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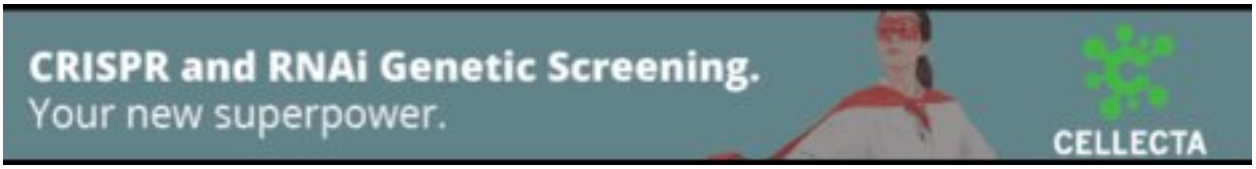

LETTER

A Contiguous High-resolution Radiation Hybrid Map of 44 Loci from the Distal Portion of the Long Arm of Human Chromosome 5

Janet A. Warrington[1] and John J. Wasmuth

Department of Biological Chemistry, College of Medicine, University of California, Irvine, California 92717

A contiguous high-resolution map of 44 loci from a 35-Mb portion of the distal region of the long arm of human chromosome 5, q21–q35, was produced using radiation hybrid (RH) mapping in conjunction with a natural deletion mapping panel. The map includes 30 genes, four sequence-tagged site (STS) loci, and 10 DNA markers. Newly mapped markers fill two gap regions that were present in previous maps, between markers FER-IL4 and IL3-IL9. Identifying the position of genes on the physical map aids in positional cloning efforts and contributes to our understanding of the overall organization of the human genome.

The distal region of the long arm of human chromosome 5 has been identified as the site of many disease genes including diastrophic dysplasia, Treacher Collins syndrome, hyperekplexia, an autosomal dominant form of limb girdle muscular dystrophy, familial adenomatous polyposis coli, acute lymphocytic leukemia, acute nonlymphocytic leukemia, 5q deletion syndrome, myelodysplastic syndrome, nonsyndromic autosomal-dominant deafness, and Boston-type craniosynostosis (Huebner et al. 1985; Le Beau et al. 1986, 1987; Sutherland et al. 1988; Grimaldi and Meeker 1989; Hästbacka et al. 1990; Groden et al. 1991; Jabs et al. 1991; Joslyn et al. 1991; Kinzler et al. 1991; Nishisho et al. 1991; Leon et al. 1992; Ryan et al. 1992a; Speer et al. 1992; Muller et al. 1993; Shiang et al. 1993; The Treacher Collins Syndrome Collaborative Group 1996). The order of and distance between 44 loci from an ~35-Mb portion of the distal region of the long arm of chromosome 5, spanning the cytogenetic region 5q21–5q35, has been determined by radiation hybrid (RH) mapping. Previous work using RH mapping in conjunction with a natural deletion mapping panel determined the order of and relative distance between 13, 18, and 12 loci, respectively (Warrington et al. 1991, 1992; Warrington and Bengtsson 1994) from 5q22 to 5q35. An RH map of the Treacher Collins region, 5q31–5q33, placed an additional gene and seven markers. In all, 23 of those gene loci—*ADRA1B, ADRB2, ANX6, CD14, CSF1R, DRD1, EGR1, FER, FGF1* (*FGFA*), *FGFR4, FLT4, GABRA1, GABRG2, GLRA1* (*GLYA1*), *GRIA1* (*GLUR1*), *GRL, IL3, IL4, IL9, IL12* (*NKSF2*), *IRF1, RPS14,* and *SPARC*—and seven of those marker loci—*D5S119, D5S207, D5S209, D5S210, D5S378, D5S379,* and *D5S519*—are included in the present map. The remaining 14 markers represent seven additional genes—*APC, CDC25C, FBN2, F12, MCC, PDE6A,* and *TCF7*—and seven DNA markers or sequence-tagged sites (STSs)—*D5S22, D5S89, D5S211, LANLSTS129, LANLSTS140, LANLSTS179,* and *LANLSTS183.* Mutations in MCC and APC genes cause colorectal cancers, and *FBN2* mutations have been implicated in a Marfan-like syndrome (Groden et al. 1991; Joslyn et al. 1991; Kinzler et al. 1991; Lee et al. 1991; Nishisho et al. 1991). *TCF7, PDE6A, F12,* and *CDC25C* encode, respectively, a T-lymphocyte transcription factor, a cGMP phosphodiesterase, human coagulation factor XII, and the human homolog of fission yeast cdc25 (Cool and MacGillivray 1987; Pittler et al. 1990; Sadhu et al. 1990; van der Wetering et al. 1991). Data from our previous maps allowed us to easily place these markers on the RH map using PCR and to construct a contiguous high-resolution

[1]Corresponding author.
E-MAIL JANET_WARRINGTON@affymetrix.com; FAX (408) 481-0422.

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map spanning ~35 Mb of the distal portion of human chromosome 5.

RESULTS

The presence or absence of each of the markers in a set of 101 radiation hybrids containing fragments of 5q, described elsewhere (Warrington et al. 1991), was determined by PCR screening. Each of the markers was nonselectively retained in 5%–32% of the hybrids. Retention frequencies of the 28 previously mapped loci ranged from 15% to 32% (Warrington et al. 1991, 1992; Loftus et al. 1993; Warrington and Bengtsson 1994). The retention frequencies for the remaining 16 loci are 21%, *APC;* 19%, *CDC25C;* 20%, *D5S22;* 12%, *D5S89;* 15%, *D5S211;* 20%, *D5S378;* 19%, *D5S379;* 16%, *F12;* 16%, *MCC;* 30%, *PDE6A;* 20%, *FBN2;* 22%, *LANLSTS129;* 15%, *LANLSTS140;* 22%, *LANLSTS179;* 21%, *LANLSTS183;* and 19%, *TCF7*. All 44 marker loci were subjected to analyses for order simultaneously, resulting in a very large number of possible orders. Of these orders, 100 have very similar likelihoods with relative likelikhoods under 30. There are six regions where markers cluster very close together. Four of the clusters consist of single marker pairs, and the remaining two clusters contain three and five markers, respectively. Because there are few breaks between the clustered markers we were unable to determine an unequivocal map order based on this method alone. However, previous mapping efforts using different map assembly methods provide some relative order information for the clusters containing more than two markers. A previously published RH map places *FLT4* and *DRD1* proximal to *FGFR4,* and fluorescence in situ hybridization (FISH) mapping places *IL4* proximal to *IL3* (Warrington et al. 1992; Saltman et al. 1993). For the rest of the map, that is, the markers not included in the six clusters, one order emerged as consistent in the 100 most likely maps and is shown in Figure 1 with the six areas of uncertainty denoted. This map is consistent with a chromosome 5 framework map produced by the National Human Genome Research Center (J. McPherson, in prep.).

lod scores of adjacent markers range from 21 to 4. Distances between adjacent loci are given

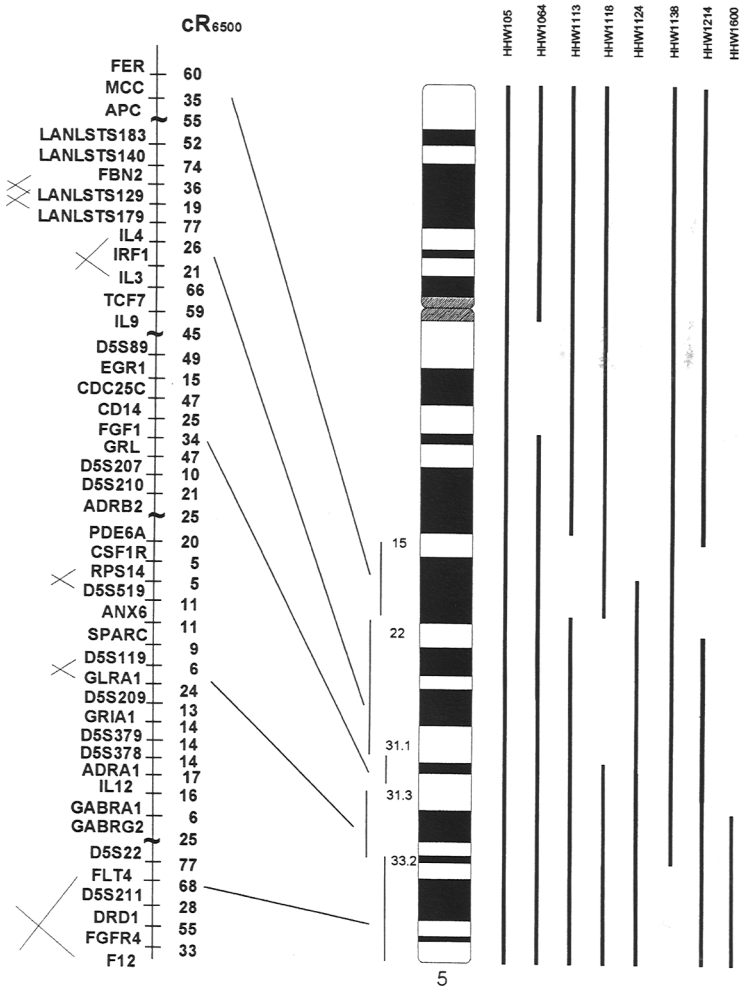

Figure 1 RH map of the distal portion of the long arm of human chromosome 5 aligned with the chromosome 5 natural deletion mapping panel. Marker order is shown on the *left* with centiray distances between markers. (~) The breakpoints in the natural deletion panel. The horizontal X to the *left* of the markers indicates those markers whose order may be inverted. The cytogenetic regions to which sets of markers are assigned are indicated by lines extending to the ideogram. The ideogram depicts a normal chromosome 5. The bars depict the region of 5 retained in each hybrid cell line. The hybrid HHW105 retains an intact human chromosome 5 as its only human material. The loci *FER, MCC,* and *APC* were in all cell lines except HHW1113 (SRO 5q15–5q21). Ten loci (*LANLSTS183–IL9*) were present in all cell lines except HHW1118 (SRO 5q22–5q31.1). Nine loci (*D5S89–ADRB2*) were present in all cell lines except HHW1600 (SRO5q31.1–5q31.3). Fourteen loci (*PDE6A–GABRG2*) were present in all cell lines (SRO 5q31.3–5q33.2), and six loci (*D5S22–F12*) were present in all cell lines except HHW1138 (SRO 5q33.3–5qter).

in RH mapping units, centirays (cR). Distances range from 5 to 77 cR_{6500}, with an average distance of 31 cR_{6500} and a median distance of 25 cR_{6500}. The relationship between a cR_{6500} and actual physical distance in kilobases of DNA for several loci in the q31–q33 region has been determined to be 21 kbp/cR (Warrington and Bengtsson 1994). Alternatively, an estimate of the distance correlation based on the comparison of the approximate physical distance from 5q21 to 5q35, 35 Mbp, and the total RH distance of 1369 cR_{6500}, provides an estimate of 26 kbp/cR.

DISCUSSION

The order of and relative distance between 44 markers on the distal portion of the long arm of human chromosome 5 was determined by RH mapping. The presence or absence of markers in cell hybrids that retain naturally occuring 5q deletions helped in ordering the loci. The combination of these methods enabled us to construct a map spanning a physical distance of ~35 million bp from 5q21 to 5q35, with an average resolution of ~800 kb. If chromosome 5 represents 5% of the human genome, or ~1.5×10^8 bp, then this 35-Mb region accounts for nearly 25% of the chromosome. The newly mapped markers filled two gap regions that were present in previous maps between the markers *FER-IL4* and *IL3-IL9* (Warrington et al. 1991, 1992). A single marker, *TCF7*, provided linkage between two markers flanking one of the gaps, IL3 and IL9. The total relative distance for this gap region was 125 cR_{6500}, or ~3 Mbp. The other gap spanned a distance of 243 cR_{6500}, or ~6 Mbp, and linkage was obtained with the markers, *LANLSTS183, LANLSTS140, FBN2, LANLSTS129,* and *LANLSTS179*. This contiguous high-resolution RH map will be useful for positional cloning efforts and sequence assembly. Establishing the position of genes on the physical map contributes to our understanding of the overall organization of the human genome.

METHODS

Isolation and Characterization of RHs

The isolation and characterization of the radiation hybrids has been described (Warrington et al. 1991). The isolation and characterization of human–Chinese hamster ovary (CHO) hybrid HHW661, the irradiated parent of the cell hybrids, has been described by Wasmuth et al. (1986). The HHW661 cell line retains a derivative human chromosome 5 [der(5)t(5;4)(5qter → 5p15.1::4p15.1 → 4pter)] as its only detectable human DNA. The nonirradiated Chinese hamster cell parent, UCW113, is an HPRT-deficient derivative of V-79 Chinese hamster lung (CHL) fibroblasts. Irradiation of the cell hybrid and refusion were performed as described previously (Cirullo et al. 1983).

PCR

The presence or absence of each of the markers in a set 101 radiation hybrids was determined using PCR as described previously (Warrington et al. 1991). Each marker was tested separately; none were multiplexed. The PCR primer sets for *APC, MCC, FBN2, TCF7, PD6EA, CDC25C, F12,* and *D5S22* were designed from published sequence data and are available in the Genome Database (GDB). PCR conditions for *ANX6, GLRA1, FER, IL4, IRF1, IL3, IL9, EGR1, CD14, FGF1, GRL, ADRB2, SPARC, RPS14, CSF1R, GLR1, IL12, GABRA1, GABRG2, FLT4, DRD1, FGFR4, ADRA1B, D5S89, D5S519, D5S378, D5S379, D5S119, D5S207, D5S209, D5S210,* and *D5S211* have been described (Warrington et al. 1991, 1992; Weber et al. 1991, Ryan et al. 1992b, Dixon et al. 1993, Loftus et al. 1993, Shiang et al. 1993, Nagarajan et al. 1994, Warrington and Bengtsson 1994). Primer sets for *LANLSTSs 129, 140, 179,* and *183* are available in the Genome Sequence Database (GSDB) under numbers L28217, L28223, L28240, and L28242 (Grady et al. 1996). Each PCR was carried out in a total volume of 25 μl using 0.25 μg of DNA in 67 mM Tris-HCl (pH 8.3), 6.7 mM $MgCl_2$, 16.6 mM ammonium sulfate, 10 mM β-mercaptoethanol, 1.25 mM each dNTP, 25 pmoles of each primer, and 1 unit of *Thermus aquaticus* DNA polymerase.

RH Mapping

The RH mapping programs RH2PT and RHMAXLIK, version 1.1 (Boehnke et al. 1991; Boehnke 1992) were used to analyze the RH data. Distances between pairs of loci are reported in cR_{6500}, where 6500 rads indicates the dosage of the X-rays used in the irradiation of the hybrids. Because the RH map distances stem from the dosage used to fragment the chromosome, it is necessary to note the dosage when reporting distances. The order of the loci was determined by first placing the markers on the cytogenetic map using a natural deletion mapping panel, a panel of five somatic cell hybrids that retain naturally occurring deletions of 5q. Locus ordering by RHMAXLIK was carried out using the stepwise ordering strategy with a machine-generated candidate order.

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