**PROCEEDINGS OF THE SECOND INTERNATIONAL CHROMOSOME 22
WORKSHOP**

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This summary outlines progress made towards constructing a physical map of human chromosome 22 at a workshop held in Montebello, Quebec from the 10-13 of September, 1991. This meeting was supported by the National Institute of Health (USA), the Medical Research Council of Canada, the Fonds de Recherche en Sante du Quebec, the National Cancer Institute of Canada, and endorsed by HUGO. The participants were:

Muriel Aubry, McGill University, Montreal

Alain Aurias, Institut Curie, Paris

Nickolas Blin, Universitat des Saarlandes, Homburg

Marcia Budarf, The Children's Hospital of Philadelphia

John Collins, Guy's Hospital, London

Pieter Dejong, Lawrence Livermore National Laboratory, Livermore

Olivier Delattre, Institut Curie, Paris

Suzanne Demczuk, McGill University, Montreal

Chris Denny, University of California at Los Angeles

Jan Dumanski, Karolinska Hospital, Stockholm

Ian Dunham, Guy's Hospital, London

Beverly S. Emanuel, The Children's Hospital of Philadelphia

Wolfgang Fiedler, University of Erlangen-Nurnberg, Erlangen

Jonathan Haines, Massachusetts General Hospital, Boston

Lyndal Kearney, St. Bartholomew's Hospital, London

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Marc Lipinski, Institut Gustave-Roussy, Villejuif
Mia MacCollin, Massachusetts General Hospital, Boston
Heather McDermid, University of Alberta, Edmonton
Anil Menon, Massachusetts General Hospital, Boston
Ketan Patel, Imperial Cancer Research Fund, London
Peter Pearson, Genome Database (GDB), Baltimore
Guy A. Rouleau, McGill University, Montreal
Martin Rutledge, Karolinska Institute, Stockholm
Marc Sanson, McGill University, Montreal
Peter J. Scambler, St. Mary's Hospital Medical School, London
Nancy Shepherd, DuPont Merck Pharmaceutical Co., Wilmington
Hiroaki Shizuya, California Institute of Technology, Pasadena
Gilles Thomas, Institut Curie, Paris
Jim Trofatter, Massachusetts General Hospital, Boston
Ellen C. Zwarthoff, Erasmus University, Rotterdam
Jessica Zucman, Institut Curie, Paris

The first scientific session addressed the construction of a genetic Map of chromosome 22. J. Dumanski (Stockholm) presented a map consisting of 45 polymorphic markers typed in CEPH pedigrees, including 35 markers generated at his institute. The total span of the map is 110 cM with an average distance of 5 cM between markers. 22 loci, including haplotypes of multiple markers at the same locus, were ordered with a probability of over 1000:1. J. Haines (Boston) described a genetic linkage map of human chromosome 22 including 28 polymorphic markers using the Venezuelan Reference Pedigree. The map extends 128 cM (sex

averaged) from D22S24 to D22S45. The sex-specific maps are of similar length, although there is variation in individual regions. J. Haines also reported the existence of 8 dinucleotide repeat polymorphisms, six of which are included in his map. These polymorphisms are: D22S156, D22S257, D22S258, PDGFB, CRYB2, CYP2D, IL2RB and TOP1P2.

The second scientific session dealt with the cytogenetic analysis of chromosome 22. L. Kearney (London) presented data on a flow sorted chromosome 22 cosmid library constructed at the ICRF in collaboration with Dr. H. Lehrach. 9216 clones have been gridded and are available for probing. Seven random clones from this library were mapped by in situ hybridization, four of which localized to chromosome 22 (approximately 60%). DNA from small numbers of sorted chromosomes (200-500) have been amplified by PCR and used to generate chromosome and region specific chromosome paints. A flow sorted normal chromosome 22 as well as a der(22) from a constitutional t(11;22)(q23.2;q11.2) and a der(9) from the Philadelphia translocation t(9;22)(q34.1;q11.2) were successfully used for whole chromosome and regional painting by fluorescent in situ hybridization (FISH). A. Aurias (Paris) presented FISH data with the precise mapping of D22S1 to the 22q11.32-q12.1 interface, VIII F2 to 22q12.1 and LIF to the 22q12.1-q12.2 interface. Testing of DiGeorge patients using FISH showed the presence of a single chromosome 22 with cosmid 506 (from Dr. P. Scambler). Cosmid 2271 (P. Scambler) displayed many loci on 22q11.2, suggesting the presence of multiple duplicated sequences in this chromosomal region. M. Budarf (Philadelphia) described the development of new fluorescent nucleotides for use in in situ hybridization experiments. These probes can be introduced into DNA by nick translation or random priming and yield signal intensities similar to that obtained by biotin-avidin. Use of these nucleotides eliminates the need for most post-hybridization steps and different fluorescent labels can be used simultaneously.

The third scientific session dealt with the presence of repeated sequences on chromosome 22. G. Rouleau (Montreal) presented data identifying six separate gamma-glutamyltransferase (GGT) loci. Three loci map to chromosome 22: one between the BCR region and the Ewing sarcoma breakpoint, and two between the centromere and the BCR region. Three additional GGT loci map each to chromosomes 18, 19 and 20. FISH confirmed the presence

of multiple copies of GGT on chromosome 22q11.2. P. Scambler (London) identified small probes which detect chromosome 22 specific repeat sequences present in 2 to 30 copies. Some of these duplications give rise to closely linked loci, while others are more dispersed throughout 22q11. He speculated that the presence of these repeats might make the region meiotically unstable, leading to the high frequency of chromosome aberrations in the region.

The fourth session dealt with chromosome 22 translocations and hybrid mapping panels. G. Thomas (Paris) described 31 well characterized somatic cell hybrids which identify 29 separate regions on chromosome 22, a level of resolution of 1-2 megabases. The hybrids described included: 2 chromosome 22 only cell lines, 3 pairs of hybrids which are derived from the same translocation but which contain the different derivative chromosomes, 12 hybrids containing a single distal fragment, 3 hybrids with a single proximal fragment, 5 hybrids with a single interstitial fragment and 3 hybrids with multiple fragments. M. Lipinski (Paris) presented data on irradiation reduced hybrids constructed using EYEF3A6. These were characterized using FISH, Southern blotting and PCR. One hybrid, DA5, contains one large and two small human chromosome fragments, one of which includes the interval from LIF to D22S29. Inter-ALU PCR was used to generate DNA from this region, and cloned into pUC18. These clones are under study. M. Budarf (Philadelphia) presented data on 22 somatic cell hybrids which subdivide chromosome 22 into 20 intervals. The highest density of breakpoints is in 22q11 which is separated into 12 regions. A number of loci have been mapped using these hybrids, including ADSL, peripheral benzodiazepine receptor, GNAZ, parvalbumin, acrosin, catach-O-methyltransferase and an expressed LINE sequence.

The fourth session was devoted to the isolation of clones from chromosome 22. J. Trofatter (Boston) isolated 3800 cosmids from three separate sources: 1) flow-sorted chromosome 22 DNA (Lawrist 5; 2700 cosmids), 2) chromosome 22-specific cell line 10888 (sCos-1; 400 cosmids) and 3) a somatic cell hybrid having chromosome 22 and some parts of chromosomes 15 and 19 (pWE15; 700 cosmids). The combined library was screened for microsatellite repeat sequences with identification of 600 (GT)_n positive clones, 140 (AG)_n positive and 40 (AAC)_n positive clones. These are being mapped to specific regions of chromosome 22 by either

FISH or a somatic cell hybrid panel. They are further screening the library with the following oligonucleotide probes: (AT)12, (AGC)7, (AAT)7, (AGG)7, (CCG)7, (AAAG)7, (AAAT)7, (AAGG)6 and (AGAT)7. P. de Jong (Livermore) presented information on two cosmid libraries constructed from sorted chromosome 22 DNA. The first was cloned into Lawrist5, arrayed and tested. It was found to be complete but only 30% of the clones were from chromosome 22. The second library has only just been completed and seems to be of 85% purity, with 10 fold redundancy for chromosome 22. These libraries have been distributed to numerous investigators.

N. Blin (Homburg) presented data on new polymorphisms for chromosome 22. J. Zucman (Paris) described a method where alu-PCR amplified DNA from defined somatic cell hybrids is used in FISH for regional chromosomal painting and for hybridization to gridded chromosome 22 cosmids. Cosmids identified by this technique were found to map in the expected regions of the chromosome. Using this method and the 31 somatic cell hybrids of G. Thomas it is possible to identify cosmids which map to any of 29 subregions of chromosome 22. M. Aubry (Montreal) described the cloning and mapping of six novel zinc-finger motif containing cosmids to chromosome 22. Two map to the short arm and four to 22q11.2. M. Sanson (Montreal) described the isolation and mapping of 45 Not1 linking clones to the long arm of chromosome 22. He estimates that this represents less than half the sites present on chromosome 22. W. Fiedler (Erlangen) described a microdissection library generated for chromosome 22q12-13.2. 27 clones were regionally mapped: 10 proximal and 17 distal to the Ewing's sarcoma translocation.

The most extensive session was devoted to the physical mapping of chromosome 22. H. McDermid (Edmonton) presented a partial long range restriction map for 22q11 which contained approximately 7,400 kb. She also presented preliminary data on the mapping of two congenital deletions of 22q13 with the proximal breakpoint near D22S29. A. Menon (Boston) presented data on the fragment sizes detected by 13 probes which are within the D22S1-D22S29 interval for Not1, Nru1, Mlu1, and other infrequently cutting enzymes. The probes D22S1 and D22S33 were found to map to the same 200 kb Sfi1 fragment. N. Blin (Homburg) showed that D22S16 and PDGFB map to the same 900kb Nru1 fragment. W. Fiedler (Erlangen) presented data on the mapping of 23 microclones from 22q12-13.1 using the enzymes Not1, Sfi1, Mlu1

and BsshII. The length of the BsshII fragments added up to approximately 5,000kb. EAN20 and LIF were located on the same NotI fragment; EAN09 and EAN78 were located on common BsshII fragments.

O. Delattre (Paris) described the use of the Lawrence Livermore cosmid library in the construction of cosmid contigs by rapid walking. A total of 1,500kb of DNA in five large contigs has been constructed in the D22S1-D22S15 interval. I. Dunham (London) and J. Collins (London) described their efforts to isolate chromosome 22 YACs. They have screened the St-Louis (average YAC size 250kb) and the ICRF (T. Monaco; average YAC size 600kb) YAC libraries using a pool of chromosome 22 probes obtained from J. Dumanski. 604 YACs have been identified by this method. They have also developed an automated system for placing 1536 YAC clones on one nylon membrane for screening by hybridization. This density will allow the entire array of chromosome 22 YACs to be included on the same filter. They have identified, by hybridization, 105 YACs for specific loc., including 51 for GGT. 26 STSs have been designed and are being used to identify cosmids. Two YACs for NAGA were found to be positive for CYP2D, suggesting a close physical association. K. Patel (London) reported the identification of a LIF YAC (200kb), a D22S15 YAC (450kb), a D22S1 YAC (900kb) and YACs for each end of the D22S1 YAC (500kb and 550kb each). This D22S1 YAC contig spans 1,800kb. These YACs were oriented by in situ hybridization using end cosmids. He also described isolation of 11 zinc finger motif containing cosmids which map, by in situ, to: 22p (2 clones), 22q11 (six clones), 22q12 (one clone) and 22q13 (2 clones). NotI linking clones are also being constructed using a PCR based strategy where Alu and NotI primers are used. Eight unique fragments have been isolated from the interval D22S1-D22S15 using irradiation reduced hybrids.

C. Denny (Los Angeles) described his investigations into the causes of co-cloning in YACs. He found that the net cross-over rate was small (3-4%), suggesting that co-ligation is the most frequent source of scrambled clones. He discussed various strategies to avoid this problem. H. Shizuya (Pasadena) presented data demonstrating the instability of cosmid clones. He then described a new cloning vector, called bacterial artificial chromosome (BAC), which is derived from the F-factor. The average insert size is 100kb and recombinant clones

containing human DNA are very stable. A library using chromosome 22 sorted DNA is being constructed. N. Shepherd (Wilmington) described a new vector system based on P1 phage. The main advantage of this system is the large insert size (average 100kb) and stability of the clones.

The seventh session was devoted to neoplasia associated with chromosome 22. B. Emanuel (Philadelphia) showed that three rhabdoid tumors and a closely related atypical teratoid tumor lost chromosome 22 by both cytogenetic and molecular methods. O. Delattre (Paris) described the cloning of the Ewing sarcoma and neuroepithelioma breakpoint. All the translocations map within a small region on chromosome 22 (a few kb) which is flanked by two HTF islands. The chromosome 22 gene had not yet been identified. M. Rutledge presented recent work with deletions in meningioma. Their findings are consistent with a meningioma locus distal to D22S28, making this a separate locus from neurofibromatosis type 2 (NF2). E. Zwarthoff (Rotterdam) presented work on a translocation in a meningioma. The translocation breakpoint is detected on pulsed field gel analysis using the probe NB129, and maps to the interval flanked by D22S1 and LIF. The same probe detected a constitutional deletion in a patient with multiple meningiomas. cDNAs are being isolated from this candidate region. The possible significance of this finding, in relation to NF2 and the more distal putative meningioma locus was discussed, though no consensus was reached. G. Rouleau (Montreal) presented genetic data which confirmed the localization of NF2 to the interval flanked by D22S1 and D22S28, and suggested that D22S32 might be a closer telomeric flanking marker. Evidence was presented strongly rejecting the hypothesis that there is non-allelic genetic heterogeneity in NF2.

The eighth and final session was devoted to the molecular definition of DiGeorge and Cat Eye syndromes. P. Scambler (London) presented arguments for reclassifying the velocardiofacial syndrome as a subtype of DiGeorge. He found deletions in 8 of 8 cases with either probe, 197 or 506, which are frequently deleted in classical DiGeorge. He also described a family where the same deletion led to DiGeorge in some family members, and velocardiofacial syndrome in others. B. Emanuel (Philadelphia) presented detailed mapping data in the DiGeorge region. The markers N25, 160B and R32, which are deleted in 14 of 14 patients, map to the interval defined by two somatic cell

hybrid breakpoints, GL5 and t(X;22). A somatic cell hybrid segregating a DiGeorge microdeletion chromosome has been constructed. Four YACs have been isolated for the locus N25 and are being studied. RFLP analysis of parents and children with DiGeorge has shown that the deletion occurred in the maternally inherited chromosome in 4 of 5 instances. S. Demczuk (Montreal) presented preliminary data on the identification of chromosome 22 homeobox containing genes as candidates for DiGeorge syndrome. H. McDermid (Edmonton) mapped a putative rearrangement breakpoint in a Cat Eye syndrome patient to within 100kb of the marker D22S72.

P. Pearson (Baltimore) described the aims and practical uses of the Genome Data Base (GDB). Many of the practical problems encountered when designing a large database which integrates genetic and physical data generated from different sources. He suggested that there should be a chromosome 22 database which is constructed so that it can interface with GDB.

Future Perspectives

It was agreed that there should be another workshop to be held in Philadelphia in one year and that Dr. B. Emanuel should be the organizer. A new set of reference markers for chromosome 22 was selected. It was felt that these loci should include very informative polymorphisms, preferably of the micro satellite type. Drs. Emanuel and Budarf volunteered to serve as a distributing lab for these new markers. (Fig. 1) The most effective method to make a complete hybrid cell panel generally available was discussed. Dr. G. Thomas agreed to make filters available of his detailed mapping panel so that any other investigator can map loci using the same reference breakpoints. G. Thomas has agreed to serve as a coordinating centre for data generated using the Lawrence Livermore cosmid library. J. Trofatter agreed to compile all data on chromosome 22 STSs. J. Collins and I. Dunham agreed to screen their YAC libraries for anyone interested in identifying a clone. The most efficient method for dissemination of information and communication within the group was discussed. There was general agreement that there should be a newsletter which would be sent to all interested persons by E-mail, preferably using the chromosome 22 bulletin board. Dr. B. Emanuel agreed to coordinate this effort.

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