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Integration of Transcript and Genetic Maps of Chromosome 16 at Near-1-Mb Resolution: Demonstration of a "Hot Spot" for Recombination at 16p12

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A single mapping resource, a mouse/human somatic cell panel with average distance between breakpoints of 1.2 Mb and a potential resolution of 1 Mb, has been utilized to integrate the genetic map and a transcript map of human chromosome 16. This map includes 141 genetic markers and 200 genes and transcripts. The localization of four genes (CHEL3, TK2, TRG1, and MMP9) reported to map to chromosome 16 could not be confirmed, and for three of these localizations to other human chromosomes are reported. A correlation between genetic and physical distance over a region estimated to be 23 Mb on the short arm of chromosome 16 identified an interval demonstrating a greatly increased rate of recombination where, in females, 1 cM is equivalent to a physical distance of 100 kb.

INTRODUCTION

Positional cloning of human disease genes has been facilitated by the construction of detailed genetic maps for the entire genome (Weissenbach *et al.,* 1992) and subsequently by more detailed chromosome-specific genetic maps. Positional cloning of disease genes can be facilitated by the construction of transcript maps to provide rapid access to candidate genes. For the transcript map to be useful it is essential that this map be integrated with the genetic map by utilizing a common mapping framework. This integration allows easy and accurate access from the genetic map to candidate genes and their transcripts.

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The variety of approaches utilized for the localization of genes and their transcripts can result in data that are difficult to merge with any accuracy into a single physical map and are unlikely to be integrated with genetic maps. We have utilized a high-resolution somatic cell hybrid panel for the entire chromosome 16 to provide a uniform framework for the integration and mapping of genes and their transcripts generated by groups cloning individual genes or generated as part of entire genome or chromosome-specific mapping efforts. Together with the physical location of microsatellite markers utilized for the genetic map to the same somatic cell hybrid panel, this provides an integrated genetic and expressed sequence map.

MATERIALS AND METHODS

Somatic cell hybrid panel. A high-resolution mouse/human somatic cell hybrid panel was constructed by fusing human cell lines with the mouse cell line A9. Selection of hybrids containing human chromosome 16 was based on the gene APRT, at 16q24.3. The majority of the human cell lines contained translocations or interstitial deletions involving chromosome 16 and were ascertained in clinical cytogenetic laboratories. The construction of this panel has been described in detail elsewhere (Callen, 1986; Callen *et al.,* 1990). A further extension of this panel has been described in Whitmore *et al.* (1994) with a complete listing of hybrids given in Table 1 of Doggett and Callen (1995). In general, each hybrid contains the region of chromosome 16 from the breakpoint to 16qter. Somatic cell hybrids with an interstitial deletion of chromosome 16 are CY180, CY160, CY138, CY130, CY125, CY127, CY113, and CY107. In each of these there are two breakpoints on chromosome 16, designated by D (distal) or P (proximal). CY18A and CY145 are complex, and each contain two fragments of chromosome 16. For the hybrids CY189 and CY3 the derivative chromosome 16 contains the region from the pter to the breakpoint because the translocations involved the X chromosome, allowing selection in tissue culture based on the gene HPRT at Xq26.

When possible, genes or cDNAs known to be on chromosome 16 were mapped to the somatic cell hybrid panel by PCR amplification

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using oligoprimers. The amplified products were visualized on ethidium bromide-stained agarose gels. Mapping was initially against a subset of the panel (hybrids CY2, CY3, CY12, CY105, CY126, and CY186, A9 mouse control, human control), with subsequent screening of additional hybrid cell lines in the identified region to achieve final localization.

Genes. Whitmore *et al.* (1994) reported genes and transcripts that were mapped to a subset of the present hybrid panel, and when necessary the localizations have been further refined by screening the additional somatic cell hybrids. Since that previous report, the following gene localizations to the somatic cell panel have been published: SLC9A5 (Klanke *et al.*, 1995), PLCG2 (Hernandez *et al.*, 1994), DNL1 (Yasuda *et al.*, 1995), MRP (Kuss *et al.*, 1994), SAH (Samani *et al.*, 1994), STM (Aksoy *et al.*, 1994), GTF3C1 (D'Arigo *et al.*, 1995), ITGAL and ITGAX (Kremmidiotis *et al.*, unpublished), and CNCG2 and CNCG3 (Ardell, Pittler *et al.*, unpublished).

The gene CBFB was mapped by probing Southern blots of somatic cell hybrid DNA with a probe showing homology to the published sequence of this gene. The gene BCGF1 was mapped by hybridizing to Southern blots of hybrid DNA a probe amplified from genomic DNA with the primers P3 and P4 (Kovanen *et al.*, 1995). All other genes were localized using PCR.

The Genome Data Base (GDB) was utilized to identify genes mapping to chromosome 16. For each gene the sequence was accessed from GenBank, and when possible oligoprimers were designed within the 3′ or 5′ untranslated regions. This reduces the possibility of PCR amplification of large introns or of a similar size band from mouse DNA. Table 1 gives details of primers designed for cloned genes that had been identified as mapping to chromosome 16. Primers were as published for the genes STP (Dooley *et al.*, 1994) and HMOX2 (Kutty *et al.*, 1994).

Transcripts. The majority of mapped transcripts were generated from the sequencing of randomly isolated cDNAs. These were initially localized to chromosome 16 by analysis of a monochromosomal hybrid panel. Details of these markers are published (Khan *et al.*, 1992; Durkin *et al.*, 1992; Polymeropoulos *et al.*, 1993; Murakawa *et al.*, 1994; Auffray *et al.*, 1995) and/or can be accessed from GDB by utilizing the appropriate D16S number. All transcripts, except those reported by Whitmore *et al.* (1994), were localized by PCR.

Genetic map. A framework genetic map (odds >1000:1) was constructed from the CEPH database (version 7, Kozman *et al.*, 1995) utilizing PCR-based microsatellite markers. This map was constructed with the BUILD option of CRIMAP. Subsequently, additional loci that mapped to physical intervals on the somatic hybrid-based physical map but that were not represented on the framework map were included. These additional loci were inserted using the ALL option of CRIMAP with genetic distances calculated by the FIXED option. The genetic markers were also physically mapped by PCR to the somatic cell hybrid panel. There are additional microsatellite markers that have been localized on the physical map but have not been placed on this genetic map.

Physical distances. A megaYAC contig consisting of 700 megaYACs and 400 STSs has been constructed for chromosome 16 (Doggett and Callen, 1995). To construct the contig, STSs were localized to the high-resolution somatic cell hybrid panel and screened against the Généthon megaYAC library. Each STS was positive for an average of 5 megaYACs and each megaYAC contained an average of 3.5 STSs. The distances between STSs in this map were estimated by using the optimal spacing of STS with the SEGMAP v2.5 program. These data were graphically represented in the SIGMA program. This permitted the positioning of somatic cell hybrid breakpoints at measured distances along the chromosome between STSs in adjacent intervals of the breakpoint map. For the purposes of the present study, genetic markers were assumed to lie midway between the breakpoints defining an interval, and if more than one marker was in an interval these were distributed evenly.

Availability of data and resources. A subset of the mouse/human somatic cell hybrids is available from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). Other hybrid cell lines are available from DFC by request. Some mapping data have been submitted to GDB as "map strings" following the Third International Workshop on Chromosome 16 (Doggett and Callen, 1995). Additional mapping information is available from the Los Alamos National Laboratory (http://www-ls.lanl.gov/masterhgp.html).

RESULTS

Figure 1 presents the integrated genetic map and transcript map of chromosome 16 based on the panel of somatic cell hybrids. This panel of over 100 unique breakpoints defines 82 intervals at an average resolution of 1.2 Mb and potentially provides an average mapping resolution over the entire chromosome of approximately 1 Mb. The density of genes varies for different regions of the chromosome. For example, only one transcript has been found to map between CY185 and CY11 (2.7 Mb estimated size) and within the interstitial deletion encompassing 16q21 that is contained in the hybrid CY130 (4.2 Mb estimated size). A high concentration of genes was found at bands 16q24.3 and in the proximal portion of 16p11.2.

The transcript D16S2555E (Bdy95g07) is homologous to the gene sequence of ATP5A1, and this therefore provides a localization for this gene. Several of the mapped transcripts from the Genexpress program (Auffray *et al.*, 1995) were found to have homology to already localized genes or to each other; see Fig. 1.

Several gene localizations that were reported to be on chromosome 16 could not be confirmed. CHEL3, butylcholinesterase-like 3, was reported to be on 16p11–q23 by *in situ* hybridization studies (Soreq *et al.*, 1987). Southern blot hybridization to a panel of somatic cell hybrid DNAs with the probe FL39 failed to show any bands consistent with a localization on chromosome 16. TK2, a mitochondrial thymidine kinase, was mapped to chromosome 16 by analysis of somatic cell hybrids (Willecke *et al.*, 1977). Primers generated from the 3′ untranslated region of the sequence (GenBank Accession No. K02581) were used to amplify DNA from the NIGMS panel of somatic cell hybrids containing single chromosomes. Results were consistent with a location of TK2 on chromosome 15. TRG1, a gene coding for a glycine tRNA, was assigned to chromosome 16 by analysis of somatic cell hybrids (McBride *et al.*, 1989). Primers generated from the sequence (GenBank Accession No. M11273), the forward primer in the 5′ untranslated region and the reverse primer in the coding sequence, failed to generate any bands specific for chromosome 16. PCR analysis of the NIGMS panel of single chromosome hybrids amplified only bands consistent with a location on chromosome 1. The tRNA glycine-like gene (TRGL1) is at 1p34–p36; however, the primers used for TRG1 showed no homology to the sequence of this gene (GenBank AM13661). MMP2 (formerly known as CLG4A) maps to the proximal long arm of chromosome 16. A second gene in this family, MMP9, has been reported to map to chromosome 16 (Collier *et al.*, 1991). Primers generated from the 5′ untranslated region of MMP9 sequence (GenBank Accession No.

TABLE 1

Primers for Genes Localized to Chromosome 16 by Analysis of the Somatic Cell Hybrid Panel

	Size product (bp)	Primers[a]	Published localization	Method[b]	Reference
SSTR5	209	GAA CAC GCT GGT CAT CTA CG CAC TGG TGA ACT GGT TGA CG	16	Hybrids	Panetta *et al.* (1993)
TPSI	250	TGT CCA AAA CAC CAC TGC TTC C CTT TAA TGA GGT CCA GCA CTC AGG	16	Not known	Moxley *et al.* (1994)
CDR2	400	CTC ATG ACG TTT GCC TCA TTG CTT T AGA CCC CTG CTT TCT CTC GAA CAT T	16p12–p13.1	FISH	Gress *et al.* (1992)
DCI	250	TTG AAA GAC ACC CTG GAG AAC ACC AGA AAA CCT TTG GGT GGA GAA CCT TCT GGT	16p13.3	FISH	Janseen *et al.* (1994)
RPS2	214	AAC TTC GGT AGG TGG TCC AC CAG GAG GGT CAG TGG TGT G	Unassigned	—	
RPS15A	236	TTG TTT TGG GTA TTG TCT CCC CAT CAA TGA TTT CAA ATT CGC	Unassigned	—	
UQCRC2	162	CAC ACA TTA CAG GAG AGA GCT GAA CG CAG AAT GTT TTA TTG ACT TTA GGT CAG CTGG	16p12	FISH	Duncan *et al.* (1993)
CA5L	500	TCG TGG GAG AGA ATG CTT TC CCT CTG CAG CGT CTG ATG AT	16	—	Nagao *et al.* (1995)
CA5	520	GAG AGA ATG GTT TGG CTG TG CCT CTG CAG CGT CTG ATG AT	16	Hybrids	Nagao *et al.* (1993)
PRKM3	260	CTC TCC CGC CAG ACT GTT AGA A TCC ATT CCA GAA CTG CCA GAG ACT G	16	Hybrids	Charest *et al.* (1993)
SGLT2	154	ACC ATA AGC CAC AGC CTC ACA GGA A TGG GAA GTG ACT GCC AAT CAG ATG	16p11.2	Hybrids	Wells *et al.* (1993)
CD19	170	TCA ACG TCT CTC AAC AGA TGG TGA GGA CCT GTT CTT CAG GC	16p11.2	FISH	Ord *et al.* (1994)
PPP2CBP	234	TAT GGT CAG ATC CAG ATG ATC GTG G CAT GAT AGC AGC CTG GTT CCC ACA A	16	Hybrids	Jones *et al.* (1993)
FUS	144	AAG ATG GAT TCC AGG GGT GAG CAC A GGT GAT CAG GAA TTG GAA GGT TAC A	16p11	Cytogenetics	Rabbitts *et al.* (1993)
CES1	270	GGC CCA GAA GCT GAA GGA CAA AGA A CCC AAA GCT GAG GTC ACA AAT AC	16q13–q22.1	Hybrids	Becker-Follmann *et al.* (1991)
POLR2C	400	CTT CAG ACT CTT CTC GTT TCT GAG TGG GAA TTT GAA GCG CTA GCA GG	16q13–q21	FISH	Acker *et al.* (1994)
CSNK2A2	410	GAC TGG AAA GCG ACG GGT CTG TTG C GTA ACT GCC GCC ATG CCA CAT ACT G	16p13.2–p13.3	FISH	Yang-Feng *et al.* (1994)
DHODH	93	CTT CGC CTC CTA CCT GAT GG GCT GAC TCC GGG TCC AGC AG	16q22	FISH	Barnes *et al.* (1993)
CYBA	316	GTG CAG TGG TGC AGC TGT GAC TCA AT GTG GTT AAG GAA CAG CCC AGC TCA G	16q24	FISH	Dinauer *et al.* (1990)
CMAR	170	TGT GAG CCG ATT GTC CTA TCT CCA GC CAA CAC TGC ATG CGT GAC TAG ACC A	16q23–q24	Linkage	Koyama *et al.* (1993)
MCIR	400	GAC GGT CCA GAG GTG TCG AAA TGT CTG CTT AGT TCA TGG TGC TGC CAG	16q24.3	FISH	Gantz *et al.* (1994)
RPL13	460	CAT GCC CGT CCG GAA CGT CGA GCT TTC TCC TTC TTA TAG ACC TA	Unassigned	—	

[a] Primers are listed in 5′ to 3′ order with forward primer listed first and then the reverse primer.

[b] Method of gene localization is by fluorescence *in situ* hybridization to metaphase chromosomes (FISH), analysis of murine/human somatic cell hybrid cell lines (hybrids), by cloning the breakpoint of a translocation involving chromosome 16 (cytogenetics), or by genetic linkage (linkage).

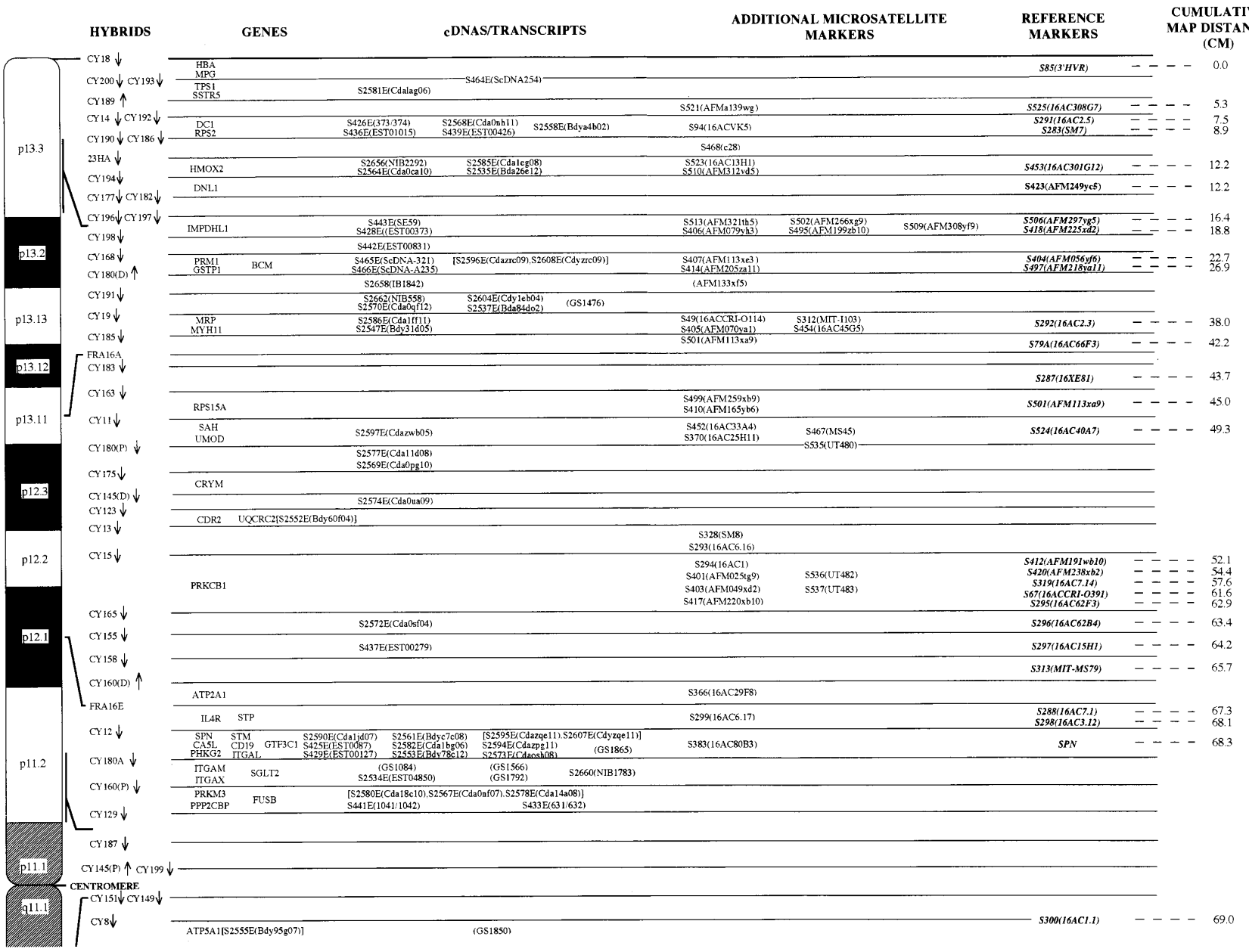
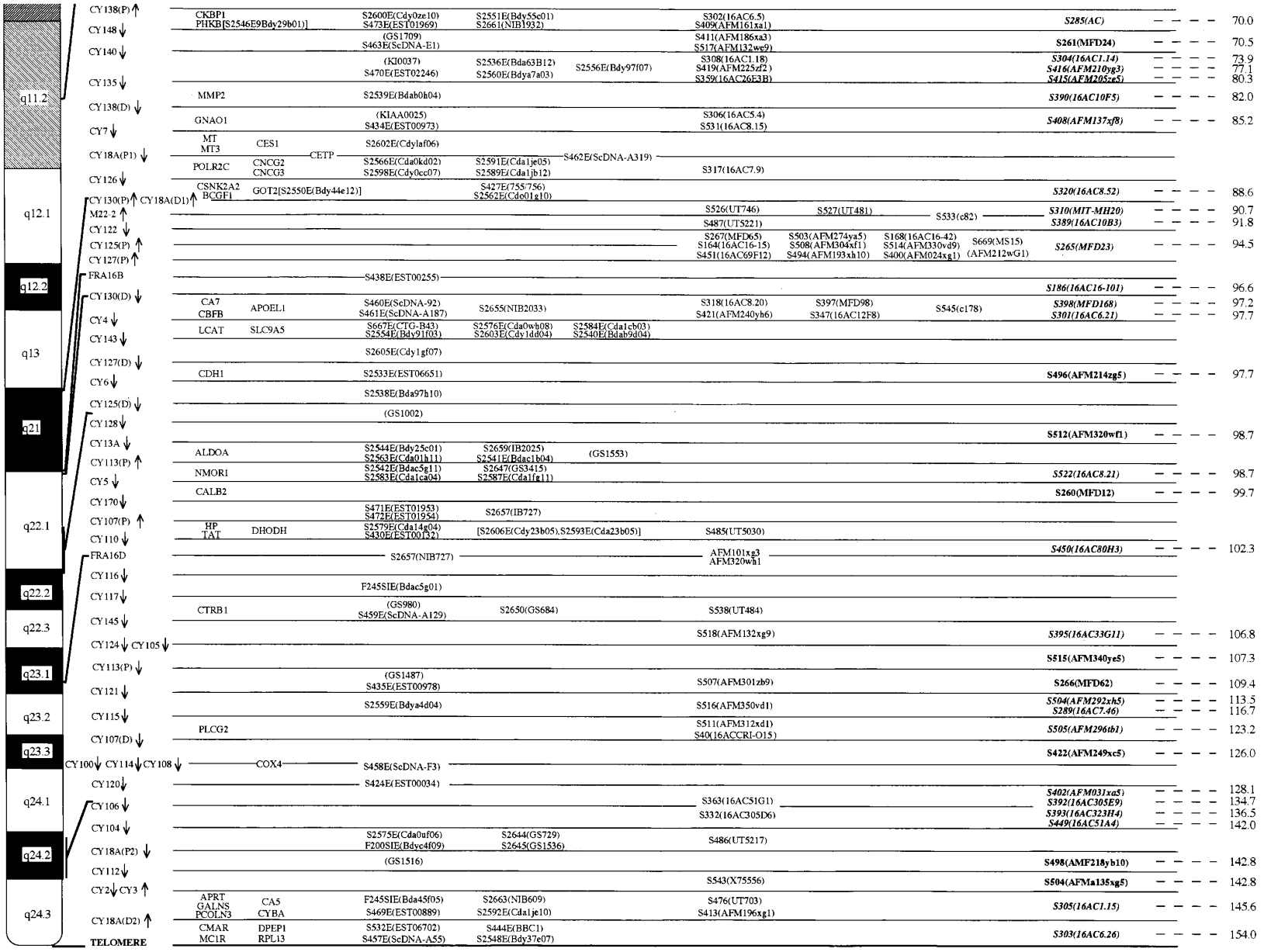


FIG. 1. Integrated genetic and physical map of genes, transcripts, and microsatellite markers on chromosome 16. To the right of the idiogram of chromosome 16 are mouse/human somatic cell hybrids and fragile sites. The horizontal lines designate the breakpoints contained in each hybrid cell line with the retained section of chromosome 16 indicated by the direction of the arrow. The relationships between breakpoints and the ideogram are designated by connecting lines and are defined by fragile sites or high-resolution FISH studies. For DNA transcripts the GDB locus designation has been abbreviated, e.g., from D16S464E to S464E. The probe name is in parentheses. For the microsatellite markers, the framework



genetic markers are presented on the right of the figure (in the column headed "Reference Markers") in italics, while other markers in this column were inserted using the ALL option of CRI/MAP (see Materials and Methods). The microsatellite markers in the column headed "Additional Microsatellite Markers" have not been integrated into the genetic map of reference markers but are located by analysis of the somatic cell hybrids. ESTs that are identical by sequence homology to each other or to a localized gene, or are ESTs derived from the same transcript, are denoted by brackets, i.e., [GOT2, S2550E].

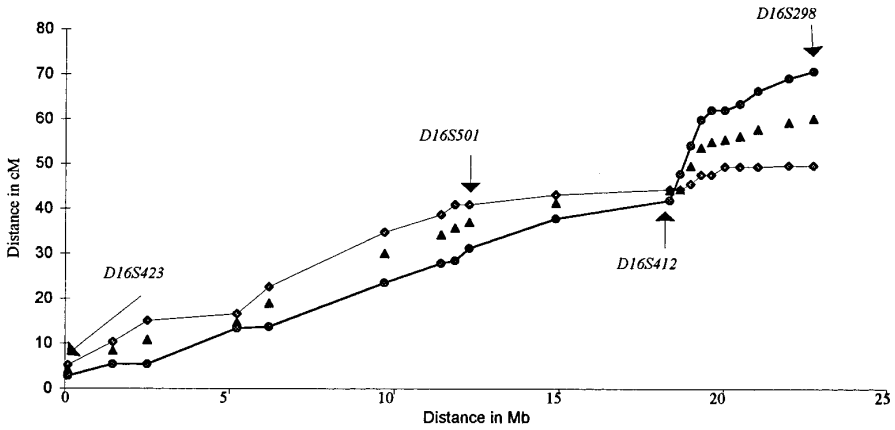


FIG. 2. Correlation between genetic distance and physical distance on the short arm of chromosome 16. The genetic distance is presented as the cumulative female (circles), male (diamonds), and sex-averaged (triangles) map distances in cM from D16S423 to D16S298. The physical distances between somatic cell hybrid breakpoints were calculated from the megaYAC contig of chromosome 16. Genetic markers were assumed to be at the midpoint of an interval, for a single marker, or where there was more than one genetic marker to be equally distributed between the hybrid breakpoints defining the physical interval.

D10051) were used to PCR amplify DNA from the NIGMS hybrid panel. Results were consistent with a localization on chromosome 20.

The genetic map provides an average distance between markers of 2.8 cM with the largest interval being 11.1 cM. Listed in Fig. 1 are the microsatellite markers included in this genetic map and additional microsatellite markers that have been localized to the physical map using somatic cell hybrids. For each marker in the short arm, from 16p13.3 to 16p11.2, the genetic distance in cM is plotted against the estimated physical distance in Mb (Fig. 2). The megaYAC contig was not sufficiently detailed to enable an estimation of physical distance for other regions of the chromosome. There was a small gap in the megaYAC contig in the region between the hybrid breakpoints CY19 and CY185, but additional evidence (unpublished) suggests that this gap is small. For the long arm, the existence of several gaps in the contig inhibited the construction of a contiguous physical map. Data were not sufficiently detailed to enable exact distances to be determined between the genetic markers. Therefore, each genetic marker was assumed to be midway between its flanking somatic cell hybrid breakpoints. Where there was more than one genetic marker in an interval these were assumed to be equidistant. For the estimated 23 Mb of the short arm represented in Fig. 2, 1 cM of the sex-averaged map is equivalent to 400 kb.

Also depicted in Fig. 2 are the sex-specific distances. The male map is 1.3-fold longer than the female map in the region from D16S423 to D16S501. Proximal to this region male recombination is greatly reduced. The frequency of recombination in the female is maintained at a relatively constant rate/unit of physical distance. An exception is between the markers D16S412 to D16S295, in the region between the hybrid breakpoints CY15 and CY165, where the recombination frequency is increased in both sexes. In females, this increase is equivalent to 1 cM representing approximately 100 kb of DNA. The relationship presented on the graph assumes equal distribution of microsatellite markers within an interval defined by hybrid breakpoints. Therefore, this may lead to an underestimate of the expansion of the genetic map in this region.

DISCUSSION

One hundred forty-one genetic markers, 76 genes, and 124 transcripts have been integrated into a physical map of chromosome 16 (Fig. 1) using a single mapping resource, a high-resolution mouse/human somatic cell panel of chromosome 16. This physical map consists of 93 breakpoints represented in mouse/human somatic cell hybrids and four fragile sites that together define 82 intervals of average size of 1.2 Mb and potentially define 93 intervals of 1 Mb average size. This integrated map will provide a resource to allow rapid selection of flanking microsatellite markers for further detailed genetic localization of a disease gene. Since the physical location of these genetic markers is known, the physical interval containing the disease gene can be defined by somatic cell hybrid breakpoints. The genes and expressed sequences in each region will then provide potential candidate genes for the mapped disease. However, use of PCR to localize transcripts does not usually allow discrimination between functional genes and pseudogenes. This can be determined only by more detailed studies of the genomic structure of a gene.

The existence of such detailed physical maps provides the opportunity to examine the density of genes in different regions of the chromosome. Evidence from FISH studies using a GC-rich isochore (Saccone *et al.*, 1992) and CpG islands (Craig and Bickmore, 1994) suggests that G-negative bands, especially those at the telomeres, are gene-rich, while G-positive bands are gene-poor. The majority of G-positive bands on chromosome 16 are relatively diffuse, and there is no obvious

evidence for lack of genes in these regions, although the hybrid breakpoints have not always been related to the G-banding pattern. An exception is band 16q21, which is an intensely staining G-positive band that maintains its integrity at high levels of banding resolution. Individuals who have a normal phenotype and are heterozygous for a deletion of this band have been described (Witt *et al.*, 1988). The somatic cell hybrid CY130 was derived from this deleted chromosome. From the megaYAC contig this band is estimated to be 4.2 Mb, and only a single transcript has been localized within the deletion contained in CY130. Therefore, 16q21 is a G-positive band that is deficient in transcripts and possibly deficient in cytogenetic breakpoints. It is likely that the properties of gene deficiency apply only to the intensely G-positive bands.

A second region, which contains a single mapped gene, is between the hybrid breakpoints CY185 and CY11 in p13.11, encompassing an estimated 2.7 Mb of DNA. This is the region that contains FRA16A, which is situated in an extensive duplicated segment that has yet to be resolved in detail (Nancarrow *et al.*, 1994).

16p13.3 is gene rich. This is not obvious from Fig. 2, since mapping in this region is underrepresented in this study. Experience from preliminary work aimed at cloning the gene for polycystic kidney disease indicated a high gene density in this region (Harris *et al.*, 1990). Regions from Fig. 1 indicating potential regions of high gene density are 16q22.1 and 16q24.3, both of which were highlighted in the studies by Saccone *et al.* (1992). An additional region containing many genes is 16p11.2. Of the genes located to this region, a number are involved in cell surface receptors.

The construction of high-resolution megaYAC contigs for chromosome 16 (Doggett and Callen, 1995) provides the opportunity to relate genetic and physical distances. It is possible that consistent deletions in megaYACs could lead to an underestimate of physical distance, but considering the density of STSs used to generate the megaYAC contig, these are unlikely to be extensive. A region of an estimated distance of 23 Mb, from 16p13.3 to 16p11.2, was the longest region of continuous megaYAC coverage. Over this interval, a sex-averaged genetic distance of 1 cM is equivalent to an estimated physical distance of 400 kb. This compares with the whole chromosome average of approximately 600 kb/cM (total length of sex-averaged genetic map 152 cM (Kozman *et al.*, 1995), total length of chromosome 95 Mb). A more detailed analysis of the sex-specific recombination shows that for the first 15 Mb of this estimated 23-Mb region, male recombination is consistently greater than that of female. However, proximal to the marker D16S501 the male rate is suppressed with respect to the female. The findings of general suppression of male recombination across the centromere but greater male recombination at the telomeres has been previously described for this chromosome (Kozman *et al.*, 1995). Of particular interest is a region of greatly increased recombination that is present in both sexes, but particularly evident in females, where 1 cM is equivalent to a estimated physical distance of 100 kb. This is a conservative estimate due to the absence of detailed physical distances between the microsatellite markers. This hot spot of recombination is unlikely to be an artifact, as the megaYAC contig is highly redundant in this region, giving a reliable estimate of physical distance, and three of the five genetic markers (D16S67, D16S295, and D16S319) have been typed in the extended set of 40 CEPH families. Hot spots of recombination have been documented within the DMD gene, where rates of 1 cM/91 kb over a 440-kb region were described (Oudet *et al.*, 1992). Together with the results of this study, it is evident that hot spots of recombination may be a general phenomenon.

In conclusion, the mouse/human somatic cell hybrid panel of chromosome 16 provides average physical mapping at a resolution of near 1 Mb, and this is an ideal resource for integrating a variety of data generated from various sources. The construction of such an integrated map containing genetic markers and gene transcripts provides a unique resource for this chromosome.

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