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Report of the second international workshop on human chromosome 5 mapping

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The Second International Workshop on Human Chromosome 5 was held on May 11-13, 1992, at the University of Chicago. Thirty-nine participants from eight countries attended the meeting, which was organized by C Westbrook, R Williamson and W Neuman. The workshop was funded by the NIH National Center for Human Genome Research, the US Department of Energy, and the UK Medical Research Council, with contributions from the Human Genome Organization, genome programs in individual countries, and Amgen, Inc. The purposes of this meeting were: to foster collaboration and scientific interchange among the chromosome 5 community; to review preliminary data and resolve discrepancies; and to combine data and prepare consensus maps for this chromosome.

Since the previous meeting in September 1990, a remarkable amount has been accomplished on chromosome 5, primarily by those attending the Workshop. The total number of markers and genes on the chromosome has increased substantially, and several areas have now been mapped to high resolution by fluorescence in situ hybridization and/or YAC contig mapping. The genetic linkage map has progressed, and there are many highly informative and PCR-formatted markers available; for most of the chromosome, these provide a framework map of close markers of known order. Most importantly, many of the physical maps have incorporated genetic markers, and the physical and genetic maps are beginning to come together.

A summary of the topics discussed, as well as the best consensus maps, will be presented here. Details of the presentations, and summaries of higher-resolution region maps appear in the Abstracts. A list of the participants is included at the end of this report. Further details are available from the participants and authors.

Disease loci on chromosome 5

The identification of disease genes has been a major driving force for human genome mapping. Since the last meeting, the cloning of the gene which is mutated to cause adenomatous polyposis coli (APC), was accomplished by two groups in August 1990. The physical map of the APC region, at 5q21 - q22, was discussed in detail. A Frischauf proposed that the previously accepted gene order is incorrect, and presented data showing that APC is proximal (centromeric) to the MCC gene; this was confirmed by radiation hybrid mapping by J Wasmuth. The discussion reinforced the difficulty in assigning chromosomal orientation (as opposed to localization) of YAC contigs, a problem which arose several times during the workshop.

M Dixon and E W Jabs updated the localization of the Treacher Collins syndrome gene (TCOF1), proposing the flanking markers D5S207/D5S210 (proximal) and SPARC (distal). J Hastbacka reported that the diastrophic dysplasia gene (DTD) also maps into this interval, which centers around 5q32-q33.

The spinal muscular atrophy (SMA) gene is located at 5q11-q13, and is being studied by a number of groups. The physical and genetic maps were discussed in detail by P Kleyn, L Simard, J Melki, B Wirth, R Daniels, A MacKenzie and J McPherson. The Limb-girdle muscular dystrophy (LGMD1) gene has been localized by M Speer to the region 5q31, flanked by the markers IL9 and D5S89.

Cri-du-chat syndrome was the only genetic disorder on 5p discussed at the workshop. J Overhauser presented unpublished data showing that some of the clinical features which make up the syndrome can be separately localized within 5p15: the characteristic "cat cry" is distal to the other features of the syndrome, including mental retardation.

Cancer-related genes on chromosome 5

In addition to the APC gene, several other chromosomal regions are under investigation as frequent sites of somatic

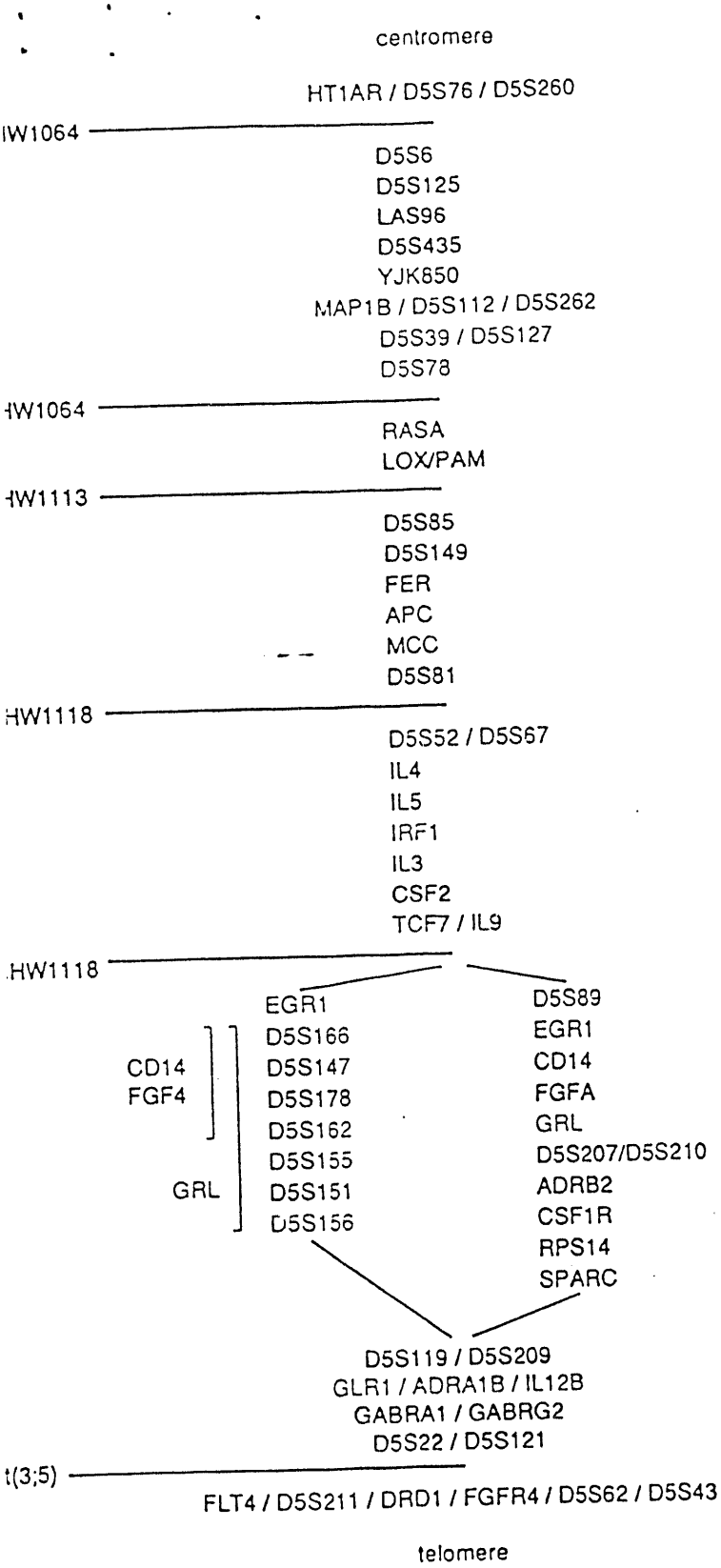

Fig. 1. Consensus order map of cloned genes, probes, and natural breakpoints on the long arm of chromosome 5. The probes and genes are listed in order from top (centromere) to bottom (telomere). Breakpoints of naturally-occurring hybrids are indicated as horizontal lines next to the name of the hybrid. One leukemia-associated translocation t(3;5)(q25;q34) is also indicated. Approximate physical locations for genetic linkages, not shown on the map are: SMA is flanked by D5S125 and D5S112/D5S262; LGMD1 is between L9 and D5S89, while DTD and TCOF1 are both within the interval demarcated by D5S207/D5S210 and SPARC.

mutations in cancer. None have been linked to familial forms of cancer. Deletions of 5q are frequently present in myeloid leukemias and myelodysplasia and are under investigation by M Le Beau, C Westbrook, W Neuman and J Boultwood. A tumor-suppressor gene is postulated to be located within the deletions. Cytogenetic studies suggest that the major area of deletion includes the gene-rich region of mid-5q (extending from EGR1 through SPARC). Another chromosomal abnormality, the t(3;5)(q25;q34) translocation in myeloid leukemias and myelodysplasia, was shown by S Morris to be distal to the marker KK5.19 (D5S121).

Genes and gene clusters on chromosome 5q

In addition to detailed mapping of the disease loci mentioned above, two gene-rich regions towards the middle of 5q have been the subject of intense mapping efforts. The first, the interleukin gene cluster at 5q23-31, contains the closely linked genes IL3, IL4, IL5, and GM-CSF; YAC contigs which cover all or part of the region were reported by D McElligot, G Dolgonav, E Frolova, and W Stock. Also localized to this contig were IRF1 and the human homolog to the mouse P600 gene (which does not year have a human gene symbol) as well as the polymorphic marker L1265 (D McElligot and G Dolgonav). The point was raised that a common basis in T-cell activity may underlie this clustering of genes.

The second gene-rich region which has been extensively mapped extends from 5q31 through 5q33, and contains EGR1, CD14, FGFA, GRL, ADRB2, CSF1R, and SPARC. Order and distance information was contributed by YAC contigs (M Lovett), fluorescence in situ hybridization (M Le Beau, W Neuman, C Westbrook and D Saltman), and radiation hybrid maps (J Wasmuth and J McPherson). There is good agreement on gene order, although there are some inconsistencies which could not be resolved with the available data.

A third region, 5q33-35, contains neurotransmitter receptors (ADRA1B, GABRA1, GABRA2, and DRD1) but map information is as yet incomplete.

New gene assignments and changes in localization

New gene assignments were reported by E W Jabs, with the details to be published elsewhere. These include peptidylglycine alpha-amidating monooxygenase to 5q14 (provisional gene symbol is PAM), IL12B (formerly NKSF2) to 5q31-33, and human apical epithelial Na+/H+ exchanger isoform to 5p15.3.

The reassignment of ARH-H9 from 5q to 1p21 by S Morris follows the reassignment to the same region of CSF1, to which it is closely linked.

Consensus physical map

A physical map for the long arm of chromosome 5 was prepared by combining data from many participants on gene/probe order, as determined by a variety of of methods including in situ hybridization, pulsed field gel electrophoresis, radiation hybrids, somatic cell panels, and YAC contigs. The map, shown in Figure 1, combines high resolution, regional data with more global efforts; in particular a large number of probes were positioned or confirmed using a radiation hybrid panel produced by J Wasmuth. The map incorporates the gene contigs, best localization for genetic disorders, and includes the breakpoints of several commonly used hybrid cell lines, and a cancer translocation. Higher resolution information for some regions, especially those surrounding disease genes, is found in the Abstracts, or will be published elsewhere. Many of the genes on chromosome 5 either are polymorphic themselves, or have been positioned relative to genetic markers, making it possible to place a number of these contigs accurately on genetic linkage maps.

Consensus genetic map

One objective of a chromosome workshop is to obtain a consensus map which has unequivocal order, with freely available, highly informative markers (formatted if possible for PCR) separated by a distance (of the order of 5 cM) which fits on both the genetic and physical map. Although the combined genetic data does not reach this high criterion as of yet, it is possible to construct a genetic map which covers much of the chromosome with ordered and informative markers at a distance of between 5-10 cM. Therefore, even though there is as yet no accepted set of index markers for this chromosome, the Workshop was confident that combining the existing data would give a good index map, which could be completed within the next year.

Two comprehensive maps were reported at this workshop, each of which placed a set of markers on the chromosome. That from the CEPH consortium was presented by R Plaetke, and contained consensus data from CEPH groups on 40 markers. It is very comprehensive, but not all order could be given unequivocally, and many of the markers were of low informativity. J Weber presented a map of 15 markers which specify highly variable short repeats of the AC-type, and had been put through a subset of the CEPH families. Additional genetic linkage maps were contributed by S Wood and M Speer.

It was felt that the various maps presented at the Workshop might be combined to produce a consensus genetic linkage map, taking advantage of the fact that these markers were typed on CEPH data. R Plaetke volunteered to complete these calculations after the workshop. To build the map, a primary map was prepared with CILINK, using markers from the CEPH database having a heterozygosity >0.67 and in which at least 100 children come from matings where at least one parent is heterozygous. To this initial map were added the probes from S Wood, and from J Weber; though the Weber and Wood probes did not always meet these criteria, especially in numbers of individuals typed, they are all PCR-formatted and are felt to be especially useful to the Chromosome 5 community. The completed map accompanies this Report as a separate publication which includes details of the map construction and of the probes used.

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Combined physical/genetic maps, radiation hybrids, and fluorescence in situ hybridization

Somatic cell hybrid panels are available for the short arm verhauser) and the long arm (J Wasmuth, M Khan and rs). As the resolution of the chromosome 5 map increases, is been possible to localize more precisely some of the kpoints of the hybrids making up these panels (J Wasmuth, ood) and also to identify small gaps outside of the deletions /ood). J Wasmuth also has a large panel of radiation-reduced id cell lines, on which many of the genetic markers and ed genes have been placed.

M Le Beau and G Landes contributed localization of a large ber of markers by fluorescence in situ hybridization (FISH). K Klinger demonstrated a new approach to preparing extended omosomes for extremely high resolution interphase mapping. umber of groups have been using dual-color FISH to order es and probes within localized regions, as discussed above. C stbrook presented the use of dual-color FISH to order probes hin 5q31 to high resolution, to facilitate the preparation of a tipoint genetic and physical linkage map.

Mouse-human homology map

S Camper prepared an updated mouse-human homology map the long arm of chromosome 5, using consensus physical er information compiled at this meeting, and unpublished data m other mouse gene mappers (not in attendance at the eting) including M Davisson, M Justice and D Stephenson. e homology map is shown in Figure 3. Murine chromosomes 13, and 18 contain genes which map to 5q. The map is now fficiently dense that long stretches of homology are apparent. veral regions which deviate from this homology, perhaps presenting deletions or inversions, can be discerned as well.

Concluding points

A large number of mapping resources have been prepared by e participants to study localized regions. Among the more obal resources are a panel of somatic cell hybrids containing letions along the length of chromosome 5, which were recently aced in the ATCC by J Wasmuth, and flow-sorted chromosome libraries prepared at Los Alamos by L Deaven. These are ailable to all participants, as are the genetic and physical arkers which were reported to the Workshop and family aterial, most of which has been lodged either with ATCC or ith the U.K. Human Genome Resource Centre.

The selection of J McPherson and L Deaven as the Genome ata Base (GDB) chromosome 5 chairs was endorsed by those tending the Workshop. There was agreement that future GDB hairs should be selected by the Workshop from its members, in rder to best serve the needs of the chromosome 5 community.

It was agreed that future workshops will alternate yearly if ossible between North America and Europe (or other non-American countries), although the orders will be reversed for 993 and 1994. The next meeting will be held in March 1993, at U C Irvine, chaired by J Wasmuth and assisted by C Westbrook as co-organizer, and it was suggested that the 1994 meeting should be in Moscow, chaired by E Frolova assisted by J Wasmuth as co-organizer. The 1995 meeting might be in Manchester (chaired by M Dixon), and 1996 might be in Canada. Further details of the 1993 meeting will be announced when the dates are fixed. In future, those wishing to attend the Workshop should submit either an abstract (for publication) or a statement demonstrating their interest in chromosome 5 mapping. Participation by mouse genome mappers, and those with expertise in informatics, would be especially welcomed.

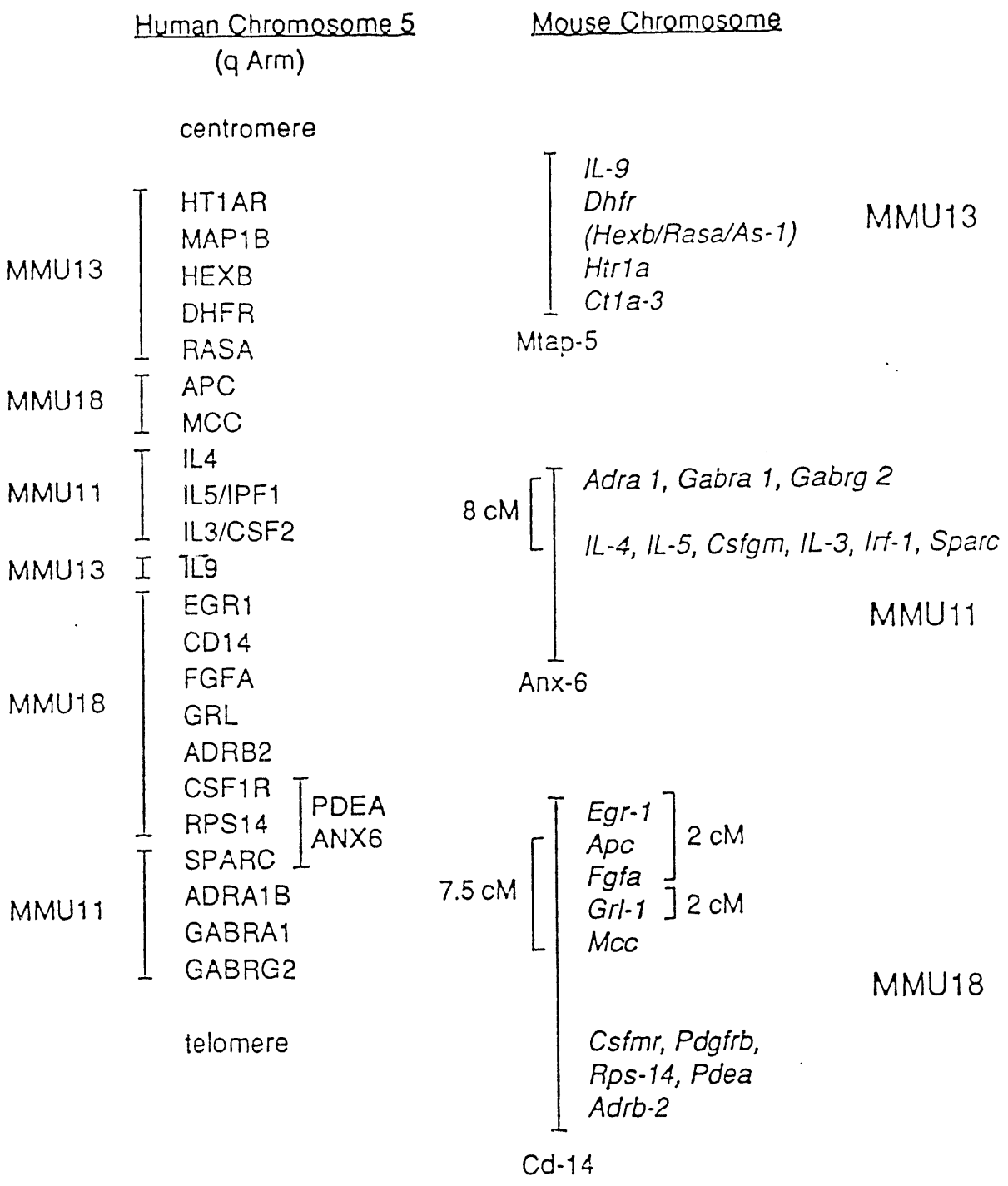

Fig. 2. Mouse-human homology map for the long arm of chromosome 5. The diagram on the left shows the order of cloned human genes for which murine homologs have been identified, from top (centromere) to bottom (telomere), and the approximate mouse chromosome segments. On the right are mouse chromosomes, showing the order of the homologous genes. Approximate genetic distances in centimorgans are shown for several of the murine genes.

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Abstracts of the second international workshop on human chromosome 5 mapping

Physical and genetic map of 8 markers within 5q31

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A map of 8 cosmid markers centered at 5q31 was prepared by a combination of genetic linkage and fluorescent *in situ* hybridization (FISH). Dual-color FISH comparisons of two probes (metaphase) or three probes (interphase) allowed a determination of chromosomal order for 7 markers; one probe, D5S151, was unsuitable for FISH. Genetic distance and likely order were determined by multipoint linkage analysis using CEPH data on all 8 markers. The orders determined by linkage and by FISH agree well except for probe, D5S147, which genetic linkage places between D5S151 and D5S156 (log_{10} likelihood 122.7) rather than between D5S166 and D5S162 (log_{10} likelihood 117.6). The latter position was confirmed by multiple independent FISH analyses; an error in CEPH genotyping is suspected, and confirmation is underway. The best consensus map, shown below, spans 37 cM. It is contained within the interval bounded by CEPH anchor markers D5S64 and D5S72, with >1000:1 odds. This region contains a number of growth factor and receptor genes, and is a common site for leukemia deletions.

Isolation of YACs for CSF-1R and CSF-1 and gene localization using FISH

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The genes encoding macrophage stimulating factor (CSF-1) and its receptor CSF-1R have been assigned to chromosome 5 in band 5q33. We have previously reported the deletion of the CSF-1R gene and the retention of the CSF-1 gene in a group of 10 patients with MDS and a 5q deletion using molecular analysis. This data suggested that the distal breakpoint of the 5q deletion was tightly localized to one chromosomal band in this group of patients. We subsequently initiated a strategy to construct a YAC contig across this region. A YAC library (ICI, UK) was screened using PCR based methods for YACs which encompassed the CSF-1 and CSF-1R YAC (15DB10) and 3 CSF-1 YACs (11HB2, 14HD10, 29FC1) were mapped using fluorescent *in situ* hybridization. The CSF-1R YAC gave, as expected, a specific signal on chromosome 5q. Each of the 3 CSF-1 YACs, however, gave a specific signal on chromosome 1p. This data is consistent with the localization of CSF-1R to chromosome 5q and confirms the recent reassignment of CSF-1 by Morris *et al* 1991 to chromosome 1p. These YACs which encompass the CSF-1 and CSF-1R genes may be a useful resource for the future analysis of human leukæmias with rearrangement or deletion of chromosomes 1p and 5q.

Reassignment of the *ARH-H9 RAS*-related gene to 1p21

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The human *ARH-H9* gene (originally *rhoC*), a member of the *RAS* gene superfamily, was previously assigned to the telomeric region of chromosome 5q(5q31-qter) based on isotopic *in situ* hybridization and its cosegregation with the *CSF1* gene locus in human × rodent somatic cell hybrids carrying partial chromosomes 5, together with other human chromosomes (Genomics 6:197, 1990). With the recent reassignment of the human *CSF1* locus to chromosome 1, region p13-p21 (Blood 78:2013, 1991), it seemed important to re-examine the localization of the *ARH-H9* gene since it segregates 100% concordantly with the *CSF1* locus in hybrid cells. Hybridization with a radiolabeled *ARH-H9* cDNA fragment containing the entire 569bp coding sequence of the gene to *Eco*R1-digested DNAs in a panel of somatic cell hybrids that included a monochromosome 5 hybrid (GM10114, Coriell Institute for Medical Research, Camden, NJ) and a hybrid carrying only chromosomes 1 and X (GM07299, Coriell Institute) demonstrated that the *ARH-H9* locus (represented by a 6kb *Eco*R1 human-specific restriction fragment) also segregates with chromosome 1. Using additional hybrids that carry partial 1p, we mapped the *ARH-H9* locus relative to other 1p loci, localizing the gene to the region 1p13-p31. Fluorescence *in situ* hybridization to metaphase chromosomes with a genomic *ARH-H9* clone, which was partially sequenced to confirm its identity, refined the gene's location to chromosome 1, band p21. Two other *RAS* superfamily genes that are distantly related to *ARH-H9*, *NRAS*(1p13-p22) and *RAP1A*(1p12-p13), have been previously localized to this chromosomal region.

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pping a 5q11.2-q13.3 deletion chromosome

Vood and L.E. Bernard
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A 5q11.2-q13.3 deletion chromosome, derived from a nced translocation carrier, was segregated into the somatic hybrid HHW1064 as the sole human chromosome (Gilliam *et* Genomics 5:940-944 1989). DNA probes absent from the tion chromosome were isolated by *Alu* PCR differential idization (Bernard *et al*, Genomics 9:241-246 1991). Probes ved from this cytogenetically defined subchromosomal region e used to develop genetic markers. The CEPH family panel typed for these markers and the loci positioned using KAGE. The markers unexpectedly mapped to three regions hromosome 5, one on 5p and two on 5q, indicating that the hybrid chromosome 5 had 3 non-contiguous deletions. ıtine banded karyotypes revealed only a deletion of 5q11.2-.3. The region of the 5p deletion was shown to be intact in the nced carrier, suggesting that this deletion may have arisen in ure. The adjacent deletions of 5q involve markers both dimal and distal to D5S76. A large deletion, distal to D5S76, olves loci from D5S6 to D5S253. The adjacent deletion, dimal to D5S76, involves the D5S260 locus. The familial slocation does not appear to involve the D5S260 locus so the dimal 5q deletion presumably also arose during culture. The sence of these microdeletions should be taken into account en HHW1064 is used in mapping panels. A highly morphic GT repeat marker, heterozygosity > 70%, was pped into each of the three deletion regions.

gh resolution genetic map of new DNA markers closely nking the SMA locus

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The proximal spinal muscular atrophies (SMA) represent the ond most common fatal recessive disorder after cystic fibrosis. e gene responsible for chronic and acute forms has been pped to chromosome 5q12-q13.2 using genetic linkage studies. and others have demonstrated by linkage analysis that all ee types of SMA map between D5S6 and D5S39. The present dy describes a high resolution genetic map using markers M4 5S6), D5S125 (EF5.15), 114ye7, MAP-1B, JK53 (D5S112) d p105-153Ra (D5S39) in 50 SMA families each containing at st two affected individuals. Pairwise linkage results for SMA h these loci gave: Z=9.81, θ=.044 (D5S6); Z=11.4, θ=.02 5S125); Z=13.75, θ=.01 (114ye7); Z=17.57, θ=.015 (MAP-); Z=11.85, θ=.02 (D5S112); Z=14.7, θ=.022 (D5S39). Multilocus analysis by the method of location score was used to establish the best estimate of the SMA gene location. our data suggest that the most likely location for SMA is between the blocks D5S125/114ye7 and MAP-1B/JK53 (location score of 30.6 in log base 10). The odds against alternative orders were >2400:1 in other cases. Thus, the SMA gene lies in a 4cM region between these blocks.

Tight linkage between D5S39 and chronic childhood onset spinal muscular atrophy in French-Canadian families

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Autosomal recessive SMA is a neurodegenerative disorder selectively affecting the α-motor neuron cells of the anterior horn. Three forms are habitually distinguished depending upon the severity and extent of muscle weakness: one acute (Type I Werdnig-Hoffman disease) and two chronic forms (Intermediate Type II and Type III Kugelberg-Wellander disease). Linkage analyses have localized the gene(s) for all three forms to 5q11.2-13.3 (1,2). We have analyzed 8 pedigrees (n=67) with 17 individuals having childhood-onset chronic SMA. DNAs were typed for 4 RFLPs at 3 loci: D5S6, D5S39 and D5S78. We found significant evidence for tight linkage between chronic SMA and D5S39; the maximum lod score (Z_{max}) was 5.47 at a recombination fraction (θ) of 2%. We did not find significant evidence for close linkage with either D5S6 (Z_{max}=0.34 at θ=0.18) or D5S78 (Z_{max} at θ=0.21). Finally, we observed a single "unaffected individual" (normal muscle biopsy and EMG demonstrating normal conduction velocities with occasional fasciculations) who had inherited the same parental haplotypes as two siblings with Type III SMA. The fact that two affected and three non-affected siblings were completely concordant for all three marker loci analyzed argues against the possibility that the SMA gene for this family is unlinked to 5q11.2-13.3. DNA samples are currently being typed for dinucleotide repeat polymorphisms (JK53 and MAP1B).

Mapping of distal 5q genes and markers relative to the breakpoint of t(3;5)(q25.1;q34) in myelodysplastic syndromes (MDS) and acute nonlymphocytic leukemia (ANLL)

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A t(3;5)(q25.1;q34) reciprocal translocation identifies a subset of cases of MDS or ANLL characterized by increased numbers of megakaryocytes and severe trilineage dysplasia. As an initial step

in characterizing this translocation, we performed Southern hybridizations with a series of polymorphic DNA markers and gene probes previously localized to distal 5q using DNA from human-hamster hybrids containing the der(3) or der(5) chromosomes to bracket the breakpoint. Loci mapping proximal to the 5q34 breakpoint included *ADRB2*, *SPARC*, *RPS14*, *CSF1R*, *PDGFRB*, *ANX6*(p68), JO157E-A(D5S36), J0205HC(D5S22), CR1-P148(D5S72), CR1-L45(D5S61), CR1-P152(D5S66), *GLUT3P1*, pHF12-65(D5S2), and cKK5.19(D5S121); loci distal to the breakpoint included CR1-L1200(D5S62), *DRD1*, *FGFR4*, and *FLT4*. These localizations relative to the 5q34 breakpoint were independently confirmed for several loci (*RPS14*, *ANX6*, *GLUT3P1*, pHF12-65(D5S2), cKK5.19(D5S121), CR1-L1200(D5S62), *DRD1*, *FGFR4*, and *FLT4*) using genomic clones in FISH of metaphases from leukemic blasts. Two color FISH using normal metaphases is in progress to order a number of the examined loci relative to one another. For example, two color FISH comparing *RPS14*, *ANX6*, *GLUT3P1*, or pHF12-65(D5S2) to cKK5.19(D5S121) established that cKK5.19 is the most distal of these loci. Our analysis to date, combined with reported genetic localizations of the polymorphic markers that we examined, suggests that the 5q34 breakpoint is most closely flanked by cKK5.19(D5S121) proximally; examination of the relative order of the distal loci is underway to determine which locus most closely flanks the breakpoint on the telomeric side.

Isolation of new clones and YACs from the region 5q11.3-13.3

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The gene for autosomal recessive proximal spinal muscular atrophy (SMA) has been localized on chromosome 5q12-q13.3 between D5S6 and D5S112, a genetic distance of about 6 cM.

In order to clone the SMA gene, the region around it has to be saturated with further markers. Three different strategies were chosen:

1. Human clones from chromosome 5 specific libraries (a *Bss*HII endclone phage library from a somatic cell hybrid and a genomic phage library from an irradiation hybrid) were isolated and mapped to somatic cell hybrids, containing the whole or parts of chromosome 5 on hamster background. Eighteen clones were mapped into the region 5q11.2-q13.3, 112 clones on 5q outside the region 5q11.2-q13.3 and 21 clones on 5p. We are currently searching for RFLPs and CA-repeats in the clones which were mapped into the region 5q11.2-q13.3. So far, the probe ECB306Bgl2.1 (D5S215) recognizes a three allele *Eco*R1 polymorphism and the clone P62 a two allele *Msp*I RFLP. Several clones contain CA-repeats.

2. Generation of human specific fragments from irradiation hybrids by inverted *Alu* and Line PCR. The *Alu* primers 517, 559 and IV6 and the Line primer L1Hs were used either alone or in combination on irradiation hybrid DNA containing overlapping fragments from the SMA region. 15 common bands in the range of 0.3-3.5 kb were isolated, subcloned and mapped back to somatic cell hybrids. Isolation of cosmids from a gridded flow sorted chromosome 5 and isolation of specific YAC clones is underway.

3. Isolation of specific YACs from the SMA region. Three YACs were isolated with the probe pM4, four YACs with the primers EF(TG/AG), one YAC with the probe JK53 and four with primers from JK53 (kindly provided by K.E. Davies) as well as one YAC with the probe p105-153 from the ICRF 4X-YAC library. All together they are covering about 2.5Mb of the region. The direction of YAC-walking from the distal side has been defined by combining genetically and physically mapping data. Isolation of polymorphic CA-repeats, especially from the proximal side is in progress.

Synteny conservation between HSA 5q and MMU 11

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Genes from the long arm of HSA 5 have been assigned to MMU 11, 18, 13 and 3. In an intersubspecific backcross, (DF/B-*df/df* × *CAS4/Rk*) × DF/B-*df/df*, three closely linked genes, Interleukin 3 (*Il-3*), granulocyte macrophage colony stimulating factor (*Csfgm*) and osteonectin (*Sparc*), have been localized on MMU 11 approximately 8 cM distal to the α_1-adrenergic receptor (*Adra-1*) (Buckwalter *et al.* Genomics 10, 515-526, 1991). These three genes are localized to HSA 5q23-q34, suggesting that a region of synteny conservation between MMU 11 and HSA 5q exists over this interval. We now report further mapping studies using the same cross. The genes for Interleukins 4 and 5 (*Il-4*, *Il-5*) and interferon regulatory factor 1 (*Irf-1*) map to the same position as *Il-3*, *Csfgm* and *Sparc* (0 recombinants in 110 animals). IRF1, IL4 and IL5 also map to the same region of HSA 5q as IL3 and GMCSF. Another gene cluster, containing the α_1 and γ_2 subunit genes of the $GABA_A$ receptor (*Gabra-1* and *Gabrg-2*) and *Adra-1*, was identified with 0/96 recombinants. This clustering is consistent with the colocalization of GABRA1 and GABRG2 on HSA 5q34-35. Thus, there are two clusters of HSA 5q genes on MMU 11, composed of *Adra-1*, *Gabra-1*, *Gabrg-2* and *Il-3*, *Il-4*, *Il-5*, *Csfgm*, *Irf-1*, *Sparc*. It is unclear whether these two groups of genes are part of a continuous segment of synteny homology or are interrupted by a non-homologous region. The absence of recombination between genes in each cluster suggests that physical mapping or analysis of additional backcross progeny will be necessary to resolve the gene order.

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ıysical map of chromosome 5q21-22

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We have constructed a physical map of Chromosome 5q21-22 ɔulsed field gel electrophoresis using 45 probes. The probes uded those obtained from other groups involved in the search the gene for APC and derivatives of those probes, new clones ived from irradiation hybrids, microclones, and clones ained by preparative pulsed field gel electrophoresis. The map ers approximately 13 Mb and consists of two unconnected ments of 10 Mb and 3.5 Mb. The segments have been ordered l orientated by genetic and somatic cell genetic data. The 10) fragment includes on its proximal side the genetically mapped rkers D5S85 (KK5.33), D5S82 (YN5.64) and S5S98 2B27). Further distal it encompasses the region containing the ıes APC and MCC which are implicated in colon cancer norigenesis. Our results suggest the order of markers around : APC gene is: Cen-D5S114 (ECB220)-D5S135 (EF5.44)- ?C-MCC-D5S81 (YN5.48)-Tel. The 3.5 Mb map segment :ludes D5S97 (ECB134) and crosses the distal breakpoint of tologically visible deletions of 2 APC patients described eviously.

Probes from: B. Vogelstein, Y. Nakamura, P. Meera Khan, R. hite

owards reducing the genetic interval of the spinal ıuscular atrophy region by linkage analysis

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The gene for autosomal recessive proximal spinal muscular ıtrophy has recently been localized by linkage analysis between D5S6 and D5S112 a distance of about 6cM on 5q12-13.3.

64 SMA families of Type I-III were analyzed with 9 ɔolymorphic markers (Lambda599 (D5S76), pM4 (D5S6), EF(TG/AG) (D5S125), RB110/111 (MAP-1B), RB104/106 MAP-1B), JK53 (D5S112), JK53CA1/2, 6741GT (D5S39), ?NCT (D5S127) all closely linked to the SMA gene.

In one SMA family Type I one affected and one healthy ɔrother showed the same haplotypes for the markers Lambda599 D5S76) and EF(TG/AG) (D5S125) and different for the other narkers. This suggests a location of the SMA gene distal to D5S125 and thus a reduction of the SMA region to about 4cM. Several other recombinations were found which placed the SMA gene proximal to MAP-1B/D5S112.

Furthermore, we found a recombination between D5S112 and JK53CA1/2 which places MAP-1B proximal to JK53CA1/2 and another one between RB110/111, the $(CA)_n$-repeat derived from an intron of the 5' end of the MAP-B1 gene and RB104/106 derived from the 3' end of the MAP-1B gene. The recombination places RB110/111 proximal to RB104/106, therefore the 5' end of the MAP-1B gene is proximal too.

We found five recombination events between D5S39 and D5S127; three of them placed D5S39 proximal to D5S127 and two the other way round. Based on haplotype and two-point linkage analysis the most probable order and distances of the loci are:

D5S76 - D5S6 - 5cM - D5S125 - 2cM - SMA - 1cM - (5' MAP-1B - 3' MAP-1B)/D5S112 - 1cM - JK53CA1/2 - 4cM - (D5S39 - 2cM - D5S127)

The genetic mapping results are in part in contradiction to the physical data. Thus the loci MAP-1B, D5S112 and JK53CA1/2 are all included in 5 overlapping YACs YJK53 and placed not further than 370kb apart from each other, while the recombination frequency between these loci are 1-4%. The same discordance was observed for D5S39 and D5S127 which are included in a YAC of 350kb (Y153), but show a recombination frequency of 2%.

Order of genes and anonymous DNA markers within chromosome band 5q31

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Interstitial deletions of the long arm of chromosome 5 are the most common structural abnormality observed in therapy-related acute myeloid leukemia (t-AML). Although the proximal and distal chromosomal breakpoints vary among patients, we have determined that band 5q31 is the smallest region deleted in all patients (critical region, CR). It is likely that the loss of a gene(s) in 5q31 is involved in the pathogenesis of t-AML. To facilitate the identification of this putative tumor suppressor gene, we are preparing a physical map of 5q31. By using fluorescence *in situ* hybridization (FISH), we localized 49 cosmid probes to 5q; 9 of these clones mapped to 5q31. Using two-color FISH, we have determined the order of these 9 cosmid probes and 13 genes within and flanking 5q31: cen-D5S150-[*CSF2/IL3-IL4/IL5*]-*IL9*-*TCF7-EGR1*-D5S166-D5S147-D5S178-D5S162-D5S155-D5S151-D5S156-WLN1-*SPARC-ADRA1*-tel. *CD14* and *FGFA* are distal to D5S166 and proximal to D5S155. *GRL* is distal to D5S166 and proximal to WLN1. Analysis of leukemia cells is in progress to define this region more precisely and to identify candidate genes.

Physical mapping of the cytokine gene cluster on human chromosome 5

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The human genes for hematopoietic growth factors IL3, IL4, IL5 and CSF2 are located in a small region of human chromosome 5 (5q23-31) that is frequently deleted in patients with myeloid disorders [del(5q)]. There is a possibility that a family of genes responsible for regulation of cell growth during hematopoiesis is located within this limited segment of chromosome 5. Precise mapping of this region is essential to the understanding of functional relationships between the genes and could reveal the genes for other growth factors and their receptors that may be located within this region.

Several hybridization probes were derived from chromosome walking on human genomic cosmid library starting from the IL3, IL4, IL5, and CSF2 genes. Using PFGE analysis with these probes the long range physical map of a cluster of genes was constructed. The IL4 and IL5 genes were placed on this map in head to head orientation at the distance of 240 kb. Basing on the following order of the four genes [cen-(IL4-IL5)-IL3-CSF2-qter] and the length of NotI fragments we proposed that the distance separating IL3 and IL-4 genes was not less than 1050 kb.

CEPH YAC library was screened by PCR-based strategy for different probes derived from the cluster and for the following polymorphic loci, D5S63, D5S52, and D5S67, which are of particular interest since they are genetically most close to the cluster. Two YACs were obtained for the regions D5S52 and D5S65 and sized by PFGE at 780 and 1050 kb. The ends of these YACs are currently being sequenced to provide STSs for a chromosome walk in the YAC library.

The YACs identified with several probes specific for different points of the cytokine gene cluster formed a contig presumably 1,9 Mb and allowed to connect the genes on physical map in the following order: [cen-IL4-IL5-IL3-CSF2-qtel]. One of the isolated YAC clones completely overlapped the region separating IL3 and IL5 genes.

Physical and genetic markers near TCOF1

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Identification of disease genes by positional cloning places a premium on attaining high resolution in physical mapping procedures. Resolution on the order of megabases can be consistently achieved with metaphase chromosomes, about 100 Kb with interphase nuclei, and about 40 Kb with pronuclei from gamete fusion products (Brandriff et at., 1992). We have developed two methods for high resolution physical mapping using fluorescent in-situ hybridization (FISH). One of these is based on the interaction of sodium butyrate with histones, while the other borrows from the histone depletion methods developed by Laemmli and colleagues. We previously mapped 58 cosmids to chromosome 5 using FISH and coarsely ordered this set of clones using contour measurements of hybridized metaphase chromosomes. Seventy-three percent of the cosmids mapped between 5q31.1-5qter. The cause for this non-random distribution is unknown, but fortuitously yielded a map containing 43 cosmids within an approximate physical distance of 50Mb. Therefore, we have begun to use these clones and the techniques of FISH and genetic linkage analysis to more accurately define the critical region around the TCOF1 locus (5q31-34) and to enhance the resolution of the genetic and physical maps for this region of the genome. We report the identification of a set of cosmids near the TCOF1 locus, and preliminary data on cosmids within this set which most closely flank the gene responsible for TCS. The marker IG52 contains an 18 allele STRP which maps distal to TCOF1 (Zmarc=9.78, θ=0.055), whilst the markers 2C7 and 2D10 map proximal to TCOF1 (Zmarc=6.70, θ=0.060; Zmarc=8.87, θ=0.031, respectively).

:port of the second international workshop on human chromosome 5 mapping: nsensus genetic map

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The map in this report combines the information from the netic maps that were presented by J.W., S.W., and R.P. at : Second International Workshop on Chromosome 5. It nsists of 33 polymorphic markers (Table I); all loci have terozygosities ≥ 0.67, except for D5S257 (0.37), D5S21 .43), D5S205 (0.46), D5S119 (0.50), and D5S208 (0.50). fteen of the markers are RFLP's. With 23 of the 33 loci we rmed a continuous map with a length of 314cM.

Building the map

We built the map using LINKAGE (Lathrop et al. 1985) nd an automated algorithm (Dumanski et al 1991) that adds :est markers" to a primary map after evaluating their ›cations with multi-point analysis.

The markers from Wood's and Weber's laboratories were dded in two steps. Since most of Weber's microsatellite narkers had been analyzed in a small subset of the CEPH-amilies (see Table I), 1-lod-unit confidence intervals were letermined for all 15 of them (Fig. 1). After adding these 15 narkers to the fixed map, we mapped the marker D5S21 to letermine the localization of the centromeric region (Fig. 2).

Results and Discussion

First a primary map consisting of 13 markers (loci marked * in Figs. 1 and 2), all from the CEPH database version 5; vas validated. We selected from more than 200 markers hose that demonstrated heterozygosities ≥ 0.67 and for which numbers of children from matings where at least one parent is ieterozygous (i.e. NIO) ≥ 100. In this primary map, all odds or inversion of adjacent loci are greater than 1000.1, except or D5S387 and D5S48 (28:1), and D5S61 and D5S198 69:1). When markers from Wood's laboratory were added, he odds for inversion of adjacent loci remained greater than 000:1, except for the two already above mentioned pairs of narkers. Finally, we determined 1-lod-unit confidence intervals for the 15 PCR-based marker from Weber's laboratory (Fig. 1), and attempted to add each of them and D5S21 to the 17-marker map. The consensus map (Fig. 2 and Table II) consists of 23 markers. Odds for inversion of adjacent loci are ≥ 1000:1 except for D5S387 and D5S48 (28:1), D5S76 and D5S118 (113:1), D5S253 and D5S107 (188:1), D5S61 and D5S198 (55:1), and D5S211 and D5S43 (617:1). Physical mapping shows that the centromere lies within the 27cM interval between D5S21 and D5S76, so the localization of D5S260 to the p- or q-arm remains unresolved. The total genetic length of this map is 314cM, and the mean distance between the markers is about 14cM. However, the markers are not equally spaced over the chromosome; some are more than 20cM apart and the q14-22 region is not covered at all.

To determine the approximate regions containing the SMA (spinal muscular atrophy) and APC (adenomatous polyposis coli) genes on our map, we analyzed two markers known flanking the SMA locus, D5S112 (JK53/PvuII) and D5S125 (EF5.15/TaqI) (see report of the Second International Workshop for Human Chromosome 5); and markers flanking the APC region, D5S135 (EF5.44/MspI) and D5S141 (L5.71/MspI) (see Kinzler et al 1991).

SMA

We were not successful in determining an interval on our map for SMA, mainly because different kinds of multi-point analyses showed discordant results. The analyses showed clearly that both SMA-linked markers fall in the region between D5S76 and D5S205; however, no CEPH-family genotyped for D5S112 and D5S125 was also genotyped for D5S118. Since two-point analysis showed that D5S125 and D5S112 are completely linked to each other (lod score = 9.0), we estimated the recombination frequencies to their adjacent loci in the map D5S76 --- D5S125/D5S112 --- D5S205. Thus the SMA region is approximately 19cM distal from D5S76 and 15cM proximal from D5S205. The odds for this location of the region are larger than 1000.1 against any other possible location in this 3-locus map.

APC

Multi-point analyses showed that the APC region lies between D5S391 and IL3. Since we could not detect any recombination between D5S135 and D5S141 with two point analysis, we assumed that those loci are totally linked to each other and determined the localization of the APC region in the map D5S391 --- D5S141/D5S1351 --- IL3. On our map, the APC gene is approximately 25cM distal from D5S391 and 14cM proximal from IL3. However, the odds of the best order versus the second best order (putting the APC region distal to IL3) are 16:1; moreover, two-point analysis showed that these two markers have lod scores of only 0.9 and 2.0 with D5S391.

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ble I Polymorphic Markers on Chromosome 5.

Locus	Probe, Enzyme	NIO	Het.	Physical Location	CEPH Contributor(s)
Markers from the University of British Columbia, Vancouver (Wood)					
D5S205*	5pcr1, TaqI	215	0.46	q11.2-q13.3	
D5S253	Alu24, pcr	305	0.77	p or q11.2	
D5S257*	Alu25, pcr	170	0.37	p or 5q11.2	
D5S260	Alu32, pcr	273	0.77	p or 5q11.2	
D5S268*	Alu62, pcr	292	0.78	p or 5q11.2	
Markers from the Marshfield Medical Research Foundation (Weber)					
D5S107	Mfd27, pcr	47	0.80	q11.2-q13.3	
D5S108	Mfd34, pcr	46	0.89	p14.1-p13.1	
D5S111	Mfd40, pcr	47	0.70	p14.1-p13.1	
D5S117	Mfd48, pcr	46	0.89	p15.3-p15.1	
D5S118	Mfd63, pcr	31	0.60	cen-q11.2	
D5S119*	Mfd6, pcr	215	0.50	q31.3-q33.3	
D5S207	Mfd43, pcr	39	0.70	q31.3-q33.3	
D5S208	Mfd88, pcr	39	0.50	p15.3-p15.1	
D5S209	Mfd116, pcr	37	0.80	q31.3-q33.3	
D5S210	Mfd122, pcr	46	0.80	q31.3-q33.3	
D5S211	Mfd154, pcr	47	0.80	q33.3-qter	
D5S357	Mfd151, pcr	32	0.59	5	
D5S385	Mfd234, pcr	153	0.78	5	
CSF1R	SE13, pcr	47	0.90	q22-q22.3 or q31.3-q33.3	
IL9	SE9, pcr	50	0.90	q22.3-q31.3	
Markers from other Laboratories (CEPH Database)					
D5S21	JO110-H-C, MspI	176	0.43	p13-p11	Weiffenbach, White
D5S43	LMS8, HinfI	85	0.88	q35-qter	Jeffreys
D5S48	CRI-L123, HAP CRI-L123, MspI CRI-L123, RsaI	149	0.68	pter-p15.3	Donis-Keller
D5S56	CRI-C44, MspI	89	0.75	p15.2-p15.1	Donis-Keller
D5S61	CRI-L45, MspI	33	0.67	q33.3-q34.1	Donis-Keller
D5S76	L599H-a, TaqI	405	0.67	cen-q11.2	White
D5S178	cEF5.12, MspI	320	0.67	q31	White
D5S198	p93-121, TaqI	333	0.74	q34	White
D5S206	16C2, HinfI	302	0.81	5p	Vergnaud
D5S347	CEB28, Hinf-Ha	317	0.95	5	Vergnaud
D5S387	pYM48-5, HAP 1pYM48-5, MspI 2pYM48-5, MspI	347	0.75	5	White
D5S391	pMN2.3, MspI	171	0.69	5	White
	IL3,IL3, HAP IL3, BglII IL3, PstI	231	0.73	q23-q31	Dean

Note.- HAP = haplotype of a group of markers. The markers that were used for haplotyping follow. NIO = number of offspring from matings where at least one parent is heterozygous, Het. = heterozygosity. CEPH contributors = listed are the names of those who submitted genotypes for the analysis. Data of loci marked with * were taken from the CEPH database. Physical locations were taken from Bernard and Wood (1991), Le Beau et al (1987), Le Beau et al (1991), Neuman et al (1991), Overhauser et al (1987), Royle et al (1988), and Weber et al (1991). For a detailed physical map of the region including IL3 and CSF2, see Frolova et al (1991).

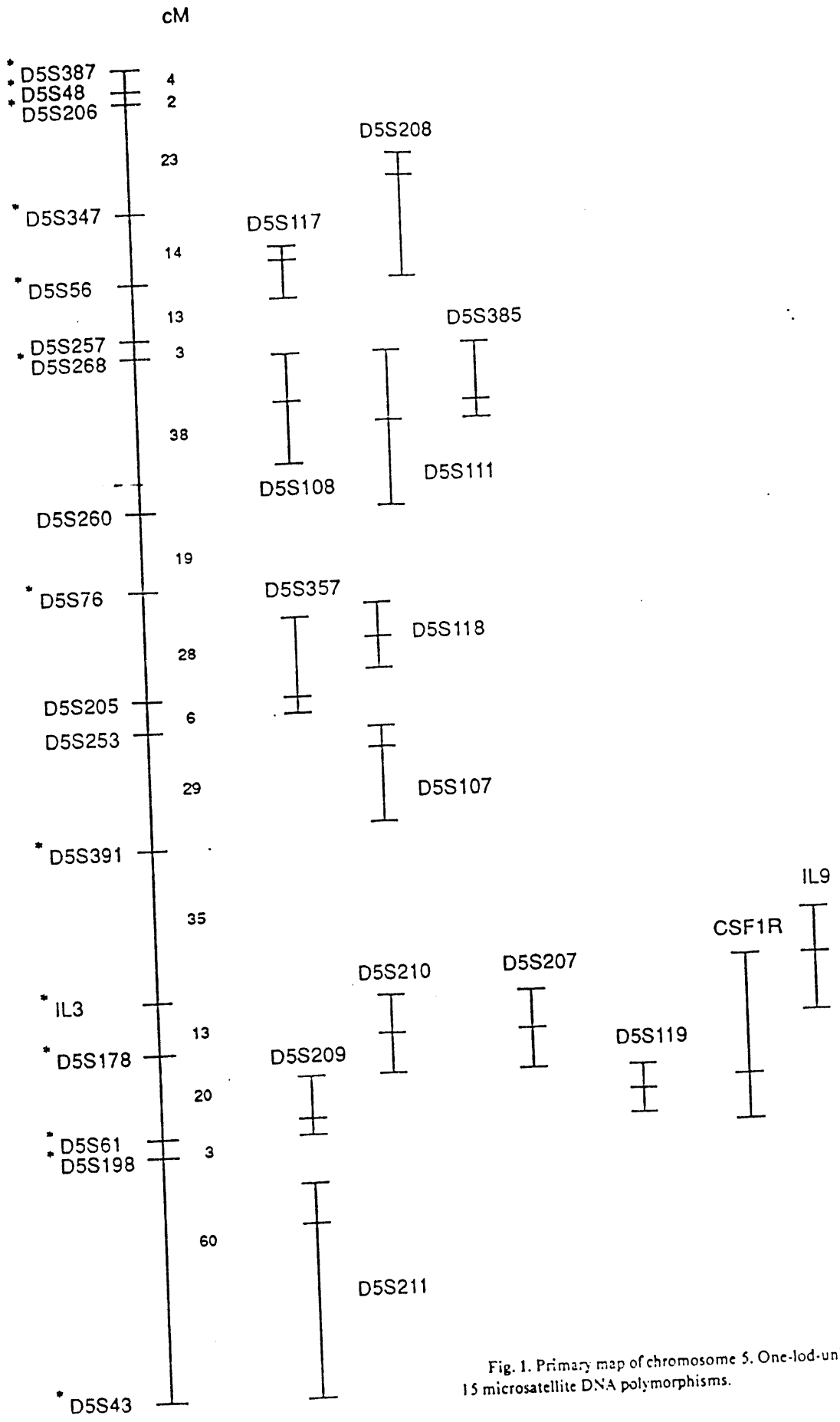

Fig. 1. Primary map of chromosome 5. One-lod-unit confidence intervals are shown for th
15 microsatellite DNA polymorphisms.

ble II Consensus Map of Chromosome 5

rker	CILINK Diff.	Theta	Morgans	Cumulative Morgans
S387	6.64	0.041	0.043	0.043
S48	14.95	0.020	0.020	0.063
S206	356.55	0.188	0.236	0.299
S347	95.54	0.121	0.139	0.438
S56	77.51	0.115	0.131	0.568
5S257	15.19	0.031	0.032	0.600
5S268	49.36	0.072	0.078	0.678
5S385	21.99	0.069	0.074	0.752
5S111	16.28	0.182	0.226	0.978
5S21	20.02	0.082	0.090	1.068
5S260	41.49	0.134	0.156	1.224
5S76	9.46	0.123	0.141	1.365
5S118	26.23	0.149	0.177	1.542
5S205	28.49	0.062	0.066	1.608
5S253	10.47	0.058	0.062	1.670
5S107	15.48	0.166	0.202	1.872
5S391	88.68	0.255	0.357	2.228
L3	23.22	0.115	0.131	2.359
5S178	131.86	0.167	0.203	2.562
5S61	8.02	0.030	0.031	2.593
5S198	56.31	0.246	0.339	2.932
5S211	12.85	0.172	0.211	3.143
5S43				

Note.- CILINK Diff. = the odds for inversion of adjacent loci in the -2ln scale. Theta = estimate of the recombination frequency. Morgans are calculated using Haldane's function.

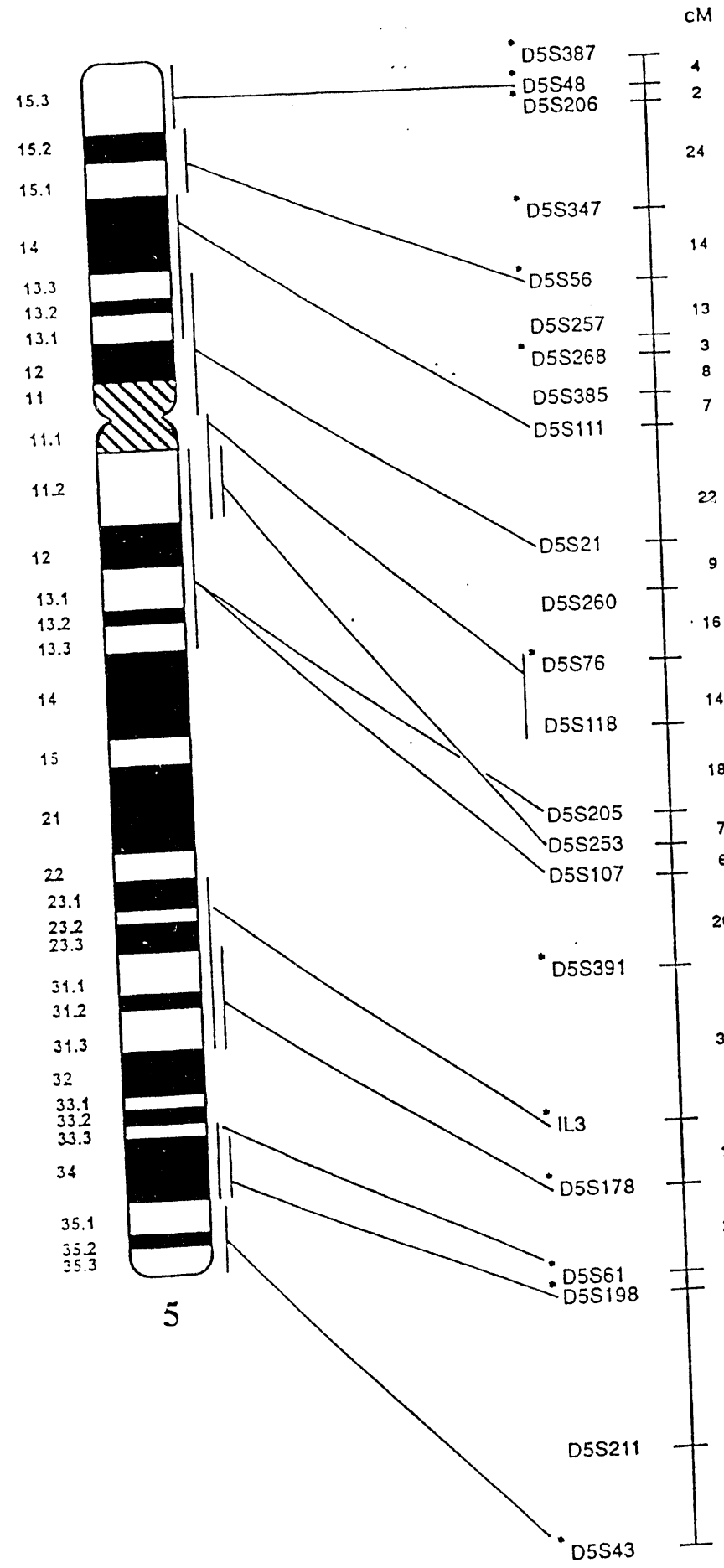

Fig. 2. Consensus genetic map of chromosome 5.

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