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Final Report

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Summary

The Third International Workshop on Human Chromosome 5 was held in Laguna Beach, California, March 5-8, 1994. The pace at which new mapping information has been published in the last year makes almost any report outdated before publication. Much of the information in this report and the most recent data from the Human Chromosome 5 Genome Center at U.C. Irvine on the physical map of chromosome 5 are accessible via a WWW server (http://chrom5.hsis.uci.edu). Additional information concerning the genetic map of chromosome 5 can be obtained via the CEPH and CHLC databases. For most loci referred to in this report that can be detected by PCR, the sequences of the oligonucleotide primers are available from either GDB or the CHLC database. In some cases, the appropriate primer sequences are provided in this report.

Genetic Maps

Shortly after this meeting, the 1993-94 Genethon Human Genetic map was both published (1) and made available electronically (http://www.genethon.fr/genethon_en.html). Their genetic map of human chromosome 5 consists of 137 markers with approximately one half of these positioned with odds >1000:1. This represents an approximately three-fold increase since the previous 1992 release. The three longest gaps are 7 cM (5p15.1, 5q13 and 5q35) with an overall sex-average length of the chromosome of 201 cM. This agrees well with an estimated physical length of the chromosome of 170 Mbp.

At this meeting, chromosome 5 genetic mapping results were presented by three groups: The Cooperative Human Linkage Center (CHLC), The Utah Genome Mapping Center and The Duke University Medical Center. These data were combined to create an integrated map shown in Figure 1. Genotypes generated from the CEPH database were extracted directly from the CHLC database (ftp), and shared by investigators at each of the other two groups. Map development was performed using CRIMAP as described elsewhere (2) without any attempt to correct genotyping errors. The map was developed by attempting to incorporate all available tetranucleotide repeat markers, followed by insertion of all known genes, followed by insertion of all available dinucleotide repeat markers. Markers were retained in the map only when their placement was supported with odds ≥1000:1 over the next most likely placement. Markers which are listed together on the same line of the map demonstrate no recombination amongst themselves. Placement of tetranucleotide repeat markers and known genes which were not supported with odds of 1000:1 are shown to the right of the map in their most likely location, and a 3 LOD unit support interval about this likely placement indicated.

The map includes 34 loci with an average spacing of about 7 cM between markers (range 0.8-18 cM). The map spans approximately 238 cM which corresponds reasonably well with the estimated sex-averaged genetic length of chromosome 5 of approximately 224 cM (3). Alternatively, persistent, undetected genotyping errors within the marker data could lead to map expansion (4) with the attendant consequence of lesser coverage of the chromosome by this map.

Physical Maps of 5q

Three groups presented data delineating the critical region involved in acute myologenous leukemia (AML) and myelodysplastic syndrome (MDS) as detected in their respective patient groups. The physical mapping efforts of these groups, and those groups attempting to clone genes associated with autosomal dominant limb-girdle muscular dystrophy (LGMD1A) and an autosomal dominant post-lingual deafness (DFNA1) were used to construct an extensive consensus map from approximately 5q23 to 5q34 (Figure 2). This consensus map spans approximately 40 Mbp and contains 61 markers. Sequences for all loci indicated are readily available from the Genome Data Base (www. and chr 5 report) with the exception of those with the prefix "LAS." The latter markers were generated at Los Alamos National Laboratory (LANL) using flow-sorted human chromosome 5 material. Sequences for the indicated "LAS" markers, and 300 more regionally assigned markers, can be obtained by contacting Drs. R.K. Moyzis and D.L. Grady at LANL. A detailed YAC contig of a portion of this region has been published (5).

In addition, two other groups (Muller et al., Bullrich et al.) described efforts to establish YAC contigs in distal 5q (q32-qter). The work of Muller et al. is part of an intensive effort to produce an integrated physical/genetic/transcriptional map of this region. Bullrich et al. used the current physical map information to identify a YAC clone that spans a translocation (2;5) breakpoint in distal 5q in patients with CD30 (Ki-1) positive lymphoma.

There was also an interesting and detailed analysis of YACs from a contig spanning ~1.2 Mb by Stoffel et al. using FISH. In an analysis of 14 YACs from three different libraries, this group found at least 3 with one or more internal deletions, at least 9 that were chimeric. These results are shown in Table 1.

Disease Loci

As evidenced by the consensus map shown in Figure 2, an important source of new mapping data are the detailed maps generated by studies directed towards cloning specific genes involved in inherited disorders.

5q- syndrome

Interstitial loss of the long arm of chromosome 5 is frequently detected in acute myelogenous leukemia (AML) and myelodysplasia (MDS) with refractory anemia. Although these disorders may be related, the current evidence indicates that different regions of mid-5q are critical in the different anomalies. It is likely that more than one gene is involved in both disorders. At this meeting, three groups reported on allele loss in their respective groups of AML patients; Nagarajan et al. observed consistent allele loss of D5S89, Williams et al. reported consistent loss of IRF1 and Le Beau et al. reported consistent loss of the segment for IL9 to D5S166. These results are summarized in Figure 3. In a set of MDS patients, Boultwood et al. have defined the critical region in this disorder as lying between FGF1 and IL12B.

Autosomal dominant corneal dystrophies

The two most common autosomal dominant dystrophies of the corneal stroma, lattice corneal dystrophy type 1 (CSD1), and granular type corneal dystrophy (CSD2), as well as a third type, Avellino (CSD3), have all been mapped to the interval IL9-FGF1 by linkage analysis. The maximal combined LOD score was 28.6 with marker D5S393 (6).

Autosomal dominant limb-girdle muscular dystrophy (LGMD1A)

Physical mapping data from the LGMD1A region (IL9-FGF1) was presented by two groups (C. Westbrook and J. McPherson) and is incorporated into the consensus map in Figure 2. A framework genetic map of the region was also presented (M. Speer) which spans 35 cM:

qcen - FBN2 - IL9 - D5S393 - D5S414 - D5S178 - D5S210/D5S207 - CSF1R - D5S119 - qter
7 2 2 5 3 7 11 cM

An interesting observation made was the apparent non-Mendelian inheritance of the marker D5S414 when using the published primers. A redesigned GT-primer was shown to correct this anomaly:

CA-primer (unchanged)	5' GGCCAGTTCAGTCAAGTG 3'
GT-primer (new)	5' TGAGGTTAAGTCCTGACC 3'

Preliminary data was presented which suggests that the LGMD1A locus may be confined to the interval D5S414-D5S178. If confirmed, this would significantly restrict the localization of LGMD1A to a 5 cM region which is estimated to be 3.5 Mbp.

Autosomal dominant post-lingual deafness (DFNA1)

DFNA1 was originally mapped to 5q31 in the Monge kindred of Costa Rita (7). Genetic and physical maps were presented to further refine its localization (E. Lynch). Two new dinucleotide markers were reported for this study:

EGR.CA	5'	3'
	TCACTCACAGGCTTCCAGG	
	TCCGGACCATTGAGATTCTC	
D5S147.CA	5'	3'
	CTCTGCAAAGACCCTCCTTG	
	CTTGCCCTCAAGACCTGGT	

The apparent interval containing DFNA1 is flanked by D5S352 and D5S147. This region is spanned by CEPH YAC 717g9.

Towards a Transcription Map of Human Chromosome 5

Of great interest to all was Michael Lovett's report of his efforts to select transcribed sequences from 5q35 using 410 regional cosmids. He reported the isolation of 114 cDNA clones which map back to the cosmids used for their selection. Approximately 40 cDNA have been sequenced to date and several of these have been converted to full length clones. Many of these cDNA represent novel sequences; however, the following homologies were observed:

Clone	Homology
T1F10	Metallothioneins/insulin-like growth factor
T1E11	C-type lectin domain protein
A5-68G5	Pro alpha 2 collagen-like
T1C4	Poly (ADP-ribose) polymerase-like
T1B12	Cell surface glycoprotein CD44-like
T1E2	Ribosomal protein?
T1A10	Caudal homeobox-like

Human-Murine Synteny Homology

The genetic mapping of two mouse genes Dat1 and Pam, whose human homologs are on 5p and 5q, respectively, was reported. Dat1 is on MMU chromosome 13, which exhibits extensive homology to several regions of chromosome 5. Pam was localized to MMU1. This represents the first demonstration of homology between chromosome 5 and MMU1. The murine homologs of genes on chromosome 5 have previously been assigned to MMU chromosomes 11, 13, 15 and 18. A summary of this information is included in Tables 2, 3, 4 and 5.

Integrated Physical/Genetic Maps of 5p

Dr. Joan Overhauser presented an impressive amount of data on assigning STSs to distinct segments of 5p by using a large panel of natural deletion hybrids. Some of this information was recently published (8) as was a YAC contig of several Mb in 5p15.2-15.3 (9). In addition, Dr. Overhauser presented a physical map in which 39 CEPH STRP markers were localized to one of 47 discrete "bins" on 5p, which are defined by natural deletion breakpoints (Figure 4). Although the orders of several sets of markers could not be distinguished by either the genetic map or the physical map, no discrepancies have been identified thus far. Given that the distal region is currently under intense investigation because of its involvement in the cri-du-chat syndrome, additional physical mapping data is likely to be forthcoming in the near future.

An Unstable Region of Proximal 5q Associated with Spinal Muscular Atrophy (SMA): Novel Chromosome 5-Specific Repetitive Elements

Several groups (Carpten et al., van der Steege et al., Carter et al.) updated published reports of regions within 5q11-13 that are unstable in both the context of genomic DNA and cloned (YAC and cosmid) segments of this region. This region of proximal 5q contains multiple copies of a novel, chromosome 5-specific repeat that is present in at least four other regions of chromosome 5, but nowhere else in the genome. In addition, an integral part of this repeat unit

contains "partially processed" pseudogenes whose functional counterparts are elsewhere in the genome and recent reports by several groups have provided more information on the unstable and repetitive nature of this region and have identified interesting candidate genes for SMA (10,11,12,13).

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