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International Workshop on Chromosome 6

Final Report

for the period June 1, 1992 - May 31, 1993

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MASTER

Progress Report

The following information is submitted in support of this application. It documents that the meeting was held, and that significant benefit accrued from the meeting of investigators with similar interests related to chromosome 6. The complete publication of the workshop report will appear shortly in the journal *Cytogenetica and Cell Genetics*. Dr Andreas Ziegler (University of Berlin) will serve as the organizer for the next Chromosome 6 workshop, with Dr. Harry Orr (University of Minnesota) the co-organizer.

The First International Workshop in Human Chromosome 6 took place in Ann Arbor, Michigan (USA) from June 7 - 9, 1992. There were 68 registered participants from eight nations including Australia, Belgium, France, Germany, Ireland, Italy, United Kingdom, and the United States. The workshop was supported by the National Institutes of Health and the Department of Energy in the United States as well as by travel funds allocated to several non-US participants by the European Community through the European Office of the Human Genome Organization.

The three major goals of the meeting were the improvement of the reference marker map (number of loci and spacing in terms of genetic distances, physical localizations), the provision of up-to-date genetic and physical maps and the encouragement to exchange reagents and cell lines between participants to enhance the mapping efforts.

After Peter Pearson (Johns Hopkins University, Baltimore, Maryland) and Bronwen Loder (HUGO, London) had presented their expectations with regard to the outcome of the workshop, the afternoon of the first day was used to provide overview reports on various aspects of chromosome 6 genetics. Andreas Ziegler (Free University of Berlin, Berlin, Germany) reported on the HGM 11 meeting in London, 1991 and stressed in particular, the necessity for an improvement of the reference marker map. Howard Cann (C.E.P.H., Paris, France) reviewed the status of the genetic linkage map, and Helen Donis-Keller (Washington University, St. Louis, Missouri) presented the latest version of the NIH Index Map for chromosome 6. Since this meeting, both the C.E.P.H. Map (Weissenbach et al., 1992) and the NIH Index Map (NIH/CEPH Collaborative Mapping Group, 1992) were recently published and investigators should consult these references for specific details of genotypic data for chromosome 6. Duncan Campbell (Medical Research Council, Oxford, United Kingdom) reviewed the physical map of the HLA region, which culminated in his suggestion that about 200 expressed genes might be part of this 4000 kb. In her description of physical mapping efforts on 6p, Huda Zoghbi (Baylor College of Medicine, Houston, Texas) mainly concentrated on the contributions of her group towards the identification of the gene for one dominant form of spinal cerebellar ataxia (SCA1). She demonstrated how the combination of translocation hybrids, radiation hybrids, YACs and pulsed field gel electrophoresis can be employed to obtain a physical map around a highly polymorphic locus (D6S89) which is closely linked to the disease locus itself. John Boyle (Paterson Institute for Cancer Research, Manchester, United Kingdom) reviewed the physical mapping efforts on 6q which have to a large extent been carried out by his and Jeffrey Trent's (The University of Michigan, Ann Arbor, Michigan) group. Using panels of conventional and radiation cell hybrids, they were able to dissect 6q into several regions, thus providing a basis for further studies. Sherman Weissman (Yale University, New Haven, Connecticut) provided an overview on diseases associated with chromosome 6, in which he focused on two recently described eye disorders (autosomal dominant retinitis pigmentosa and North Carolina macular dystrophy) which have been mapped to 6p and 6q, respectively. The very likely occurrence of tumor suppressor genes on chromosome 6 were mentioned by him as well.

The evening session was organized by Francis Collins (The University of Michigan, Ann Arbor, Michigan). It was devoted to the discussion of novel strategies for long range physical mapping and the identification of transcribed sequences. Dean Nizetic (Imperial Cancer Research Fund, London, United Kingdom) gave a description of the work at the ICRF aimed at making gridded YAC and chromosome 6-specific cosmid libraries available. These libraries will surely speed up the physical mapping efforts on this chromosome. The procedures and applications of chromosome microdissection techniques were then detailed by Jeffrey Trent and Paul Meltzer (The University of Michigan, Ann Arbor, Michigan), mainly with regard to 6q (meeting/abstract 46). David Burke (The University of Michigan, Ann Arbor, Michigan) presented an excellent summary on the construction, analysis and pitfalls of YAC libraries, and Francis Collins concluded

this extremely informative session by an in-depth description of the various possibilities available today to identify transcribed regions in cloned genomic sequences.

During the remainder of the Workshop, a series of focused meetings and poster presentations were made regarding physical mapping of 6p and 6q, resources (particularly somatic cell hybrids), genetic mapping of chromosome 6, and disease loci mapping to 6. A brief summary of each of these aspects of the program is provided below.

Report on Resources of Chromosome 6 (E. Meese, University of Saar, Germany)

The significant increase in both genes and D-segments on chromosome 6 requires additional tools for regional assignment on this chromosome. Recently, two somatic cell hybrid panels have been published including both radiation and translocation hybrids. The recently published panels from Boyle et al. (1992) and Meese et al. (1992) combine to allow the differentiation of 15 regions on the long arm of chromosome 6. Also, H. Zoghbi reported on a panel of 65 radiation reduction hybrids developed by her and her co-workers which divides 6p into ten mapping intervals (meeting/abstract 20). Another radiation hybrid panel of 40 lines with comparatively small human chromosomal fragments was described by A. Volz and colleagues which they have employed to map genes to 6p (meeting/abstract 7, 18). Boyle et al. reported on the generation of a further radiation panel consisting of 75 hybrids (meeting/abstract 31), and Trent and co-workers also reported the development of a panel of radiation reduction hybrids for 6q (meeting/abstract 45).

A second major resource needed for mapping chromosome 6 is the generation of chromosome specific libraries. Besides two ATCC libraries (LL06NS01 and LA06N501), there is a gridded cosmid library established by Lehrach and co-workers (pers. com.). Shortle et al. reported the construction of a cosmid library from the human/hamster hybrid 4J4 that contains HLA class I and 6q27 sequences (meeting/abstract 4). Zoghbi et al. established a library for the short-arm of chromosome 6 (pers. com.). At present, there are no chromosome 6 specific YAC libraries available.

In addition, physical mapping on chromosome 6 will be facilitated by somatic cell mutants bearing various DNA-rearrangements. Panels of somatic cell mutants have been reported by Figaira et al. (meeting/abstract 30) and Ziegler and co-workers (Volz et al., 1992).

In summary, there is a significant shortage of chromosome 6 specific libraries. However, there are now somatic cell hybrid mapping panels that have recently been developed and allow differentiating 10 regions on 6p and 15 regions on 6q. In addition, a large number of radiation hybrids have very recently been established and will contribute to more detailed mapping intervals.

6p Physical Mapping Panel (D. Campbell, Oxford; A. Ziegler, Berlin)

Fifteen abstracts (no. 1-6, 7-14, 18) dealt with mapping of genes in the HLA complex or its close vicinity. Several groups have now developed overlapping YACs covering most of the HLA region and the search for new genes has begun, in particular in the class I region. The mapping of an expressed tubulin β -chain gene (TUBB) between HLA-C and HLA-E was reported (meeting/abstract 18). The gene for hemochromatosis (HFE) has still not been found despite considerable effort. One approach has been to map the region around HLA-A and define novel transcribed sequences, another to look for candidate genes with available probes. One of these, FTHP1, has been mapped centromeric of HLA and thus cannot be regarded as a potential disease gene anymore. However, it appears possible that another ferritin heavy chain gene on 6p exists (meeting/abstract 9).

As mentioned previously, groups in the USA and Europe have tried to localize (meeting/abstract 7, 15-17, 19-21) SCA1 indirectly by physical mapping of other closely linked loci. For a further locus involved in a

neurological disorder, juvenile myoclonic epilepsy (EJM1), no physical mapping data is currently available, but the locus has definitely been linked to HLA, most likely telomeric. Attempts to generate a physical map of 6p centromeric of HLA have also been initiated (meeting/abstract 35). The results further strengthen the well established synteny between parts of 6p and mouse chromosome 17. Apart from the molecular analysis of retinitis pigmentosa, some regions of 6p could also be involved in specific translocations in some tumors as reported at this meeting (meeting/abstract 27, 28).

6q Physical Mapping Panel (J. Boyle, Paterson Institute for Cancer Research)

A major focus of this meeting was to provide interaction on new probes, and refinements in probe localization to 6q. This is because historically the primary knowledge base on physical mapping has focused on 6p (due in large part to the presence of the HLA region). Table I lists the summary of new and reassigned probes on 6q. Briefly, chromosome 6q acquired one new disease locus (MCDR1, North Carolina macular dystrophy) at 6q14-q16.2 and one new surface antigen (M6P2 defined by monoclonal antibody MARB15) at 6q12-q14. Several other genes were located or regionally refined by in situ hybridization, including the cannabinoid receptor (CNR) at 6q14-q15, MYB at 6q23.3, ESR at 6q25.1 and SOD2 at 6q25.2. Thirteen RFLP loci were either newly localized or relocated. Seventeen new dinucleotide microsatellites were mapped to 6q by hybrids, including one spanning the centromere region 6p21.1-q12. Twelve cosmid contigs derived from a radiation reduced hybrid, 4J4, were mapped (1 to 6q16-q21 and 11 to 6q27) by in situ hybridization using representative clones from each contig. Region 6q27 now contains more than 100 reported markers.

Genetic Mapping (H. Zoghbi, Baylor University; H. Cann, C.E.P.H.)

The genetic linkage subcommittee reviewed the status of the genetic map of chromosome 6. The genetic map of chromosome 6 is based on genotypic data analyzed in the C.E.P.H reference families. At the time of the meeting, approximately 87 polymorphisms were mapped to chromosome 6 and had been analyzed in these C.E.P.H. families. Approximately 20 of these markers have heterozygosities approaching or exceeding 70%, and 13 of these polymorphisms are of the dinucleotide repeat type. Forty-four markers with more than 200 informative meioses had been genetically mapped using multipoint linkage analysis. The genetic map of the short-arm of chromosome 6 has a resolution of approximately 10 cm or less. The genetic map of 6q on the other hand has major gaps in the 6q14-q24 region. This latter problem should be corrected as nine new dinucleotide repeat polymorphisms have been recently localized by multipoint linkage analysis (Wilkie et al., 1992). Data on these markers have been deposited in the C.E.P.H. data base and efforts to localize many of these markers on 6q are in progress. The reader is again referred to the two recent publications of the C.E.P.H. (Weissenbach et al., 1992) and NIH Index Map (NIH/CEPH Collaborative Mapping Group, 1992) for the most current genetic mapping information on chromosome 6. The consensus map for chromosome 6, at the time of the meeting, is provided in Table III.

Table I. New markers or reassignments to 6q

Location	Locus	Probe	Method	Previous Assignment	Reference	Comment
p21.1-q12		p47	Hybrids	-	Abstract 32	CA (n)
q12	D6S150	cC16-28	In situ	6	Abstract 36	RFLP
q12-q14	M6P2	-	Hybrids	-	Abstract 37	150kD surface protein defined by monoclonal antibody MARB15
q13		mfd 183	Hybrids	-	Wilkie et al.	CA(n)
q14-q15	CNR	HSKR6p5	In situ	-	Abstract 39	Cannabinoid receptor
		p8	Hybrids	-	Abstract 32	CA(n)
		p11	Hybrids	-	Abstract 32	CA(n)
		p22	Hybrids	-	Abstract 32	CA(n)
q14-q16.2		mfd131	Hybrids	-	Wilkie et al.	CA(n)
		mfd171	Hybrids	-	Wilkie et al.	CA(n)
		mfd97	Hybrids	-	Wilkie et al.	CA(n)
	NCMD (mcdrl)		Linkage	-	Abstract 24	North Carolina Macular Dystrophy
q15-q21		p1	Hybrids	-	Abstract 32	CA(n)
		p3	Hybrids	-	Abstract 32	CA(n)
q16.21		4J4 VS-152	In situ	-	Abstract 40	Cosmid contig C4J4 VS-11
		4J4 VS-228	In situ	-	Abstract 40	Cosmid contig C4J4 VS-11
q21-q25.2	ARG1	mfd91	Hybrids	q23	Wilkie et al.	CA(n)
	D6S87	mfd47	Hybrids	-	Wilkie et al.	CA(n)
		mfd118	Hybrids	-	Wilkie et al.	CA(n)
q21-qter		mfd226	Hybrids	-	Wilkie et al.	CA(n)
q22.3	D6S32	p3-B10	In situ	6	Abstract 36	RFLP
q23.3	MYB	pCM8	In situ	q22-q23	Abstract 36	RFLP
q24	D6S33	CRI-L994	In situ	q21-q27	Abstract 36	RFLP
	D6S85	CRI-LF0246	In situ	q22-q23	Abstract 36	RFLP
q25.1	ESR	pYAC4ESR	In situ	q24-q27	Abstract 32	CA(n) from pYAC4ESR
		ESR 1	In situ	-	Abstract 32	
q25	SOD2	pHnS04	In situ Hybrids	q21	Church et al.	
q25.2-qter		mfd181	Hybrids	-	Wilkie et al.	CA(n)
q25.3	D6S43	Zb 6-1	In situ	q21-q27	Abstract 36	RFLP
q26	D6S37	pJc230	In situ	q27	Abstract 36	RFLP

Table continued on next page.

Table I (continued)

Location	Locus	Probe	Method	Previous Assignment	Reference	Comment
q27	D6S2	p2-2	In situ	p21-qter	Abstract 36	RFLP
	D6S22	CRI-1077	In situ	6q	Abstract 36	RFLP
	D6S25	CRI-T22	In situ	6q	Abstract 36	RFLP
	D6S201	cC16-184	In situ	6	Abstract 36	RFLP
	D6S148	cC16-23	In situ	6	Abstract 36	RFLP
	D6S170	cC16-63	In situ	6	Abstract 36	RFLP
	D6S21	CRI-L1065	In situ	q27	Abstract 36	Confirmed locations RFLP
	D6S39	pJHH-5	In situ	q27	Abstract 36	Confirmed locations RFLP
	D6S44	pYNZ132	In situ	q27	Abstract 36	Confirmed locations RFLP
	D6S48	pMC0B12	In situ	q27	Abstract 36	Confirmed locations RFLP
	D6S132	CEB-3	In situ	q27	Abstract 36	Confirmed locations RFLP
	D6S133	CEB-4	In situ	q27	Abstract 36	Confirmed locations RFLP
		p12	Hybrids	-	Abstract 32	CA(n)
	11 Cosmid Contigs		In situ	-	Abstract 40	From radiation hybrid 4J4
	<u>Contig #</u>	<u>Clone #'s</u>				
	c4J4VS-1	4J4VS-50,60, 66,117,148,229	"	-	"	"
	c4J4VS-3	4J4VS-16,19, 49,59,282	"	-	"	"
	c4J4VS-5	4J4VS-34,35, 56,37,61,278	"	-	"	"
	c4J4VS-6	4J4VS-265,281	"	-	"	"
	c4J4VS-7	4J4VS-22,23	"	-	"	"
	c4J4VS-9	4J4VS-45,175	"	-	"	"
	c4J4VS-10	4J4VS-1,115 135,156,224, 227,249,261	"	-	"	"
	c4J4VS-12	4J4VS-133,251, 255	"	-	"	"
	c4J4VS-13	4J4VS-100,161	"	-	"	"
	c4J4VS-18	4J4VS-118,123	"	-	"	"

Table II. Chromosome 6 STRP linkage Map*

Locus ^a	Marker	Sex-equal cM ^b	Cytogenetic position ^c	Het.
D6S105	Mfd61	0	p	0.80
	Mfd183	43.1	q13	0.66
	Mfd131	51.7	q14-q16.3	0.78
	Mfd 171	60.6	q14-q16.3	0.63
	Mfd97	64.8	q14-q16.3	0.51
ARG1	Mfd91	94.1	q21-q23.3	0.45
D6S87	Mfd47	96.9	q21-q23.3	0.61
	Mfd118	106.5	q21-q23.3	0.86
	Mfd226	124.2	q24-25.2	0.75
	Mfd181	135.3	q25.2-qter	0.66

^aD-numbers are in the process of being assigned for remainder of markers.

^bCRIMAP was used to construct linkage maps. All orders were supported by odds of 1,000:1 or greater. All markers were typed through 40 C.E.P.H. families.

^cBased on hybrids described in Meese, et al. (1992), Genomics 12:542-548.

*(from Wilkie et al., 1992)

Table IIIA. 6p consensus map.

#	Locus	Probe	Enzyme	Het	Lab #	Theta	Odds
1	D6S7	p7H4	EcoRV	.30	20,2	.162	
2	F13A1	F13A1	Haplotype	.90	23,2	.123	
3	EDN1		PCR(CA) _n			.076	
4	D6S89	D6S89	PCR(CA) _n	.91	23	.071	
5	D6S109	LR15	PCR(CA) _n	.79	65	.026	4:1
6	D6S88	1-10-2	EcoRI	.59	65	.085	
7	D6F14S1	OL43	EcoRI	.25	20	.05	
8	D6S8		MSPI			.02	
9	D6S105					.02	
10	HLA-A	HLA	A	.82	2	.008	
11	HLA-B	HLA	B	.95	2	.010	
12	HLA-DRB1	HLA	DR	.85	2	.042	
13	D6S29	pHHH157	BamHI	.49	20,23	.058	
14	GLO1	GLO1	protein	.58	20,2	.062	

Table continued on next page.

Table IIIA (continued)

#	Locus	Probe	Enzyme	Het	Lab #	Theta	Odds
15	D6S19	CRI-L171	TaqI	.65	23,1	.090	
16	TCTE1	TCTE1	GT	.83	23	.040	
17	D6S90	HZ71	BclI	.37	23	.052	2:1
18	D6S41	pEFD6	PstI	.30	20	.001	3:1
19	D6S41	pEFD6	BglII	.06	20	.001	
20	D6S23	CRI-R368	MspI	.52	1	.001	4:1
21	D6S23	CRI-R368	TaqI	.41	1	.029	
22	(D6S4,MUT)	4c11,mmcn	Haplotype		2,20,23		
23	PGK1P2	pGK4	EcoRV	.55	2	.037	
24	KRAS1P	J819	TaqI	.56	2,1,20	.047	
25	D6Z1	308	TaqI	.50	63	.001	
26	D6Z1	308	BamHI	.49	63		

Table IIIB. 6q consensus map.

#	Locus	Probe	Enzyme	Het	Lab #	Theta	Odds
25	D6Z1	308	TaqI	.50	63		
26	D6Z1	308	BamHI	.49	63	.001	
27	CNR					.123	
28	D6S30	B3.6	MspI	.55	20	.054	
29	D6S32	p3B10	MspI	.65	20	.377	
30	MYB	pHM2.6	EcoRI	.61	24,20	.029	
31	TCP1	pB14hum	PstI	.25	2	.305	
32	PLG	VI-56-5	SacI	.39	2	.001	1:1
33	SOD2	phMnSOD4	TaqI	.41	20	.035	
34	D6S133	CEB4	HinfI	.99	46	.112	
35	TCP10	T66	PvuII	.58	40	.011	
36	TCP10	2T66	PvuII	.33	40	.001	1:1
37	D6S37	pJCZ30	HindIII	.73	20	.025	9:1
38	D6S83	LILA-2	PstI	.46	20		
39	D6S2	p2-2	PvuII	.60	20	.011	
40	D6S21	CRI-L1065	RsaI	.74	1	.099	
41	D6S44	pYNZ132	TaqI	.69	20	.017	
42	D6S132	CEB3	HinfI	.94	46	.048	
43	D6S39	pTHH5	HindIII	.55	20	.012	
44	D6S48	pMCOB12	TaqI	.76	20	.241	

A vertical line indicates polymorphic markers contained within the same locus

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