Gene mapping and medical genetics

Journal of Medical Genetics 1986, **23**, 193–199

Molecular genetics of human chromosome 4

JAMES A GUSELLA, T CONRAD GILLIAM, MARCY E MACDONALD, SHIRLEY V CHENG, AND RUDOLPH E TANZI

From the Neurogenetics Laboratory, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114, USA.

SUMMARY The recent discovery that the gene causing Huntington's disease (*HD*) resides on chromosome 4 has generated increased interest in this autosome. Chromosome 4 contains two of the more informative conventional genetic markers, *GC* and *MNS*, but most loci have been assigned to it by recombinant DNA techniques. There are currently more anonymous DNA fragments detecting restriction fragment length polymorphisms (RFLPs) on chromosome 4 than on any other autosome. In addition, most of the cloned genes from this chromosome detect useful RFLPs. A genetic linkage map including both conventional and DNA markers should soon span the entire chromosome and will undoubtedly lead to the localisation of other inherited disorders.

Chromosome 4 comprises 6.5% of human genomic DNA and therefore encodes perhaps 5000 to 10000 genes. As with all human chromosomes, only a very small minority of the genes on chromosome 4 have been identified. Taking together all genes, pseudogenes, cell surface antigens, fragile sites, and anonymous DNA segments given locus symbols, 69 loci had been officially assigned to the chromosome at the Human Gene Mapping Workshop 8.¹ These loci have been mapped by many different techniques including somatic cell genetics, dosage studies, and family studies, but recombinant DNA methods have produced the bulk of the assignments. The chromosome is second only to the X chromosome and chromosome 21 in the number of cloned sequences available.² At least 16 of the cloned segments represent known genes or pseudogenes (table), while the remainder are anonymous DNA fragments with no known function. The vast majority of DNA segments assigned to chromosome 4 detect restriction fragment length polymorphisms (RFLPs). With the great number of new markers now available, it is expected that a genetic linkage map encompassing the entire chromosome will soon be

constructed. This development will set the stage for the systematic pursuit of family studies to identify additional loci causing human genetic disease, as well as facilitate the regional localisation of newly cloned genes.

Genes mapped to chromosome 4

Thirty-one genes or pseudogenes have been mapped to chromosome 4 by a variety of methods and they are listed in the table. Many of the genes were assigned purely by traditional methods, such as family studies, dosage in cells with chromosomal aberrations, and somatic cell genetics. The loci encoding albumin (*ALB*), vitamin D binding protein (*GC*), and the fibrinogens (*FGA*, *FGB*, *FGG*) were originally mapped to chromosome 4 by linkage analysis or dosage experiments, but when each of these genes was cloned it was possible to confirm the assignments directly by recombinant DNA techniques.^{3–15} Several of the genes on chromosome 4, such as interleukin 2 (*IL2*) and epidermal growth factor (*EGF*), were mapped only after cloning of cDNAs permitted DNA hybridisation to panels of somatic cell hybrids or in situ hybridisation.

Two of the genes that have not yet been isolated, *PGM2* and *PEPS*, encode the biochemical activities

TABLE Genes mapped to chromosome 4*.

Gene symbol	Description	Location	Cloned DNA	References
<i>HD</i>	Huntington's disease	p16, linked to <i>D4S10</i>		60 68
<i>R112</i>	Processed pseudogene of raf oncogene	p15→pter	+	32
<i>PGM2</i>	Phosphoglucomutase 2	p14→q12		16 18
<i>PLPS</i>	Peptidase S	p11→q12		18 21
<i>PP1T</i>	Phosphoribosylpyrophosphate amidotransferase	pter→q21		22
<i>MT2P1</i>	Metallothionein 2 processed pseudogene	p11→q21	+	33 35
<i>AFP</i>	Alphafoetoprotein	q11→q13	+	6 23
<i>ALB</i>	Albumin	q11→q13	+	3 7 23
<i>HPAFP</i>	Hereditary persistence of alphafoetoprotein	Linked to <i>ALB</i>		53
<i>GC</i>	Group specific component, vitamin D binding protein	q12→q13	+	3 8 10
<i>ASSP8</i>	Argininosuccinate synthetase pseudogene 8	q21→qter	+	36
<i>DG11</i>	Dentinogenesis imperfecta	Linked to <i>GC</i>		57 59
<i>ADH1</i>	Alcohol dehydrogenase, class I alpha chain	q21→q25	+	25
<i>ADH2</i>	Alcohol dehydrogenase, class I beta chain	q21→q25	+	24 25
<i>ADH3</i>	Alcohol dehydrogenase, class I gamma chain	q21→q25	+	24
<i>ADH5</i>	Alcohol dehydrogenase, class III chi chain	q21→q25		26
<i>FDH</i>	Formaldehyde dehydrogenase	q21→q25		73 76
<i>AG1</i>	Aspartylglucosaminidase	q21→qter		54 55
<i>EGF</i>	Epidermal growth factor	q25→q27	+	40 42
<i>IL2</i>	T cell growth factor, interleukin 2	q26→q28	+	38 39
<i>FGA</i>	Fibrinogen alpha chain	q26→q28	+	13 15
<i>FGB</i>	Fibrinogen beta chain	q26→q28	+	13 15
<i>FGG</i>	Fibrinogen gamma chain	q26→q28	+	11 13 15
<i>MNS</i>	MNS blood group, glycoporphins A & B	q28→q31		27 30 45
<i>Sf</i>	Stoltz/fus blood group	Linked to <i>MNS</i>		46 47
<i>TYS</i>	Sclerostylosis	Linked to <i>MNS</i>		56
<i>CD2</i>	Leucocyte surface antigen 5 (CD2p50)	Not regionally mapped		77
<i>MD11</i>	T cell antigen detected by monoclonal Ab	Not regionally mapped		78
<i>MIC16</i>	ALL antigen detected by monoclonal Ab	Not regionally mapped		79
<i>QDPR</i>	Quinoid dihydropteridine reductase	Not regionally mapped	+	49 51
<i>NA</i>	c-kit proto-oncogene	Not regionally mapped	+	37

NA = not assigned.

* The list does not include anonymous DNA sequences or fragile sites which can be found in references 1 and 2.

most frequently used as markers for the presence of chromosome 4 in somatic cell hybrid experiments.¹⁶⁻²¹ The *PPAT* locus, also not yet cloned, was mapped by complementation in auxotrophic mutant hamster cells deficient in the enzyme phosphoribosylpyrophosphate amidotransferase.²² This makes *PPAT* a potential selectable marker for directed somatic cell genetic studies of chromosome 4.

The approximate locations of the known genes that have been regionally mapped on chromosome 4 are shown in the figure. Several of the genes appear to cluster into families of related sequences. Below the centromere, *ALB* and *AFP* encode related proteins and are separated by only a few thousand base pairs.²³ Moreover, the cloning of *GC* which is tightly linked to the *ALB* locus has yielded sequence

data suggesting that it is a member of the same gene family.¹⁰ Further down the long arm, there is a set of genes encoding alcohol dehydrogenases.²⁴⁻²⁶ It is likely that when these are fully sequenced and characterised they will consist of related sequences tightly clustered on the chromosome. The *MNS* blood group locus results from polymorphisms in glycoporphin A (MN) and glycoporphin B (Ss), two related proteins encoded by tightly linked DNA sequences.²⁷⁻³⁰ A third glycoprotein from red blood cell membranes, glycoporphin C, might well be encoded in the same region.³¹ Finally, towards the bottom of the long arm there is a cluster of three fibrinogen genes which are contiguous in a 50 kilobase segment.¹⁵

In contrast to the clustering of the functional gene families, there are three loci on chromosome 4 that

FIGURE Regionally localised genes on chromosome 4. The order and regional localisations are only approximate.

each represent a processed pseudogene, whose functional counterpart is located on an entirely different chromosome. *RAF2*, *MT2P1*, and *ASSP8* are processed pseudogenes homologous to the *RAF1* proto-oncogene (*RAF1*) on chromosome 3, the metallothionein-2 gene (*MT2*) on chromosome 16, and the argininosuccinate synthetase gene (*ASS*) on chromosome 9, respectively.³²⁻³⁶

The recent mapping of the human homologue of v-kit, the viral oncogene from the Hardy-Zuckerman 4 feline sarcoma virus (HZ4-FeSV), to chromosome 4 is the first indication of a potential oncogene on this autosome.³⁷ The only previous implication of chromosome 4 in carcinogenesis has been the observation of a translocation of the long arms of chromosomes 4 and 11 in some cases of acute lymphocytic leukaemia. This translocation joins the *ETSI* oncogene locus from chromosome 11 and a chromosome 4 sequence that has not yet been identified.⁴³

Conventional genetic markers, GC and MNS

Two of the loci mapped to chromosome 4 by conventional methods, *GC* and *MNS*, show considerable polymorphism, placing them among the more informative expressed genetic markers. *GC*, group specific component or vitamin D binding protein, displays polymorphism that is detectable as differences in electrophoretic mobility of the protein. It has a polymorphism information content (pic) of 0.33.⁴⁴

The *MNS* blood group marker actually results from two tightly linked genes.²⁷⁻³⁰ The first, encoding glycophorin A, the major red cell surface protein, displays a sequence polymorphism at its 5' end that determines the M or N specificities. This protein is highly glycosylated, with the presence of the appropriate sugar residues being essential for detecting the appropriate specificities.³⁰ Glycophorin B, a less abundant red cell surface protein, has an identical 5' amino acid sequence to the N form of glycoprotein A. The remaining sequence diverges, however, and a polymorphism in this region results in either S or s alleles, again detected as antigenic differences. Since the sequences encoding the two polymorphisms are close together in the genome, the resultant haplotypes are used, in practice, as alleles for a single locus. Less frequent variants at these two loci have produced proteins that lack either M or N activity, or that appear to be fusions between glycoproteins A and B.⁴⁵ The *MNS* marker has a pic of 0.64, making it second only to HLA in informativeness among the expressed markers.⁴⁴

A third expressed polymorphism mapping to chromosome 4 is the Stoltzfus blood group marker.⁴⁶⁻⁴⁷ Like *MNS*, Stoltzfus (*SF*) is detected as differences in the antigenic determinants on red cell membranes. It is genetically linked to the *MNS* marker and is not frequently used in genetic linkage studies.

Regional localisation of the *GC* and *MNS* markers by dosage studies in families with rearranged chromosomes places them towards opposite ends of the long arm. Despite this apparently great physical separation, very extensive family studies have demonstrated statistically significant but distant linkage between the two loci. This linkage is detectable in male meioses, presumably reflecting a greater frequency of recombination on the long arm of chromosome 4 in females than in males.²⁸⁻⁴⁸

Disease genes mapped to chromosome 4

In general, genetic diseases can be mapped to chromosomes either by family studies to detect coinheritance with a polymorphic marker or by

demonstrating an alteration in a gene product from a known locus. The latter is more common, since infrequent variants have been described for many well characterised proteins. For chromosome 4, both approaches have contributed only a few genetic defects to the map.

The *QDPR* locus encoding dihydropteridine reductase is the presumed site of the defect in type II phenylketonuria where patients do not respond to a phenylalanine free diet.⁴⁹⁻⁵¹ This enzyme is involved in the production of bipterin, a necessary cofactor for the enzyme phenylalanine hydroxylase, which is defective in type I phenylketonuria. Analbuminaemia is a rare condition in which subjects fail to produce albumin from the *ALB* locus on the long arm of chromosome 4.⁵² A second condition, hereditary persistence of alpha-fetoprotein (*HPAFP*), is closely linked to the *AFP* and *ALB* loci and may represent a mutation in regulatory sequences at the alpha-fetoprotein locus. Recently, the *AGA* locus encoding aspartylglucosaminidase, the enzyme deficient in the lysosomal storage disorder aspartylglucosaminuria, has been assigned to the long arm of chromosome 4.⁵⁴⁻⁵⁵

Genetic linkage studies have been modestly successful on chromosome 4, although the high quality of *GC* and *MNS* as markers makes it somewhat surprising that this approach has not identified more disease loci. In fact, only two disease genes have been located using these markers. In 1968, sclerotosis (*TYS*), an autosomal dominant skin disorder, was provisionally linked to *MNS*, although this assignment has never been confirmed.⁵⁶ In 1982, Ball *et al* showed linkage of dentinogenesis imperfecta (*DGII*), a defect in dentition, to *GC*.⁵⁷⁻⁵⁸ This linkage has been confirmed by Conneally *et al*.⁵⁹

DNA markers on chromosome 4

The advent of recombinant DNA techniques for directly detecting polymorphisms in DNA has produced the potential for detecting loci by linkage anywhere in the genome. The discovery of a DNA marker linked to the Huntington's disease (*HD*) locus on chromosome 4 was the first successful use of this approach for an autosomal disease.⁶⁰ *HD* is a late onset neurodegenerative disorder that shows autosomal dominant transmission. The initial linkage has now been confirmed in several families, although the possibility of non-allelic heterogeneity in *HD* has not yet been excluded.⁶¹⁻⁶³ The linked marker, *D4S10*, is detected using the anonymous single copy DNA probe G8. Although originally two polymorphic *HindIII* sites were used to generate four haplotypes for this locus, the discovery of several additional RFLPs with other enzymes has made this a highly

informative locus displaying greater than 90% heterozygosity.⁶⁴ The DNA marker is linked to *HD* at a distance of 4 to 5% recombination (1-loc confidence interval 1-8%).¹

The two loci have recently been mapped to the terminal band of the short arm, 4p16.⁶⁵⁻⁶⁸ Gusella *et al*⁶⁵ showed that in patients with Wolf-Hirschhorn syndrome, a congenital defect caused by the heterozygous deletion of this region, the *D4S10* locus is also deleted. This result has been confirmed by in situ hybridisation by several groups.⁶⁶⁻⁶⁸ In one study it was possible using patients with interstitial deletions of the short arm to narrow the assignment of *D4S10* to the terminal half of 4p16.1, one of three sub-bands seen in prometaphase banding experiments.⁶⁸ The location of *D4S10*, and by inference *HD*, to the terminal part of the short arm explains the failure to detect the disease locus by linkage to either *GC* or *MNS* on the long arm.⁶⁰

The mapping of *HD* to chromosome 4 has provided the impetus to generate large numbers of anonymous DNA markers for this autosome. These now represent more than half of all the assigned loci on chromosome 4. A few markers have been mapped using somatic cell hybrid panels after they were identified in searches for RFLPs using DNA probes chosen at random from genomic DNA.⁶⁰⁻⁶⁹⁻⁷¹ The majority of the anonymous DNA sequences, however, have come from libraries enriched for chromosome 4 sequences by flow sorting of metaphase chromosomes.⁷¹⁻⁷² Subsequent screening for RFLPs has revealed that most of these can be successfully used as genetic markers in linkage studies. A total of 37 anonymous DNA loci had been described for chromosome 4 by Human Gene Mapping Workshop 8, and 30 of these represent useful polymorphic markers.¹² Many additional anonymous DNA loci are currently being characterised (Gusella *et al*, unpublished results).

Of the cloned genes and pseudogenes from chromosome 4, 12 detect RFLPs useful in linkage analysis, although this number is slightly misleading due to the tight clustering of some related genes¹ (Gusella *et al*, unpublished results). Overall, including both anonymous sequences and cloned genes, there are currently more DNA markers for chromosome 4 than for any other autosome.³

No additional markers linked to *HD* have yet been identified from this pool of DNA loci, perhaps because of its terminal location on the short arm. An intensive effort is now under way to saturate 4p16 with new DNA markers. These are needed to increase the accuracy and applicability of any presymptomatic or prenatal diagnostic test procedures in *HD* which could potentially be based on linkage analysis. They will also be required to refine

genetic mapping of the *HD* locus in anticipation of cloning and characterising the defect.

A detailed linkage map of 4p16 will not be the only byproduct of the search for the *HD* gene. This effort has already produced a large number of anonymous DNA markers from both the long and short arms. These RFLPS, along with those detected by cloned genes from chromosome 4, are currently being traced through large reference pedigrees by several groups. This combined effort will soon generate a complete linkage map spanning all of chromosome 4. The vast array of as yet unidentified genes on chromosome 4 undoubtedly include several involved in genetic disease. The availability of a selection of spaced polymorphic markers for this autosome will permit directed family studies with more efficient and powerful data analysis leading to the eventual localisation of these defects.

Preparation of this manuscript was supported by NIH grants NS16367 and NS20012. JFG is supported by the Searle Scholar Program/Chicago Community Trust. TCG and MEM are recipients of the Anna Mitchell Fellowship and the Grace E Neuman Fellowship, respectively, from the Hereditary Disease Foundation. SVC received a fellowship from the National Huntington's Disease Association.

References

- Kidd KK, Gusella JF. Report of the committee on the genetic constitution of chromosomes 3 and 4. HGM8. *Cytogenet Cell Genet* 1985;**40**:107-27.
- Willard HF, Scolnick MH, Pearson PL, Mandel JL. Report of the committee on human gene mapping by recombinant DNA techniques. HGM8. *Cytogenet Cell Genet* 1985;**40**:360-489.
- Weitkamp LR, Renwick JH, Berger J, et al. Additional data and summary for albumin-Gc linkage in man. *Hum Hered* 1970;**20**:1-7.
- Kao FT, Hawkins JW, Law ML, Dugaiczky A. Assignment of the structural gene for albumin to human chromosome 4. *Hum Genet* 1982;**62**:337-41.
- Kurnit DM, Wallner Philipp B, Bruns GAP. Confirmation of the mapping assignment of human serum albumin using a cloned human albumin gene. *Cytogenet Cell Genet* 1982;**34**:282-8.
- Harper ME, Dugaiczky A. Linkage of the evolutionarily related serum albumin and alpha fetoprotein genes within q11-22 of human chromosome 4. *Am J Hum Genet* 1983;**35**:565-72.
- Murray JC, Mills KA, Demopoulos CM, Hornung S, Motulsky AG. Linkage disequilibrium and evolutionary relationships of DNA variants (restriction enzyme fragment length polymorphisms) at the serum albumin locus. *Proc Natl Acad Sci USA* 1984;**81**:3486-90.
- Mikkelsen M, Jacobsen P, Henningsen K. Possible localization of Gc-system on chromosome 4. Loss of long arm 4 material associated with father child incompatibility within the Gc-system. *Hum Hered* 1977;**27**:105-7.
- Van der Hagen C, Olaisen B, Berg K. Confirmation of provisional assignment of the *GC* locus to chromosome 4. *Cytogenet Cell Genet* 1979;**25**:214.
- Naylor SL, McCombs JL, Moore CM, Yang F, Parker J, Bowman BH. Mapping of human group-specific component to chromosome 4. *Cytogenet Cell Genet* 1985;**40**:710.
- Olaisen B, Teisberg P, Gedde-Dahl T, Mevag B. Fibrinogen gamma chain locus is on chromosome 4 in man. *Cytogenet Cell Genet* 1984;**37**:560.
- Olaisen B, Teisberg P, Gedde-Dahl T. Fibrinogen gamma-chain locus is on chromosome 4 in man. *Hum Genet* 1982;**61**:24-6.
- Henry I, Uzan G, Weil D, et al. The genes coding for A alpha-, B beta-, and gamma- chains of fibrinogen map to 4q2. *Am J Hum Genet* 1984;**36**:760-8.
- Humphries S, Imam A, Robbins T, et al. The identification of a DNA polymorphism of the alpha fibrinogen gene, and the regional assignment of the human fibrinogen gene to 4q26-qter. *Hum Genet* 1984;**68**:148-53.
- Kant JA, Fornace AJ, Saxe D, Simon MI, McBride OW, Crabtree GR. Evolution and organization of the fibrinogen locus on chromosome 4: gene duplication accompanied by transposition and inversion. *Proc Natl Acad Sci USA* 1985;**82**:2344-8.
- Wijnen LMM, Grzeschik KH, Pearson PL, Meera Khan P. The human *PGM-2* and its chromosomal location in man-mouse hybrids. *Hum Genet* 1977;**37**:217-78.
- Sparkes RS, Mohandas T, Sparkes MC, Shulkin JD. Regional localization of human phosphoglucomutase-2 locus on chromosome 4. *Exp Cell Res* 1978;**111**:492-5.
- Bocian M, Mohandas T, Sparkes RS, Funderburk S. Peptidase S (*PEPS*) and phosphoglucomutase 2 (*PGM*) loci on the short arm of human chromosome 4. *Cytogenet Cell Genet* 1979;**25**:138.
- Shows TB, Brown JA, Eddy RL, et al. Assignment of peptidase S (*PEPS*) to chromosome 4 in man using somatic cell hybrids. *Hum Genet* 1978;**43**:119-25.
- Brown S, Lalley PA, Minna JD. Assignment of the gene for peptidase S (*PEPS*) to chromosome 4 in man and confirmation of peptidase D (*PEPD*) assignment to chromosome 19. *Cytogenet Cell Genet* 1978;**22**:167-71.
- Schmutz SM, Simpson NE. Suggested assignment of peptidase S (*PEPS*) to 4p11-4q12 by exclusion using gene dosage, accounting for variability in fibroblasts. *Hum Genet* 1984;**64**:134-8.
- Stanley W, Chu EHY. Assignment of the gene for phosphoribosylphosphate amidotransferase to the pter-q21 region of human chromosome 4. *Cytogenet Cell Genet* 1978;**22**:228-31.
- Urano Y, Sakai M, Watanabe K, Tamaoki T. Tandem arrangement of the albumin and alpha-fetoprotein genes in the human genome. *Gene* 1984;**32**:255-61.
- Smith M, Povey S, Arredondo-Vega F, et al. Mapping of human class I alcohol dehydrogenase. *Cytogenet Cell Genet* 1984;**37**:586.
- Smith M, Duester G, Carlock L, Wasmuth J. Assignment of *ADH1*, *ADH2* and *ADH3* genes (class I ADH) to human chromosome 4q21-4q25, through use of DNA probes. *Cytogenet Cell Genet* 1985;**40**:748.
- Carlock L, Hiroshige S, Wasmuth J, Smith M. Assignment of the *ADH5* gene coding for class III ADH to human chromosome 4: 4q21-4q25. *Cytogenet Cell Genet* 1985;**40**:598.
- Cook PJ, Lindenbaum R, Salonen R, et al. The MNSs blood groups of families with chromosome 4 rearrangements. *Ann Hum Gen* 1981;**45**:39-47.
- Bootsma D, McAlpine P. Report of the committee on the genetic constitution of chromosomes 2, 3, 4 and 5. HGM5. *Cytogenet Cell Genet* 1979;**25**:21-31.
- Furthmayr H. Structural comparison of glycoporphins and immunochemical analysis of genetic variants. *Nature* 1978;**271**:519-24.
- Furthmayr H, Metaxas MN, Metaxas-Buhler M, Mg and Mc: mutations within the amino terminal region of glycoporphin A. *Proc Natl Acad Sci USA* 1981;**78**:631-5.
- Dahr W, Beyreuther K, Kordowicz M, Kruger J. N-terminal amino acid sequence of silaoglycoprotein D (glycophorin C) from human erythrocyte membranes. *Eur J Biochem* 1982;**125**:57-62.

- ³² Bonner T, O'Brien SJ, Nash WG, Rapp UR, Morton CC, Leder P. The human homologs of the *raf* (*mil*) oncogene are located on human chromosomes 3 and 4. *Science* 1984;223:71-4.
- ³³ Karin M, Richards RL. Human metallothionein genes—primary structure of the metallothionein-II gene and a related processed gene. *Nature* 1982;299:797-802.
- ³⁴ Schmidt CJ, Hamer DH, McBride OW. Chromosomal location of human metallothionein genes: implications for Menkes' disease. *Science* 1984;224:1104-6.
- ³⁵ Lieberman HB, Rabin M, Barker PE, Ruddle FH, Varshney U, Gedamu L. Human metallothionein-II processed gene is located in region p11 q21 of chromosome 4. *Cytogenet Cell Genet* 1985;39:109-15.
- ³⁶ Su TS, Nussbaum RL, Airhart S, et al. Human chromosomal assignments for 14 argininosuccinate synthetase pseudogenes: cloned DNAs as reagents for cytogenetic analysis. *Am J Hum Genet* 1984;36:954-64.
- ³⁷ Barker PE, Besmer P, Ruddle FH. Human *c-kit* oncogene on human chromosome 4. *Am J Hum Genet* 1985;37:143A.
- ³⁸ Seigel LJ, Harper ME, Wong-Staal F, Gallo RC, Nash WG, O'Brien SJ. Gene for T-cell growth factor: location on human chromosome 4q and feline chromosome B1. *Science* 1984;223:175-8.
- ³⁹ Shows R, Eddy R, Haley L, et al. Interleukin 2 (*IL2*) is assigned to human chromosome 4. *Somatic Cell Mol Genet* 1984;10:315-8.
- ⁴⁰ Brissenden JE, Ullrich A, Francke U. Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor. *Nature* 1984;310:781-4.
- ⁴¹ Zabel BU, Eddy RL, Lalley PA, Scott J, Bell GI, Shows TB. Chromosomal locations of the human and mouse genes for precursors of epidermal growth factor and the beta subunit of nerve growth factor. *Proc Natl Acad Sci USA* 1985;82:469-73.
- ⁴² Morton C, Bell G, Shows T. Epidermal growth factor (*EGF*) is located at q25 q27 on human chromosome 4. *Cytogenet Cell Genet* 1985;40:702-3.
- ⁴³ Guerts van Kessel AHM, Hagemeijer A, Kersey J, Drabkin HD, Patterson D, Papas TS. *Hu-ets-1* and *Hu-ets-2* genes are transposed in acute leukemias with (4;11) and (8;21) translocations. *Science* 1986;231:379-82.
- ⁴⁴ Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980;32:314-31.
- ⁴⁵ Mawby WJ, Anstee DJ, Tanner MJ. Immunochromatographic evidence for hybrid sialoglycoproteins of human erythrocytes. *Nature* 1981;291:161-2.
- ⁴⁶ Bias WB, Meyers DA. Segregation and linkage analysis of the Stoltzfus blood group. *Cytogenet Cell Genet* 1979;25:137.
- ⁴⁷ Bias WB, Meyers DA. Further data on the linkage between the MNS and Stoltzfus blood group systems. *Cytogenet Cell Genet* 1982;32:254.
- ⁴⁸ Falk C, Huss J. Estimates of recombination frequencies between the chromosome 4 markers *MNS*, *GC*, and *PLG*. *Cytogenet Cell Genet* 1985;40:626.
- ⁴⁹ Kaufman S, Holtzman NA, Milstein S, Butler II, Krumholz A. Phenylketonuria due to a deficiency of dihydropteridine reductase. *N Engl J Med* 1975;293:785-90.
- ⁵⁰ Kuhl P, Olek K, Wardenbach P, Grzeschik KH. Assignment of a gene for human quinoid-dihydropteridine reductase (QDPR) to chromosome 4. *Hum Genet* 1979;53:47-9.
- ⁵¹ Lockyer JM, Kidd VJ, Ledley FD, Milstein S, Kaufman S, Woo SLC. Molecular cloning of human dihydropteridine reductase. *Am J Hum Genet* 1985;37:165A.
- ⁵² Murray JC, Demopolous CM, Lawn RM, Motulsky AG. Molecular genetics of human serum albumin: restriction enzyme fragment length polymorphisms and analbuminemia. *Proc Natl Acad Sci USA* 1983;80:5951-5.
- ⁵³ Ferguson-Smith MA, Yates JRW, Kelly D, et al. Hereditary persistence of alpha-fetoprotein maps to the long arm of chromosome 4. *Cytogenet Cell Genet* 1985;40:628.
- ⁵⁴ Pollitt RJ, Jenner FA, Mersky H. Aspartylglucosaminuria. An inborn error of metabolism associated with mental defect. *Lancet* 1986;ii:253-5.
- ⁵⁵ Aula P, Astrin KH, Francke U, Desnick RJ. Assignment of the structural gene encoding human aspartylglucosaminidase to the long arm of chromosome 4 (4q21-4qter). *Am J Hum Genet* 1984;36:1215-24.
- ⁵⁶ Deminatti M, Delmas-Marsalet Y, Mennecier M, Marquet S, Agache P, Huriez C. Etude du linkage probable entre une genodermatose a transmission autosomale dominante et le systeme de groupe sanguin MNSs. *Ann Genet (Paris)* 1986;11:217-24.
- ⁵⁷ Ball SP, Cook PJL, Mars M, Buckton KE. Linkage between dentinogenesis imperfecta and Gc. *Ann Hum Genet* 1982;46:35-40.
- ⁵⁸ Corney G, Ball S, Noades JE. Linkage studies on dentinogenesis imperfecta. *Cytogenet Cell Genet* 1984;37:439-40.
- ⁵⁹ Conneally PM, Bixler D, Horton-Kelly S, Daugherty L. Confirmation of linkage between dentinogenesis imperfecta and Gc. *Cytogenet Cell Genet* 1984;37:438.
- ⁶⁰ Gusella JF, Wexler NS, Conneally PM, et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 1983;306:234-8.
- ⁶¹ Gusella JF, Tanzi RE, Anderson MA, et al. DNA markers for nervous system disorders. *Science* 1984;225:1320-6.
- ⁶² Folstein S, Phillips J, Myers D, et al. Huntington's disease: two families with differing clinical features show linkage to the G8 probe. *Science* 1985;229:776-9.
- ⁶³ Harper PS, Youngman S, Anderson MA, et al. Genetic linkage between Huntington's disease and the DNA polymorphism G8 in South Wales families. *J Med Genet* 1985;22:447-50.
- ⁶⁴ Gusella JF, Gibbons K, Hobbs W, et al. The G8 locus linked to HD. *Am J Hum Genet* 1984;36:1395.
- ⁶⁵ Gusella JF, Tanzi RE, Bader PL, et al. Deletion of the Huntington's disease linked G8 (*D4S10*) locus in Wolf-Hirschhorn syndrome. *Nature* 1985;318:75-8.
- ⁶⁶ Zabel BU, Naylor SL, Sakaguchi AY, Gusella JF. Regional localization of a DNA polymorphism (*D4S10*) linked to Huntington's disease. *Cytogenet Cell Genet* 1985;40:787.
- ⁶⁷ Wang HS, Greenberg CR, Kalousek D, Gusella J, Horsman D, Hayden MR. Subregional assignment of the linked marker (*D4S10*) for Huntington disease by in situ hybridization. *Cytogenet Cell Genet* 1985;40:772.
- ⁶⁸ Magenis E, Gusella J, Weliky K, Haight G, Sheehy B. Huntington disease-linked (*HD*) restriction fragment length polymorphism localized to band p16 of chromosome 4 by in situ hybridization. *Cytogenet Cell Genet* 1985;40:685.
- ⁶⁹ Kao FT, Hartz JA, Law ML. Assignment of a random human DNA segment D4ER11 to the long arm of human chromosome 4. *Cytogenet Cell Genet* 1984;37:506.
- ⁷⁰ Gusella JF. Recombinant DNA techniques in the diagnosis of inherited disorders. *J Clin Invest* (in press).
- ⁷¹ Gilliam TC, Scambler P, Robbins T, Ingle C, Williamson R, Davies KE. The position of three restriction fragment length polymorphisms on chromosome 4 relative to known genetic markers. *Hum Genet* 1984;68:154-8.
- ⁷² Gilliam TC, Healey S, Tanzi R, Stewart G, Gusella J. Polymorphic DNA probes from a human chromosome 4 library. *Cytogenet Cell Genet* 1985;40:641.
- ⁷³ Meera Khan P, Wijnen LMM, Hagemeijer A, Pearson PL. Human formaldehyde dehydrogenase (*FDH*) and its assignment to chromosome 4. *Cytogenet Cell Genet* 1984;38:112-5.
- ⁷⁴ Van Cong N, Gross MS, Jegou-Foubert C, Frezal J. Formaldehyde dehydrogenase and chromosome 4. *Cytogenet Cell Genet* 1985;40:765-6.
- ⁷⁵ Hiroshige S, Carlock L, Wasmuth J, Smith M. Regional assignment of human formaldehyde dehydrogenase (*FDH*) 4q21-4q25. *Cytogenet Cell Genet* 1985;40:651-2.
- ⁷⁶ Van der Goes R, Geurts van Kessel A, Hagemeijer A, Wijnen

- LMM, Meera Khan P. Localization of human *FDH* to 4q21-qter. *Cytogenet Cell Genet* 1985;**40**:766.
- ⁷⁷ Geurts van Kessel AHM, Stoker K, Claas FHJ, van Agthoven AJ, Hagemeijer A. Assignment of the leukocyte group 5 surface antigens to human chromosome 4. *Tissue Antigens* 1983;**21**: 213-9.
- ⁷⁸ Peters PM, Kamarck ME, Hemler ME, Strominger JL, Ruddle FH. Genetic and biochemical characterization of human lymphocyte cell surface antigens. *J Exp Med* 1984;**159**:1441-54.
- ⁷⁹ Katz F, Povey S, Parkar M, *et al*. Chromosome assignment of monoclonal-antibody defined determinants on leukemic cells. *Eur J Immunol* 1983;**13**:1008-13.

Correspondence and requests for reprints to Dr J F Gusella, Neurogenetics Laboratory, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.