experiment are shown in Tables 3 and 4.

Probes pPW228C, pPW237D, and pPW245D each detected BamHI fragments that were only present in hybrid lines containing chromosome 21 and were never present in cell lines lacking this chromosome. Probe pPW236B detected two EcoRI fragments in all positive cell lines, one at 0.9 kb and a second at

TABLE 3-MAPPING OF PROBES USING SOMATIC CELL HYBRID PANEL #1

	Probes				Chromosomes																						
Hybrids	228C	237D	245D	235D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	TL*
WIL-13	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	
WIL-15	+	+	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	
WIL-14	-	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	
WIL-2	+	+	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	
XER-7	-	-	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	+	
ICL-15	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	+	+	-	-	
ICL-15 CSB-1	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	
REW-8D CSAz4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	
ATR-13	-	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	-	+	+	-	-	-	-	-	5/X
REW-8D CSAz3	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	
ITW	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
%DISCORDANCY	228C,237D and 245D				82	64	82	73	73	73	73	73	73	73	55	55	73	82	73	64	45	64	73	45	0	45	73	
	235D				82	82	64	73	73	73	73	55	91	55	91	55	73	64	73	100	9	64	91	82	36	64	55	

228C, 237D, and 245D were hybridized to BamHI digested hybrid DNAs; 235B was hybrydized to HindIII digests. Chromosomes of hybrid cells were karyotyped and banded by Giemsa-trypsin staining. Enzyme markers assigned to each chromosome except the Y have been tested on each hybrid, confirming the chromosome analysis. Translocations have been described previously (26). % discordancy indicates the lack of cosegregation of the marker and a specific chromosome. No discordancy demonstrates chromosome assignment.

either 1.9kb or 2.9 kb depending on the hybrid line. The latter two fragments correspond to alternate alleles of the EcoRI RFLP detected by this probe. The presence of both the 0.9 kb fragment and the RFLP-related fragments correlated exactly in the cell hybrids with the presence of chromosome 21. The fifth probe, pPW235D, detected a single HindIII fragment, but the presence of this band did not correlate unequivocally with any single human chromosome (Table 3). The data was not inconsistent with the presence on chromosome 21 of a segment hybridizing to the probe, but suggested that similar segments may exist on other human chromosomes. Since pPW235D detects variable TaqI fragments, a filter containing TaqI-digested somatic cell hybrid DNAs was prepared to clarify this issue. Table 4 shows the results for each hybrid

TABLE 4-MAPPING OF PROBES WITH SOMATIC CELL HYBRID PANEL #2

	235D TaqI	236B EcoRI	Chromosomes																							
Hybrids			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	TL*
REW-8D CSAz3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	
MH-21	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	
WIL-2	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	+	-	-	-	+	-	+	
WIL-14	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+	
WIL-15	+	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	
ICL-4	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	
ICL-6	-	-	+	-	-	+	+	-	-	-	-	+	-	+	+	-	-	-	+	+	-	+	-	-	-	
ATR-13	-	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	5/X
ATR-15 BSAg	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	+	-	-	+	+	-	-	-	+	-	
DUM-23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	
REX-26	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	
SIR-8	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	
VTL-21	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	-	-	+	+	-	+	-	+	-	+	
VTL-22	+	+	-	-	+	-	+	-	-	+	-	-	-	+	-	-	+	+	+	-	+	-	+	-	-	
VTL-23	+	+	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	-	-	+	-	
VTL-24	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	-	+	-	
%DISCORDANCY	235D		69	38	44	56	44	50	44	50	50	44	25	31	63	56	50	31	50	69	56	69	0	50	31	
	236B		69	38	44	56	44	50	44	50	50	44	25	31	63	56	50	31	50	69	56	69	0	50	31	

* Translocation chromosome (26). Probe 236B was scored for the presence of both 1.9 kb and 0.9 kb EcoRI fragments. Probe 235D was scored for the presence of either the 4.9 kb or the 4.0 kb allele of the TaqI RFLP. The other TaqI bands detected with this probe did not correlate with any single human chromosome.

line scored by presence or absence for one of the alternate alleles represented by the 4.9 and 4.0 kb fragments. These correlated exactly with chromosome 21 indicating the presence of the locus from which our clone was derived. Neither the constant 8.0 kb fragment, nor the several variable fragments detected by the probe showed a complete correlation with any single chromosome, including chromosome 21 (data not shown). It is likely therefore that in addition to the locus on chromosome 21, probe 235D detects hybridizing fragments from several other chromosomes and additional restriction enzymes will be required to distinguish these loci.

DISCUSSION

Chromosome 21 comprises approximately 1.7% of the human genome or approximately $5x10^7$ base pairs of DNA (24). We have isolated DNA clones containing 580 kilobase pairs or about 1% of human chromosome 21. All of the DNA from this chromosome could be contained in less than 1500 nonoverlapping cosmid clones. The cloning of an entire chromosome is therefore within the realm of possibility. It is more likely however that effort will be expended to clone particular regions of the chromosome which contain individual genes of interest, such as the 21q22 region which in the trisomic state results in Down Syndrome. The construction of a complete genetic linkage map of chromosome 21 using RFLPs would allow the localization of any disease genes mapping to this chromosome using linkage to a standardized battery of DNA probes.

This report describes the characterization of 11 new RFLPs that will be useful for genetic linkage analysis and for studies of chromosomal nondisjunction. In addition, seven other variants might prove to be useful DNA markers when they are better characterized. Four of the DNA probes have been unequivocally mapped to human chromosome 21, while a fifth detects an RFLP on 21 as well as sequences on other chromosomes. The remaining polymorphic DNA markers are presumed to reside on chromosome 21 since it was the only detectable human material in the hybrid cell used to construct this recombinant library. Final proof that they do indeed derive from this chromosome will await the generation of linkage data that indicates they all form part of a single linkage group. Alternately, physical mapping by in situ hybridization and/or somatic cell hybrid panels could be used to provide this confirmation. Ultimately, obtaining both types of data will allow a direct comparison of the physical and genetic maps for this chromosome and an estimation of the relationship between physical distance on the chromosome and

frequency of recombination.

The entire human genome consists of approximately 3300 centimorgans. Chromosome 21 would therefore represent approximately 50 cM if recombination events are randomly distributed in the genome. If they are not, this estimate could be quite inaccurate. Preliminary linkage data from our probes indicates that they are not all confined to a small genetic region of the chromosome but rather span at least 50cM (R. Tanzi, P. Watkins and J. F. Gusella, manuscript in preparation). These polymorphic markers will therefore provide the foundation for a preliminary linkage map of chromosome 21.

In addition to the 11 new markers described here, several RFLPs mapping to chromosome 21 have been identified using libraries constructed using flow-sorted chromosomes (25). Furthermore, a cDNA clone coding for superoxide dismutase 1 which maps to chromosome 21 has also revealed RFLPs (Gusella, J.F., Tanzi, R. et al.,unpublished observations). It should also be straightforward to generate many more anonymous DNA sequence RFLPs using the approach described here. Many of the phage clones that we have isolated could be screened following digestion with enzymes other than EcoRI to identify single-copy segments. Furthermore, many single copy fragments that we have already detected have not yet been subcloned or fully screened for polymorphism. Finally, the capacity of our library to provide more phage clones with inserts of chromosome 21 DNA has not yet been exhausted. The continued pursuit of this strategy should therefore culminate in the capacity to construct a very detailed linkage map spanning all of chromosome 21.

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