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An Informative Panel of Somatic Cell Hybrids for Physical Mapping on Human Chromosome 19q

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Summary

A panel of 22 somatic cell hybrids divides the q arm of human chromosome 19 into 22 ordered subregions. The panel was characterized with respect to 41 genetic markers. In most cases, a single fragment of chromosome 19 was present in each hybrid. In two cell lines the presence of multiple fragments of the chromosome was demonstrated by segregation of these fragments in subclones. On the basis of the results of marker analysis in this panel, the most likely order of the markers tested is MANB-D19S7-PEPD-D19S9-GPI-C/EBP-TGFB1-(CYP2A,BCKDHA,CGM2,NCA)-PSG1-(D19S8,XRCC1)-(ATP1A3,D19S19)-(D19S37,APOC2)-CKM-ERCC2-ERCC1-(D19S116,D19S117)-(D19S118,D19S119, D19S63,p36.1,D19S112,D19S62,D19S51,D19S54, D19S55)-pW39-D19S6-(D19S50,TNNT1)-D19S22-(HRC,CGB,FTL,PRKCG)-qter. This gene order is generally consistent with published physical and genetic mapping orders, although some discrepancies exist. By means of a mapping function that relates the frequency of cosegregation of markers to the distance between them, estimates were made of the sizes, in megabases, of the 19q subregions. The relative physical distances between reference markers were compared with published genetic distances for 19q. Excellent correlation was observed, suggesting that the physical distances calculated by this method are predictive of genetic distances in this region of the genome and, therefore, are just as useful in estimating relative positions of markers.

Introduction

Several somatic cell hybrid panels for physical mapping on human chromosome 19 have been described. The earliest of these panels consisted of only a few cell lines and divided chromosome 19 into three zones—the p and q terminal regions and the large central region p13.2-q13.2, which contained the majority of assigned genes (Worwood et al. 1985). Two of these early hybrids were derived from a single X;19 translocation and contained the derivative chromosomes (Xpter→Xq22::19q13.3→qter) and (Xqter→Xq23-25::19q13.3→19pter). The third hybrid was derived from a translocation involving 19p13, a common breakpoint region in acute lymphocytic leukemia. This hybrid contains the derivative chromosome (Xqter→Xcen-q13::19p13→qter).

More recently, additional informative translocations involving chromosome 19 have been described, and hybrids useful for physical mapping have been constructed (Hulsebos et al. 1986; Lusis et al. 1986; Brook et al. 1987, 1991; Boyd et al. 1988; Korneluk et al. 1989*b;* Schonk et al. 1989), which, when taken together, identify a total of 17 independent breakpoints. However, because of the nonrandom distribution of the breaks relative to the markers used to characterize them, these hybrids divide the chromosome into only nine subregions (reviewed by Brook et al. [1991]).

Somatic cell hybrids made between DNA repair-deficient Chinese hamster ovary (CHO) cell mutants and human cells (Thompson 1989) contain spontaneously

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generated fragments of human chromosome 19 (Siciliano et al. 1986) that are large enough to enable ordering of regions by inspection (as in translocation hybrids) yet, when taken as a panel, have enough randomly distributed breakpoints to enable ordering of many markers at distances of one to a few megabases and to estimate the physical size of the regions by a technique similar to that used in radiation hybrid mapping. These hybrids have been used to assign several markers regionally on chromosome 19 (Stallings et al. 1988; Bachinski and Siciliano 1989; Thompson et al. 1989*b*; McCauliffe et al. 1990; Hendricks-Taylor et al. 1992). The panel has been expanded by the addition of cell lines 908K1 (Hulsebos et al. 1986), GM89A9C9-7 (Hellkuhl and Grzeschik 1978), and 2F5 (Brook et al. 1992). Here, we present the results of characterizing the panel with a total 41 chromosome 19 q-arm markers and using the results to define marker order and to estimate physical distances in the region.

Material and Methods

Chromosome 19 Regional Mapping Panel

Somatic cell hybrids made between DNA repair–deficient CHO cell mutants and human lymphocytes or fibroblasts were constructed by L.H.T., at Lawrence Livermore National Laboratory. Hybrids designated "9HL" are derived from the strand-break mutant EM9 (Siciliano et al. 1986). Hybrids designated "5HL" and "135HL" are derived from the excision-repair mutants UV5 and UV135, respectively (Thompson et al. 1987, 1989*b*). Hybrids designated "1SHL" are derived from CHO cell mutant irs1SF (Fuller and Painter 1988). These hybrids represent fusions of the CHO cell mutants with normal human lymphocytes. In addition, hybrids designated "41XP" and "20XP" (Thompson et al. 1985*a*, 1985*b*) were derived from fusions between excision-repair mutants UV41 and UV20, respectively, and various xeroderma pigmentosum (XP) fibroblasts. Hybrids are named descriptively so as to indicate fusion partners, independent clone number, and, where applicable, subclone. For example, hybrid 20XP3542-1-4 is derived from a fusion between UV20 and the XP line GM3542; it is subclone 4 of the independent clone 1.

The human chromosome 19 content of these hybrids was determined by testing them by hybridization, amplification, and isozyme analyses, for the presence or absence of over 40 known markers. A total of 35 hybrids were identified in which chromosome 19 was broken, as evidenced by the presence of some but not all of the markers tested. Of these, 18 hybrids were informative for the q arm of chromosome 19. In addition to the somatic cell hybrids derived from CHO DNA-repair mutants, translocation-derived hybrids 908K1 (Hulsebos et al. 1986) and GM89A9C9-7 (Hellkuhl and Grzeschik 1978) and hybrid 2F5 (Brook et al. 1992), which contains 1–2 Mb of DNA from 19q13.3 and is a radiation hybrid derived from 20XP3542-1-4 (Stallings et al. 1988), were added, bringing the total size of the 19q panel to 22 cell lines.

Analysis of Markers in Hybrids

A. Southern blotting procedures.—Genomic DNA samples for Southern blot analysis were prepared in 80-μl agarose blocks containing 5.0×10^5 cells/block and were processed according to published procedures (Carle et al. 1986; van Ommen and Verkerk 1986). For each sample digested, one-half block was rinsed four times for 15 min each time in water and was equilibrated for 30 min in 500 μl of 1× restriction buffer with spermidine (2 mM) and BSA (0.1%). Buffer was removed, blocks were melted briefly at 65°C, and restriction digestion was carried out for 3 h with 40 units of enzyme (*Hin*dIII, *Eco*RI, or *Pst*I). Molten samples were then loaded into slots in a 20 × 20-cm^2 0.8% agarose gel and electrophoresed at 30 V for 18 h.

DNA was transferred to Zetabind hybridization membranes (AMF Cuno, Meridian, CT) according to the manufacturer's suggested procedures. Hybridizations were performed at 65°C in a Hybaid hybridization oven. Hybridization buffer and washing were according to the method of Amasino (1986). Autoradiography was on Kodak X-AR film for 1–10 d at −70°C. Probes used for Southern blot analysis of these hybrids are described in table 1.

B. PCR and verification of results.—Slow troponin T (*TNNT1*), histidine-rich calcium-binding protein (*HRC*), and pKE0.6 (*D19S117*) were mapped by PCR. Hybrid DNA for PCR was obtained by washing the 80-μl agarose blocks in water, as described above, melting them at 65°C, and diluting with water to 500 μl. DNA amplification with *Taq* DNA polymerase was performed on 10-μl aliquots of diluted block, and products were visualized on 1% agarose gels.

Primers for determination of *HRC* were designed from a published cDNA sequence (Hofmann et al. 1991). The sense primer represented nucleotides 515–538, the antisense primer nucleotides 636–655, priming amplification of a 140-bp fragment. Primer sequence for pKE0.6 (*D19S117*) was provided by Dr. R. Korne-

Table 1

Probes Used in Marker Analysis for Determination of Gene Order on Chromosome 19

Probe Name	Gene	Gene Name	Reference
LDLR-2HH1	LDLR	Low-density-lipoprotein receptor	Yamamoto et al. 1984
pHP450(1)	CYP2A	Cytochrome P450	Phillips et al. 1985
pBas	TGFB1	Transforming growth factor beta	Derynck et al. 1985
APOC2-cDNA	APOC2	Apolipoprotein C2	Jackson et al. 1984
pHMCK	CKM	Creatine kinase muscle form	Perryman et al. 1986
pBR322-cβHCG	CGB	Chorionic gonadotropin beta	Fiddes and Goodman 1983
pLF108	FTL	Ferritin light chain	Boyd et al. 1985
pATP1A3	ATP1A3	Na-K ATPase alpha subunit	Yang-Feng et al. 1988
E1a	BCKDHA	Branched-chain keto acid dehydrogenase	Crabb et al. 1989
pcD4a4	ERCC1	Excision-repair cross-complementing 1	Westerveld et al. 1984
pE12-12	ERCC1	Excision-repair cross-complementing 1	van Duin et al. 1986
pKER2	ERCC2	Excision-repair cross-complementing 2	Weber et al. 1988
pER2-6	ERCC2	Excision-repair cross-complementing 2	Weber et al. 1988
pXR1-30	XRCC1	X-ray repair cross-complementing 1	Thompson et al. 1990*b*
CGM2	CGM2	CEA gene family member 2	Thompson et al. 1989*a*
Clone 9	NCA	Nonspecific cross-reacting antigen	Zimmermann et al. 1988
PSG1a	PSG1	Pregnancy-specific glycoprotein 1a	Zimmermann et al. 1989
C/EBP	C/EBP	CAAT/enhancer-binding protein	Hendricks-Taylor et al. 1992
PRKCG	PRKCG	Protein kinase C-gamma subunit	Ohno et al. 1988
pBam34	D19S6	Anonymous DNA sequence	Yamaoka et al. 1985
p4.1H2	D19S7	Anonymous DNA sequence	Shaw et al. 1986
p17.1SH1	D19S8	Anonymous DNA sequence	Shaw et al. 1986
p1J2	D19S9	Anonymous DNA sequence	Shaw et al. 1986
LDR152	D19S19	Anonymous DNA sequence	Bartlett et al. 1987
pEFD4.2	D19S22	Anonymous DNA sequence	Nakamura et al. 1988
pα1.4	D19S37	Anonymous DNA sequence	Korneluk et al. 1989*b*
pEWRB1.1	D19S50	Anonymous DNA sequence	Korneluk et al. 1989*a*
p134c	D19S51	Anonymous DNA sequence	Johnson et al. 1989
pD8	D19S62	Anonymous DNA sequence	Brook et al. 1990*b*
pD10	D19S63	Anonymous DNA sequence	Brook et al. 1990*a*
pX75B	D19S112	Anonymous DNA sequence	Hermens et al. 1991
pKE2.1	D19S116	Anonymous DNA sequence	Tsilfidis et al. 1991*a*
pKE0.6	D19S117	Anonymous DNA sequence	Shutler et al. 1991
pKEX0.8	D19S118	Anonymous DNA sequence	Tsilfidis et al. 1991*b*
pKBE0.8	D19S119	Anonymous DNA sequence	Korneluk et al. 1991
p36.1	Unassigned	Anonymous DNA sequence	Harley et al. 1991
p219	D19S54	Anonymous DNA sequence	Johnson et al. 1990
p242	D19S55	Anonymous DNA sequence	Johnson et al. 1990
pW39	Unassigned	Anonymous DNA sequence	Present report

luk and resulted in amplification of a 510-bp fragment. Primers for *TNNT1* were provided by Dr. L. Kedes, of University of Southern California. After visualization of amplification products on 1% agarose gels, the DNA was transferred to Zetabind membranes and hybridized to the labeled control amplification product in order to verify identity of the products obtained from the hybrid cells.

C. Isozyme analysis.—Isozyme analysis was carried out to detect the presence of the human form of *MANB* (α-mannosidase, ß-subunit), *PEPD* (peptidase-D), and *GPI* (glucose-phosphate isomerase), after starch gel electrophoresis as described elsewhere (Siciliano and White 1987).

Mathematical Analysis of Breakage Frequency

The algorithm chosen to generate a distance metric is that of Chakravarti and Reefer (1992) and is derived from that used by Goss and Harris (1975) and by Cox et al. (1990). Since the gene order in these hybrids had already been determined, the formula was applied to the calculation of distance between adjacent marker

groups only. According to Chakravarti's mapping function, an additive distance metric, θ, is derived by the formula $\theta = 1-e^{-w}$, where w is equal to the number of independent breaks between any two markers. This mapping function was derived from the Poisson distribution and is similar to functions used for genetic mapping. θ can vary from a value of 0 (no breaks and therefore complete linkage) to 1 (an infinite number of breaks and therefore no linkage). Values of θ were calculated for one to five breaks (the numbers seen in these panels).

The frequency of breakage (number of breaks divided by number of cell lines counted) between each adjacent marker pair was calculated and multiplied by the appropriate θ to arrive at a probability for breakage at that point. These probabilities were normalized over the approximate physical length of the region under consideration (19q) to arrive at a megabase estimate for each interval. The approximate physical length was calculated on the basis of estimated karyotypic measurements, which found chromosome 19 to be 2.2% of the haploid genome (Hulten 1974). The approximate physical length, in megabases, of the region was calculated by multiplying the genome size (3,300 Mb) by the relative length of chromosome 19 (2.2%), resulting in a size of about 72.6 Mb for the whole chromosome. Chromosome 19 is roughly metacentric, so the q arm was estimated to be about 50% of the chromosome, or about 36.3 Mb. Since the scope of the distance analysis includes only the markers *D19S7* (19q12) through *D19S22* (q13.4), the distance involved is slightly less than the total length of the arm. Use of 36.3 Mb as a total distance would thus have resulted in an overestimate of the physical distances. An arbitrary correction of 12% (or about one-half a chromosome band) was applied, and the distances were calculated on the basis of a total length of 32.2 Mb. All calculations were done on a Compaq computer using the program Lotus 123, release 2.3.

Results

Marker Analysis to Determine Gene Order on Chromosome 19

The somatic cell hybrid panel was tested for the presence of five DNA markers assigned to proximal 19q—*CYP2A, TGFB1, C/EBP, D19S9,* and *D19S7*—and for the isozymes *MANB, PEPD,* and *GPI*. The results of these determinations are presented in table 2. Although the panel was not informative for markers located on 19p, the marker *LDLR* (19p13.2-p13.1) was included in this table to demonstrate segregation of markers on 19p from those on proximal 19q in this panel. These results helped define the position of *MANB* as the most proximal q-arm marker tested. The breakpoints distal to *MANB* define seven subregions in 19cen-q13.1. In sev-

Table 2

Segregation of Markers in Region 19cen-19q13.1

	Presence or Absence of Marker Loci in Hybrids[a]								
Cell Line	LDLR	MANB	D19S7	PEPD	D19S9	GPI	C/EBP	TGFB1	CYP2A
1SHL9	+	+	+	+	+	−	−	−	−
9HL6	−	+	+	+	+	+	ND	+	+
9F350	−	+	+	UD	+	+	+	+	+
1SHL25	−	+	+	+	+	+	+	+	+
20HL-21-4-25	−	+	+	UD	+	+	+	+	+
9HL9	−	−	+	+	+	+	ND	+	+
135HL30	−	−	+	+	+	+	+	+	−
20XP3542-2	−	−	−	+	+	+	+	+	+
20XP3542-1	−	−	−	+	+	+	+	−	−
41XP3542-2-20	−	−	−	UD	+	+	−	−	−
20XP3542-2-10	−	−	−	+	+	−	−	−	−
1SHL31	−	−	+	+	−	−	ND	−	−
9HL5	−	ND	+	UD	+	+	+	+	+
20XP2991-3-1	−	ND	−	+	+	+	ND	−	−
9HL1-1	−	ND	−	−	−	−	−	+	+

[a] A plus sign (+) denotes that the marker is present in hybrid; a minus sign (−) denotes that the marker is absent; UD = undetectable; and ND = not done.

eral cases, *PEPD* (tested by isozyme analysis) was negative, although *D19S7* and *D19S9* were positive. The evidence that these markers flank *PEPD* comes both from this study and from others (Hulsebos et al. 1986; Brook et al. 1987; Schonk et al. 1989). It must be concluded that a negative *PEPD* result by isozyme analysis does not necessarily mean that the gene is not present, merely that it is not expressed at a level detectable in the hybrid cell. This is indicated in the table as "UD" (undetectable).

Twelve DNA markers assigned to 19q13.1-13.2 were tested: *CYP2A, BCKDHA, NCA, CGM2, PSG1, XRCC1, D19S8, D19S19, ATP1A3, D19S37, APOC2,* and *CKM*. Results for those hybrids that were informative for the region are shown in table 3. The letter "B" in the table indicates that a hybrid contains a chromosome 19 that is broken in the region covered by that probe. Six classes of breakpoints are seen, defining four additional regions.

Although all members of the pregnancy-specific glycoprotein (PSG) gene family detected by the PSG1a probe have been mapped to an approximately 500-kb region of chromosome 19 (Ropers et al. 1992), hybrid 20XP3542-2 showed an altered hybridization pattern (many missing bands) with this probe, whereas normal patterns were seen with the closely linked probes *NCA* and *CGM2* (Thompson et al. 1989*a*, 1990*a*). The results are shown in figure 1. In addition, the hybrid 5HL9-4, which is monochromosomal for human 19, showed the same pattern of hybridization bands as did the human control, when probed with PSG1a. These results suggested that the chromosome fragment containing this marker is truncated in hybrid 20XP3542-2, with the PSG gene family marking the most telomeric end of the region. This placement of *PSG1* is consistent with that recently reported by the International Workshop on Human Chromosome 19 (Ropers et al. 1992). The exact nature of the deletion or rearrangement present in this hybrid has not been determined; nevertheless, the altered hybridization pattern must represent some physical rearrangement and thus mark the most distal boundary of the contiguous fragment containing the markers from this region that are present in this line.

Fourteen markers from the region surrounding the myotonic dystrophy locus at 19q13.3 were tested. These included *ERCC2, ERCC1, D19S116, D19S117, D19S118, D19S119, D19S54, D19S55, D19S63, D19S62, D19S112, D19S51,* and unassigned markers p36.1 and pW39. Results for informative hybrids are shown in table 4. Breakpoints define five regions distal to *CKM*.

Table 5 shows the results of marker analysis in the 19q13.4 region. DNA markers included *D19S6, D19S50, D19S22, FTL, CGB,* and *PRKCG*. The marker groups represented by *D19S51* and pW39 (19q13.3) are included in this table to illustrate that the q13.4 region of the chromosome is separated from more proximal regions in all of these hybrids. Five more breakpoint classes, defining four additional regions, are seen in this table.

The most conservative interpretation of the segrega-

Table 3

Segregation of Markers in Region 19q13.1-13.2

	Presence or Absence of Marker Loci in Hybrids[a]					
Cell Line	CGM2 NCA BCKDHA CYP2A	PSG1	D19S8 XRCC1	D19S19 ATP1A33	D19S37 APOC2	CKM
9HL1-1	+	+	+	+	+	+
908K1	+	+	+	+	+	B
20XP3542-2	+	B	−	+	+	+
20XP3542-2-10	−	−	−	+	+	+
20XP2992-1	−	−	−	−	+	+
20XP0435-2	−	−	−	−	+	+
20XP3542-1-4	−	−	−	−	+	+
GM89A9C9-7		−	−	−	−	B

[a] Markers segregating together are grouped across the top. A plus sign (+) denotes that the marker is present in hybrid; a minus sign (−) denotes that the marker is absent; and B = cell line is broken in the region.

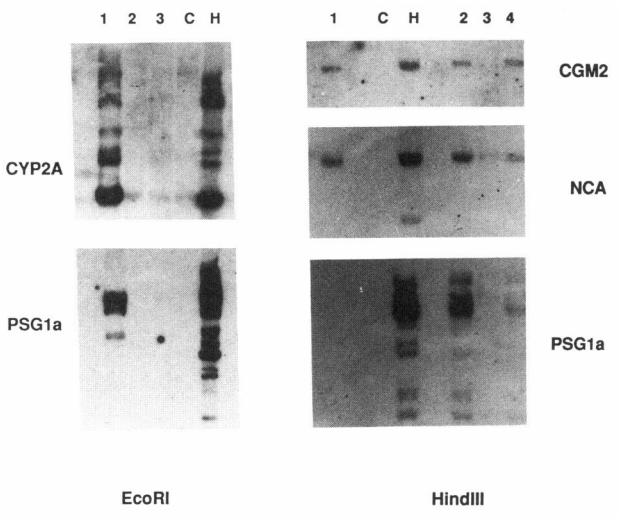

Figure 1 *Left, Eco*RI-cut DNA probed with CYP2A (*top*) and PSG1a (*bottom*). Lane H, Human control HeLa. Lane C, Hamster control CHO. Lane 1, Hybrid 20XP3542-2 showing a normal human pattern with the CYP2A probe but an abnormal pattern (on the same filter) with the PSG1 probe. Lanes 2 and 3, Hybrids that do not contain this region of the chromosome. *Right, Hin*dIII-cut DNA probed consecutively with PSG1a, NCA, and CGM2. Lane 1, Hybrid 20XP3542-2. This hybrid exhibited the normal human chromosome 19 pattern with the NCA and CGM2 probes (when compared with the patterns seen for the monochromosomal 19 hybrid 5HL9-4) but showed essentially no hybridization to PSG1a. Lane 2, Monochromosomal 19 hybrid, 5HL9-4. This hybrid does not show the smaller band seen in HeLa with probe NCA. It must be concluded that this band does not represent a sequence on chromosome 19. Hybrid 5HL9-4 shows the same hybridization pattern for the CGM2 and PSG1a probes as does HeLa, although less intensely. Lanes 3 and 4, Hybrids only weakly positive for all three markers.

tion data demands that only a single piece of the chromosome be present in each hybrid. In the case of hybrids 20XP3542-1 and 20XP3542-2, however, this did not appear to be the case (see table 4). It was not possible to reconcile the pattern of marker retention in these hybrids with the assumption that a single piece of the chromosome is retained. These two hybrids were subcloned by limiting dilution, and subclones (8 for 20XP3542-1 and 11 for 20XP3542-2) were retested for a number of markers. A comparison of the markers in the parent line with those retained in the subclones revealed the segregation of chromosomal fragments in subclones (data not shown), confirming that the original hybrid was probably a mixed culture in which individual cells retained different chromosome 19 fragments.

Figure 2 depicts the most probable chromosome 19 content of the most informative cell lines. In all, the somatic cell hybrids described here divide human chromosome 19 into 23 subregions, or intervals, on the basis of marker cosegregation. As many as 22 of these regions are on the q arm. The position of the centromere, with respect to the most proximal markers, is not certain, but solely on the basis of the pattern of marker cosegregation seen in this panel, the following order for the markers themselves is suggested: *MANB–D19S7–PEPD–D19S9–GPI–C/EBP–TGFB1–(CYP2A, BCKDHA,CGM2,NCA)–PSG1–(D19S8,XRCC1)–(ATP1A3,D19S19)–(D19S37,APOC2)–CKM–ERCC2–ERCC1–(D19S116,D19S117)–(D19S118,D19S119, D19S63,*p36.1,*D19S112,D19S62,D19S51,D19S54, D19S55)*–pW39–*D19S6–(D19S50,TNNT1)–D19S22–(HRC,CGB,FTL,PRKCG)*–qter.

Estimation of Physical Distances, in Megabases, on 19q

By means of the panels described above, the physical distances between markers on human chromosome 19 were estimated on the basis of the frequency of independent breaks in somatic cell hybrids. In order not to violate the assumption that the chromosome involved in the breakage event is unrearranged, only hybrids derived by direct fusion with *normal* human cells were included in the panel used to estimate distance. Thus, the two hybrids 908K1 and GM89A9C9-7 derived from translocation chromosomes and the radiation hybrid 2F5 were not included in this break count. In order to avoid counting the same break twice, only independent breaks were counted; that is, a hybrid and its derived subclone were both counted only if they exhibited a *different* segregation pattern for a marker pair; otherwise, only the parent line is included in the count. Since no hybrids were informative for 19p (data not shown), and since a number of cell lines were not tested for *MANB,* the scope of the distance measurements is limited to the q arm proceeding distally from *D19S7* located at 19q12. For purposes of counting breaks, cell lines positive for *D19S7* and *D19S9* were presumed to contain *PEPD* as well. Cell lines not expressing *PEPD* but containing only one of the flanking markers were not included in the analysis, since no conclusion concerning the position of the breakpoint could be drawn.

The breakage frequencies used for the distance estimates, as well as the results of these calculations, are shown in table 6. A megabase estimate was calculated for each marker pair (interval), on the basis of the predicted size of 32.2 Mb for this portion of 19q, as described in Material and Methods. A comparison was

Table 4

Segregation of Markers in Region (19q13.3)

	Presence or Absence of Marker Loci in Hybrids[a]					
Cell Line	CKM	ERCC2	D19S116 ERCC1	D19S55 D19S54 D19S51 D19S112 p36.1 D19S63 D19S119 D19S117	D19S118	pW39
GM89A9C9-7	B	+	+	+	+	+
2F5	−	−	+	+	+	+
20XP0435-2	+	+	+	+	+	−
20XP2992-1	+	+	+	+	−	−
9HL5	+	+	+	+	−	−
20XP3542-2	+	+	+	−	−	−
908K1	B	−	−	−	−	−

[a] See footnote to table 3.

made between these estimates of physical distance (based on chromosome breakage) and the estimated genetic distances (based on recombination) presented as consensus distances (male-female averages) at Human Gene Mapping Conference 10 (HGM10) (Keats et al. 1989; LeBeau 1989). These consensus distances have been accepted as the current standard by the Committee on the Genetic Constitution of Chromosome 19 (Keats et al. 1991; Ropers and Pericak-Vance 1991). One reference marker per interval was chosen for each comparison. Because the total genetic distance from *D19S9* (the most proximal 19q marker reported by the

Table 5

Segregation of Markers in 19q13.4

	Presence or Absence of Marker Loci in Hybrids[a]					
Cell Line	D19S55 D19S54 D19S51 D19S112 p36.1 D19S63 D19S119 D19S118	pW39	D19S6	D19S50 TNNT1	D19S22	PRKCG CGB FTL HRC
9HL-1	+	+	−	−	−	−
20XP0435-2	+	−	−	−	−	−
9HL-5	+	−	−	−	−	−
20XP3542-1-4	+	+	−	−	−	−
20XP3542-1	+	+	−	+	+	+
9HL-13	+	+	+	+	+	−
20XP3542-2-10	−	−	+	+	−	−
20XP3542-2	−	−	+	+	+	+

[a] Symbols are as defined in tables 2–4.

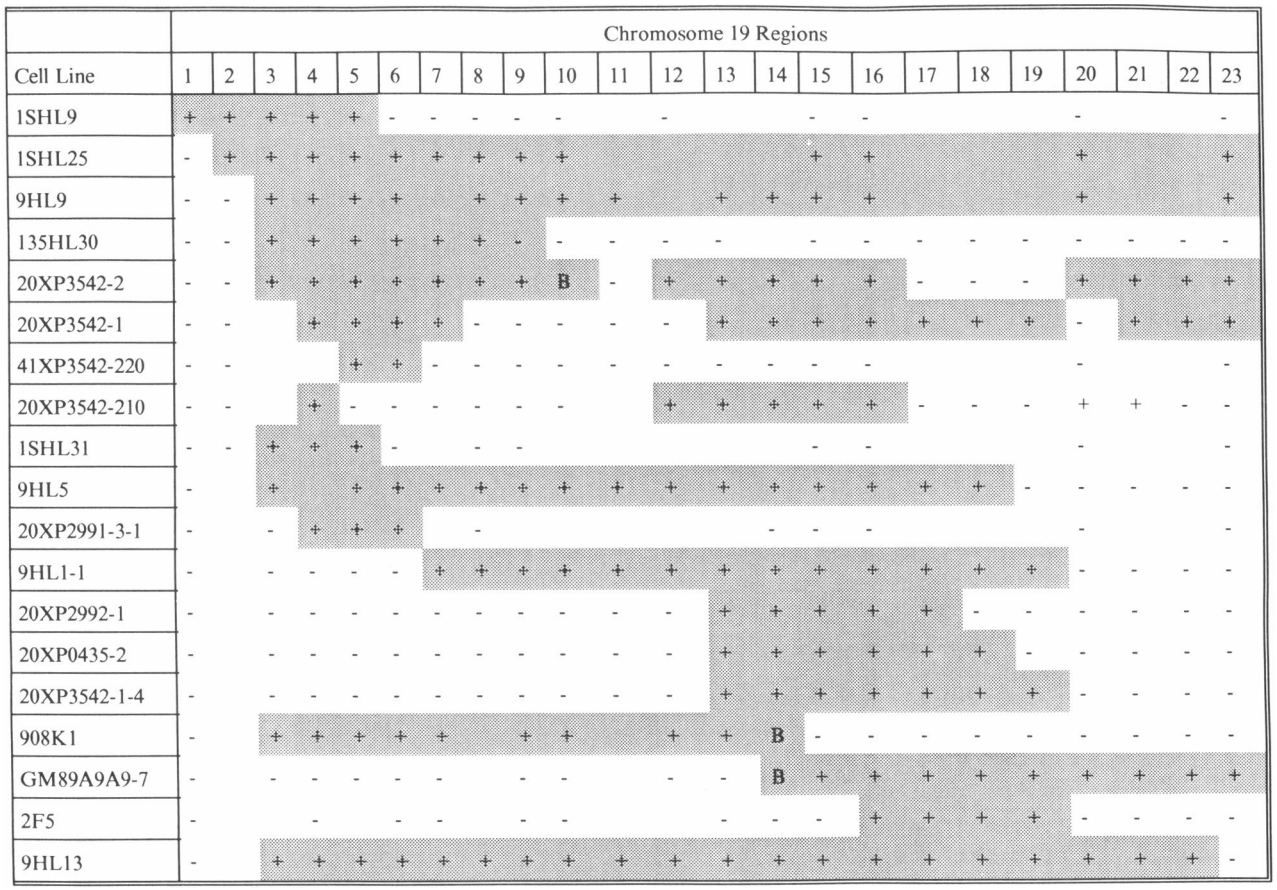

Figure 2 *Shaded sections,* Chromosomal regions presumed to be present in the most informative cell lines. A plus sign (+) denotes that the marker was tested and found present; a minus sign (−) denotes that the marker was found to be absent; a blank space denotes that the marker was not tested; and B = break in the designated region. Markers included in the numbered regions are LDLR (1); MANB (2); D19S7 (3); PEPD (4); D19S9 (5); GPI (6); C/EBP (7); TGFB1 (8); CYP2A, BCKDHA, NCA, and CGM2 (9); PSG1 (10); D19S8 and XRCC1 (11); D19S19 and ATP1A3 (12); D19S37 and APOC2 (13); CKM (14); ERCC2 (15); ERCC1 and D19S116 (16); D19S55, D19S54, D19S51, D19S112, p36.1, D19S63, D19S119, and D19S117 (17); D19S118 (18); pW39 (19); D19S6 (20); D19S50 and TNNT1 (21); D19S22 (22); and PRKCG, CGB, FTL, and HRC (23).

committee) to *D19S22* (the most distal) is estimated as 30.7 cM, and because the present study estimated the total physical distance to be 32.2 Mb or slightly less, this region can be seen to conform generally to the genomic average of 1 cM/Mb. Table 7 compares the genetic and physical estimates for each interval in the region where this information is available.

A regression analysis was performed on these data, and a highly significant correlation (R^2 = .968213 with 4 df was observed between the estimates of physical distance, calculated by using this algorithm, and the consensus genetic distances. This result indicates that these two statistics are directly predictive of each other and represent equally good estimates of chromosomal distance in this region of the genome.

Discussion

The conclusions concerning gene order that are derived from this panel are based on the conservative assumption that a single fragment of chromosome 19 is present in each hybrid and that this fragment represents the native order of markers. Where hybrids appeared to violate this assumption, subcloning and subsequent reanalysis produced results consistent with the presence of multiple fragments segregating in these cell populations. In most cases the position of each marker within the order is determined by multiple independent cosegregation events in the panel members. In only a few cases is the suggested marker order dependent on a break in a single hybrid. Specifically, *TGFB* is placed

Table 6

Use of Somatic Cell Hybrid Panel to Estimate Distances on 19q

	Data for Interval[a]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of lines counted	13	13	14	14	13	13	12	12	12	12	12	12	13	14	14
No. of breaks	2	2	2	4	1	1	1	3	1	2	1	3	1	1	1
Frequency	.154	.154	.143	.286	.077	.077	.083	.250	.083	.167	.083	.250	.077	.071	.071
Frequency × θ	.151	.151	.124	.309	.049	.049	.072	.158	.072	.105	.072	.158	.049	.045	.045
Estimated size (in Mb)[b] ..	3.020	3.020	2.470	6.175	.972	.972	1.441	3.160	1.441	2.107	1.441	3.160	.972	.903	.903

[a] Intervals are as follows: 1 = D19S7-PEPD; 2 = PEPD-D19S9; 3 = D19S9-GPI; 4 = GPI-TGFB1; 5 = TGFB1-CYP2A; 6 = CYP2A-D19S8; 7 = D19S8-D19S19; 8 = D19S19-CKM; 9 = CKM-D19S117; 10 = D19S117-D19S51; 11 = D19S51-pW39; 12 = pW39-D19S6; 13 = D19S6-D19S50; 14 = D19S50-D19S22; and 15 = D19S22-CGB.

[b] Based on a total length of 32.2 Mb for the region.

proximal to *CYP2A*, on the basis of a break in 135HL30; *PSG* is placed distal to *CYP2A*, on the basis of the altered hybridization pattern in 20XP3542-2; (*ATP1A3/D19S19*) is placed distal to *D19S8* and *XRCC1*, on the basis of the retention of a fragment in 20XP3542-2-10; and pW39 is placed distal to the myotonic dystrophy region, on the basis of segregation in hybrid 20XP0435-2. The relative positions of markers in the myotonic dystrophy region have all been confirmed by physical mapping based on cloned DNA (Brook et al. 1990*a*, 1990*b*; Harley et al. 1991; Hermens et al. 1991; Korneluk et al. 1991; Shutler et al. 1991; Tsilfidis et al. 1991*a*, 1991*b*) and on pulsed-field electrophoresis (Smeets et al. 1990). There were no violations of the internal consistency of marker order in any of these hybrids. In addition, the derived order is consistent, in general, with results of other investigators using the panels previously described. The panel described here, however, has much greater resolving power than does any other chromosome 19 panel published to date.

Table 7

Physical versus Genetic Distances on Human Chromosome 19q

Interval	Estimated Size (Mb)	Distance from D19S9[a] Genetic (cM)	Distance from D19S9[a] Physical (Mb)	Ratio (genetic distance/physical distance)
D19S7-PEPD	3.020	...	...	...
PEPD-D19S9	3.020	...	...	...
D19S9-GPI	2.470	...	2.470	...
GPI-TGFB1	6.175	...	8.645	...
TGFB1-CYP2A	.972	5.3	9.617	.551
CYP2A-D19S8	.972	10.1	10.589	.954
D19S8-ATP1A3	1.441	11.5	12.030	.956
ATP1A3-CKM	3.160	17.7	15.190	1.165
CKM-D19S117	1.441	...	16.243	...
D19S117-D19S51	2.107	...	18.349	...
D19S51-pW39	1.441	...	19.933	...
pW39-D19S6	3.160	...	23.092	...
D19S6-D19S50	.972	26.7	24.064	1.110
D19S50-D19S22	.903	30.7	24.967	1.212
D19S22-CGB	.903	...	25.870	...

[a] To the most distal marker in each interval.

The single difference between the gene order presented here and that presented by the Second International Workshop on Human Chromosome 19 (Ropers et al. 1992) is the position of the gene *ATP1A3*. That report places *ATP1A3* between the *CGM2/NCA* cluster and the PSG subfamily, to which *D19S8* has now been assigned. The reported order (*CGM2–ATP1A3–PSG,D19S8*) is slightly different from the order suggested by the data presented here (*CGM2–PSG–D19S8–ATP1A3*). The relative position of *ATP1A3* is determined in this panel by only a single hybrid, 20XP3542-2-10. The order suggested by the workshop is less likely under our assumption of the fewest possible fragments being present in any hybrid. If the order proposed by the workshop is indeed correct, then this hybrid must either have an additional chromosome 19 fragment containing the region of the ATPase gene or have undergone a rearrangement of this region. The possibility of inferring a false gene order because of rearrangements in somatic cell hybrids is the chief objection to their use in mapping. The consistency of the inferred order over a panel of multiple hybrids is a safe-

guard against this type of error. This safeguard is lost in any region where the order depends on a single hybrid, as it does in the case of *ATP1A3*.

Several new orderings are made on the basis of the panel presented here. *MANB* (previously unordered with respect to the other markers) is seen to be proximal to *D19S7* and *PEPD*. The position of *D19S7* proximal to *PEPD* is consistent with published results (Brook et al. 1987; Schonk et al. 1989). *TGFB1*, also previously unordered within this region, is seen to be proximal to *CYP2A* and to the carcinoembryonic antigen (CEA) gene family (represented by *NCA*, *CGM2*, and *PSG*). *PSG* is seen to be distal to the other members of the CEA gene family that were tested. Because this gene family is located on a single 750-kb *Sac*II fragment (Thompson et al. 1989*a*, 1990*a*), this finding orients the group. *XRCC1* and *D19S8* are placed between the *CYP2A/CEA* group and the *ATP1A3/D19S19* group. The consensus genetic map published by the chromosome 19 committee of HGM10 (Le Beau et al. 1989) suggests that *D19S8* is distal to *ATP1A3*. The International Workshop on Human Chromosome 19 supports this order. However, more recent genetic studies support the order presented here (Harley et al. 1991). This question remains to be resolved. Last, the q13.4 region of the chromosome is divided into four subregions. No other published panel is informative for marker order in this region, although genetic studies support the order *D19S50–D19S22–PRKCG* seen here (Johnson et al. 1988; Korneluk et al. 1989*a*).

If the chromosome-breakage pattern seen in these hybrids is random, the frequency with which breaks occur between any two markers depends only on the distance separating them. It is possible, then, to estimate relative physical distances between markers by using the frequency of breaks as the basis for a unit of measure. This very basic principle in physical mapping was originated by Goss and Harris (1975). A number of studies have been reported in which radiation was used to produce chromosomal breaks for this purpose (Goss and Harris 1977*a*, 1977*b*; Cox et al. 1990; Falk 1990; Chakravarti and Reefer 1992), and algorithms have been proposed to relate these breaks to physical distance.

The mapping function used in this analysis, like mapping functions used in recombination mapping, is more accurate (i.e., has more resolving power) at small distances, since θ approaches the limiting value of 1 at about the level of three breaks. Although the θ values are additive, the apparent distances are foreshortened when calculated between widely separated markers, in a manner similar to the apparent shortening of genetic distances that is due to multiple recombination events.

In comparing these estimates of physical distances with estimates of genetic distance in the same region, it was seen that, although the overall ratio of genetic to physical distance is close to 1 cM/Mb, the regional ratio is lowest near the centromere (0.551 cM/Mb in 19q12-13.1) and rises steadily to its highest value near the telomere (1.212 cM/Mb in 19q13.4). This divergence is not unexpected, since the region around a centromere engages in less crossing-over than does the rest of the chromosome, so that genes in the vicinity of the centromere appear to be more clustered while distal genes appear to be more widely separated. Since gene density is presumed to be higher in Giemsa light regions, it is tempting to relate these genetic/physical distance ratios to cytogenetic features such as chromosome bands; caution must be observed, however, because chromosome 19 is relatively devoid of such features and because the number of breaks observed for individual regions in these hybrids is too low to allow a statistically significant result to be obtained.

The hybrids differed considerably in their breakage patterns, depending on fusion partners and selection conditions. Number and size of fragments were characteristic for hybrids of different types. In general, hybrids made with normal human fusion partners were most likely to exhibit breaks when fused with X-ray–sensitive CHO mutants such as EM9 and irs1SF. Hybrids made using various XP cell lines as human fusion partners had the most chromosomal breakage, often retaining multiple small human fragments. In spite of this heterogeneity, the excellent agreement between the consensus recombination distances and the megabase estimates obtained using Chakravarti's mapping function suggests that the use of spontaneously generated chromosome breaks for purposes of estimating distance is a valid approach and does not lead to gross distortion of the map.

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References

Amasino RM (1986) Acceleration of nucleic acid hybridization rate by polyethylene glycol. Anal Biochem 152:304–307

Bachinski LL, Siciliano MJ (1989) A comprehensive gene order for human chromosome 19q. In: Cantor C, Caskey T, Hood L, Kamely D, van Ommen G (eds) UCLA Symposia on Molecular and Cellular Biology, new ser. Alan R Liss, New York, pp 29–38

Bartlett RJ, Pericak-Vance MA, Yamaoka L, Gilbert J, Herbstreith M, Hung W-Y, Lee JE, et al (1987) A new probe for the diagnosis of myotonic muscular dystrophy. Science 235:1648–1650

Boyd D, Vecoli C, Belcher DM, Swatantra KJ, Drysdale JW (1985) Structural and functional relationships of human ferritin H and L chains deduced from cDNA clones. J Biol Chem 260:11755–11761

Boyd Y, Cockburn D, Holt S, Munro E, van Ommen GJ, Gillard B, Affara N, et al (1988) Mapping of 12 translocation breakpoints in the Xp21 region with respect to the locus for Duchenne muscular dystrophy. Cytogenet Cell Genet 48:28–34

Brook JD, Harley HG, Rundle SA, Walsh KV, Shaw JD (1990*a*) RFLP for a DNA clone which maps to 19q13.2-19qter (D19S63). Nucleic Acids Res 18:1085

Brook JD, Knight SJL, Roberts SH, Harley HG, Walsh KV, Rundle SA, Freyne K, et al (1991) The physical map of chromosome 19q: some new assignments confirmations and reassignments. Hum Genet 87:65–72

Brook JD, Skinner M, Roberts SH, Rettig WJ, Almond JW, Shaw JD (1987) Further mapping of the markers around the centromere of human chromosome 19. Genomics 1:320–328

Brook JD, Walsh KV, Harley HG, Rundle SA, Shaw JD (1990*b*) A polymorphic DNA clone which maps to 19q13.2-19qter (D19S62). Nucleic Acids Res 18:1086

Brook JD, Zemelman BV, Haddingham K, Siciliano MJ, Crow S, Harley HG, Rundle SA, et al (1992) Radiation reduced hybrids for the myotonic dystrophy locus. Genomics 13:243–250

Carle G, Frank M, Olson MV (1986) Electrophoretic separation of large DNA molecules by periodic inversion of the electric field. Science 232:65–68

Chakravarti A, Reefer JE (1992) A theory for radiation hybrid (Goss-Harris) mapping: application to proximal 21q markers. Cytogenet Cell Genet 59:99–101

Cox DR, Burmeister M, Price ER, Kim S, Myers RM (1990) Radiation hybrid mapping: a somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. Science 250:245–250

Crabb DW, Deaven LL, Luedemann M, Zhang B, Harris RA (1989) Assignment of the gene for the E1a subunit of branched chain a-ketoacid dehydrogenase to chromosome 19. Cytogenet Cell Genet 50:40–41

Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, et al (1985) Human transforming growth factor-B complementary DNA sequence and expression in normal and transformed cells. Nature 316:701–705

Falk CT (1990) A simple method for ordering loci using data from radiation hybrids. Genomics 9:120–123

Fiddes JD, Goodman HM (1983) The cDNA for the beta-subunit of human chorionic gonadotropin suggests evolution of a gene by readthrough into the 3′ untranslated region. Nature 286:684–687

Fuller LF, Painter RB (1988) A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. Mutat Res 193:109–121

Goss SJ, Harris H (1975) New method for mapping genes in human chromosomes. Nature 255:680–684

——— (1977*a*) Gene transfer by means of cell fusion. J Cell Sci 25:17–37

——— (1977*b*) Gene transfer by means of cell fusion. II. The mapping of 8 loci on human chromosome 1 by statistical analysis of gene assortment in somatic cell hybrids. J Cell Sci 25:39–57

Harley HG, Walsh KV, Rundle S, Brook JD, Sarfarazi M, Koch MC, Floyd JL, et al (1991) Localisation of the myotonic dystrophy locus to 19q13.2–13.3 and its relationship to twelve polymorphic loci on 19q. Hum Genet 87:73–80

Hellkuhl B, Grzeschik K-H (1978) Partial reactivation of a human inactive X chromosome in human-mouse somatic cell hybrids. Cytogenet Cell Genet 22:527–530

Hendricks-Taylor LR, Bachinski LL, Siciliano MJ, Feritta A, Trask B, de Jong PJ, Ledbetter DH, et al (1992) The CAAT/enhancer binding protein (C/EBP) gene maps to human chromosome 19q13.1 and the related nuclear factor NF-IL6 gene maps to human chromosome 20q13.1. Genomics 14:12–17

Hermens R, Coerwinkel M, Brunner H, Smeets H, Wieringa B (1991) MspI RFLP at 19q13.3 identified by the anonymous DNA sequence pX75B (D19S112). Nucleic Acids Res 19:1726

Hofmann SL, Topham M, Hsieh CL, Francke U (1991) cDNA and genomic cloning of HRC a human sarcoplasmic reticulum protein and localization of the gene to human chromosome 19 and mouse chromosome 7. Genomics 9:656–669

Hulsebos T, Wieringa B, Hochstenbach R, Smeets D, Schepens J, Oerlemans F, Zimmer J, et al (1986) Toward early

diagnosis of myotonic dystrophy: construction and chrar-acterization of a somatic cell hybrid with a single human der(19) chromosome. Cytogenet Cell Genet 43:47–56

Hulten M (1974) Chiasma distribution in males. Hereditas 76:52–58

Jackson CL, Bruns GAP, Breslow JL (1984) Isolation and sequence of a human apolipoprotein CII cDNA clone and its use to isolate and map to human chromosome 19 the gene for apolipoprotein CII. Proc Natl Acad Sci USA 81:2945–2949

Johnson KJ, Jones PJ, Spurr N, Nimmo E, Davies J, Creed H, Weiss M, et al (1988) Linkage relationships of the protein kinase C gamma gene which exclude it as a candidate for myotonic dystrophy. Cytogenet Cell Genet 48:13–15

Johnson K, Shelbourne P, Davies J, Buxton J, Nimmo E, Anvret M, Bondeulle M, et al (1989) Recombination events that locate myotonic dystrophy distal to APOC2 on 19q. Genomics 5:746–751

Johnson K, Shelbourne P, Davies J, Buxton J, Nimmo E, Siciliano MJ, Bachinski LL, et al (1990) A new polymorphic probe which defines the region of chromosome 19 containing the myotonic dystrophy locus. Am J Hum Genet 46:1073–1081

Keats B, Ott J, Conneally M (1989) Report of the Committee on Linkage and Gene Order. Cytogenet Cell Genet 51:459–502

Keats B, Sherman SL, Oh J (1991) Report of the Committee on Linkage and Gene Order. Cytogenet Cell Genet 58:1077–1102

Korneluk RG, MacKenzie AE, Nakamura Y, Dube I, Jacob P, Hunter AGW (1989*a*) A reordering of human chromosome 19 long-arm DNA markers and identification of markers flanking the myotonic dystrophy locus. Genomics 5:596–604

Korneluk RG, MacLeod HL, McKeithan TW, Brook JD, MacKenzie AE (1989*b*) A chromosome 19 clone from a translocation breakpoint shows close linkage and linkage disequilibrium with myotonic dystrophy. Genomics 4:146–151

Korneluk RG, Tsilfidis C, Shutler G, Mahadevan M, Bailly J, Surh LC (1991) A three allele insertion polymorphism is identified by the human chromosome 19q13.3 probe pKBE08 (D19S119). Nucleic Acids Res 19:1157

LeBeau MM, Ryan D, Pericak-Vance MA (1989) Report of the Committee on the Genetic Constitution of Chromosomes 18 and 19. Cytogenet Cell Genet 51:338–357

Lusis AJ, Heinzmann C, Sparkes RS, Scott J, Knoff TJ, Geller R, Sparkes MC, et al (1986) Regional mapping of human chromosome 19: organization of genes for plasma lipid transport (APOC1 -C2 and -E and LDLR) and the genes C3 PEPD and GPI. Proc Natl Acad Sci USA 83:3929–3933

McCauliffe DP, Lux FA, Lieu TS, Sanz I, Hanke J, Newkirk MM, Bachinski LL, et al (1990) Molecular cloning expression and chromosome 19 localization of a human Ro/SS-A autoantigen. J Clin Invest 85:1379–1391

Nakamura Y, Fujimoto E, Ballard L, O'Connell P, Leppert M, Lathrop GM, Lalouel J-M (1988) Isolation and mapping of a polymorphic DNA sequence pEFD42 on chromosome 19 (D19S22). Nucleic Acids Res 16:1228

Ohno S, Akita Y, Konno Y, Imajoh S, Suzuki K (1988) A novel phorbol ester receptor/protein kinase nPKC distantly related to the protein kinase C family. Cell 53:731–741

Perryman MB, Kerner SA, Bohlmeyer TJ, Roberts R (1986) Isolation and sequence analysis of a full-length cDNA for human M creatine kinase. Biochem Biophys Res Commun 140:1–989

Phillips IR, Shephard EA, Ashworth A, Rabin BR (1985) Isolation and sequence of a human cytochrome P-450 cDNA clone. Proc Natl Acad Sci USA 82:983–987

Ropers H-H, Pericak-Vance MA (1991) Report of the Committee on the Genetic Constitution of Chromosome 19. Cytogenet Cell Genet 58:751–784

Ropers H-H, Pericak-Vance M, Siciliano M, Mohrenweiser H (1992) Report on the Second International Workshop on Human Chromosome 19. Cytogenet Cell Genet 60:87–95

Schonk D, Coerwinkel-Dreissen M, van Dalen I, Oerlemans F, Smeets B, Schepens J, Hulsebos T, et al (1989) Definition of subchromosomal intervals around the myotonic dystrophy gene region at 19q. Genomics 4:384–396

Shaw DJ, Meredith AL, Sarfarazi M, Harley HG, Huson SM, Brook JD, Bufton L, et al (1986) Regional localisations and linkage relationships of seven RFLPs and myotonic dystrophy on chromosome 19. Hum Genet 74:262–266

Shutler G, Tsilfidis C, Leblond S, Korneluk RG (1991) RFLP identified by the probe pKE06 (D19S117) at human chromosome 19q13.3. Nucleic Acids Res 19:1158

Siciliano MJ, Carrano AV, Thompson LH (1986) Assignment of a human DNA-repair gene associated with sister-chromatid exchange to chromosome 19. Mutat Res 174:303–308

Siciliano MJ, White BF (1987) Isozyme identification of chromosomes in interspecific somatic cell hybrids. In: Gottesmann MM (ed) Methods in enzymology. Academic Press, New York, pp 169–173

Smeets B, Bachinski L, Coerwinkel M, Schepens J, Hoeijmakers J, van Duin M, Grzeschik K-H, et al (1990) A long-range restriction map of the human chromosome 19q13 region: close physical linkage between CKMM and the ERCC1 and ERCC2 genes. Am J Hum Genet 46:492–501

Stallings RL, Olson E, Strauss AW, Thompson LH, Bachinski LL, Siciliano MJ (1988) Human creatine kinase genes on chromosomes 15 and 19, and proximity of the gene for the muscle form to the genes for apolipoprotein C2 and excision repair. Am J Hum Genet 43:144–151

Thompson J, Barnert S, Berling B, von Kleist S, Kodelia V, Lucas K, Mauch E-M, et al (1989*a*) Structure expression and evolution of the human and rat carcinoembryonic antigen (CEA) gene families. In: Yachi A, Shively JE (eds) The

carcinoembryonic gene family. Elsevier Science, Amsterdam, pp 65–74

Thompson J, Koumari R, Wagner K, Barnert S, Schleussner C, Schrewe H, Zimmermann W, et al (1990*a*) The human pregnancy-specific glycoprotein genes are tightly linked on the long arm of human chromosome 19 and are coordinately expressed. Biochem Biophys Res Commun 167: 848–859

Thompson LH (1989) Somatic cell genetics approach to dissecting mammalian DNA repair. Environ Mol Mutagen 14:264–281

Thompson LH, Bachinski LL, Stallings RL, Dolf G, Weber CA, Westerveld A, Siciliano MJ (1989*b*) Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: a third repair gene on human chromosome 19. Genomics 5:670–679

Thompson LH, Brookman KW, Jones NJ, Allen SA, Carrano AJ (1990*b*) Molecular cloning of the human XRCC1 gene which corrects defective DNA strand break repair and sister chromatid exchange. Mol Cell Biol 10:6160–6171

Thompson LH, Carrano AV, Sato K, Salazar EP, White BF, Stewart SA, Minkler JI, et al (1987) Identification of nucleotide-excision repair genes on human chromosomes 2 and 13 by functional complementation in hamster-human hybrids. Somat Cell Mol Genet 13:539–551

Thompson LH, Mooney CL, Brookman KW (1985*a*) Genetic complementation between UV-sensitive CHO mutants and xeroderma pigmentosum fibroblasts. Mutat Res 150:423–429

Thompson LH, Mooney CL, Burkhart-Schultz K, Carrano AV, Siciliano MJ (1985*b*) Correction of a nucleotide excision repair mutation by human chromosome 19 in hamster-human hybrid cells. Somat Cell Mol Genet 11:87–92

Tsilfidis C, Shutler G, Leblond S, Korneluk RG (1991*a*) An SstI RFLP detected by the probe pKE21 (D19S116) localized to human chromosome 19q13.3. Nucleic Acids Res 19:1158

Tsilfidis C, Shutler G, Mahadevan M, Korneluk RG (1991*b*) A frequent HincII polymorphism identified by the human chromosome 19q13.3 probe pKEX08 (D19S118). Nucleic Acids Res 19:1157

van Duin M, de Wit J, Odijk H, Westerveld A, Yasui A, Koken MHM, Hoeijmakers JHJ, et al (1986) Molecular characterization of the human excision repair gene ERCC1: cDNA cloning and amino acid homology with the yeast DNA repair gene RAD10. Cell 44:913–923

van Ommen GJB, Verkerk JMH (1986) Restriction analysis of chromosomal DNA in a size range up to two million base pairs by pulsed field gradient electrophoresis. In: Davies KE (ed) Human genetic diseases: a practical approach, IRL Press, Oxford, pp 113–133

Weber CA, Salazar EP, Stewart SA, Thompson LH (1988) Molecular cloning and biological characterization of a human gene ERCC2 that corrects the nucleotide excision repair defect in CHO UV5 cells. Mol Cell Biol 8:1137–1146

Westerveld A, Hoeijmakers JHL, van Duin M, de Wit J, Odijk H, Pastink A, Wood RD, et al (1984) Molecular cloning of a human DNA repair gene. Nature 310:425–429

Worwood M, Brood JD, Cragg SJ, Hellkuhl B, Jones BM, Perera P, Roberts SH, et al (1985) Assignment of human ferritin genes to chromosomes 14 and 19q13.3-19qter. Hum Genet 69:371–374

Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW (1984) The human LDL receptor: a cysteine-rich protein with multiple alu sequences in its mRNA. Cell 39:27–38

Yamaoka LH, Bartlett RJ, Ross DA, Fey HG, Ledbetter DH, Bruns G, Pericak-Vance MA, et al (1985) Localization of cloned unique DNA to three different regions of chromosome 19: screen for linkage probes for myotonic dystrophy. J Neurogenet 2:403–412

Yang-Feng TL, Schneider JW, Lindgren V, Shull MM, Benz EJ, Lingrel JB, Francke U (1988) Chromosomal localization of human NaK-ATPase alpha and beta subunits. Genomics 2:128–138

Zimmermann W, Weber B, Ortlieb B, Rudert F, Schempp W, Fiebig H-H, Shively JE, et al (1988) Chromosomal localization of the carcinoembryonic antigen gene family and differential expression in various tumors. Cancer Res 48:2550–2554

Zimmermann W, Weiss M, Thompson JA (1989) cDNA cloning demonstrates the expression of pregnancy-specific glycoprotein genes a subgroup of the carcinoembryonic antigen gene family in fetal liver. Biochem Biophys Res Commum 163:1197–1209