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Molecular genetics of human chromosome 16

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SUMMARY The major diseases mapped to chromosome 16 are adult polycystic kidney disease and those resulting from mutations in the α globin complex. There are at least six other less important genetic diseases which map to this chromosome. The adenine phosphoribosyltransferase gene allows for selection of chromosome 16 in somatic cell hybrids and a hybrid panel is available which segments the chromosome into six regions to facilitate gene mapping. Genes which have been mapped to this chromosome or which have had their location redefined since HGM8 include *APRT*, *TAT*, *MT*, *HBA*, *PKD1*, *CTRB*, *PGP*, *HAGH*, *HP*, *PKCB*, and at least 19 cloned DNA sequences. There are RFLPs at 13 loci which have been regionally mapped and can be used for linkage studies.

Chromosome 16 is not one of the more extensively mapped human autosomes. However, it has a number of features which make it attractive to the gene mapper. It contains the gene for adenine phosphoribosyltransferase (*APRT*), which is a selectable marker in tissue culture. It has at least three fragile site loci which can be used to segment the chromosome for in situ hybridisation studies, it can be recognised without the need for chromosome banding, it can be sorted to reasonable purity, and it carries the genes for two important genetic diseases, the α thalassaemias and adult polycystic kidney disease.

It is not the intention of this brief review to relist all the genes and DNA segments which have been mapped to chromosome 16, since this has been done well elsewhere.¹ It is proposed to concentrate on the developments which have occurred since the time of the Eighth Human Gene Mapping Workshop in 1985,² which include the fine mapping of the α globin and metallothionein complexes, the haptoglobin gene, the remapping of the adenine phosphoribosyltransferase gene, new RFLPs which have been detected, and new DNA fragments which

have been cloned and mapped to this chromosome. Brief mention will be made of a hybrid cell panel which allows for an efficient regional localisation of genes on chromosome 16.

Disease genes mapped to chromosome 16

The report of HGM8² lists 31 genes and markers mapped to chromosome 16, of which nine existed in the form of cloned DNA segments. Only a small number of these genes are known to produce human genetic diseases (table 1). Possibly the most significant of these is the α globin gene cluster associated with α thalassaemia and the haemoglobin H/mental retardation syndrome. Another gene of major significance closely linked to the α globin cluster is that for adult polycystic kidney disease. Rare genetic diseases are associated with abnormalities or deficiencies of aldolase A (which is inconsistently mapped to chromosomes 16 and 22), adenine phosphoribosyltransferase (*APRT*), and lethicin cholesterol acyltransferase (*LCAT*), which is associated with Norum disease and is mapped to 16q22. The gene for tyrosine aminotransferase (*TAT*) which is associated with tyrosinaemia II has been mapped to chromosome 16 since HGM8.

TABLE 1 Disease genes on chromosome 16.

Disease	Gene	Location	McKusick No
Aldolase A deficiency	<i>ALDOA</i>	16 or 22	10385
APRT deficiency	<i>APRT</i>	16q24	10260
Cystathioninuria	<i>CTH</i>	16	21950
α thalassaemia	<i>HBA</i>	16p13	14180
Hb H/mental retardation	?	16p13	14175
Norum disease	<i>LCAT</i>	16q22	24590
Tyrosinaemia II (Richner-Hanhart syndrome)	<i>TAT</i>	16q22-1→q22:2	27660
Congenital cataract, cerulcan type	<i>CCA1</i>	?16q	11670
Polycystic kidney disease (adult type)	<i>PKD1</i>	16p13	17390

Genes for which there has been new information since HGM8

APRT

To somatic cell geneticists this is perhaps the most interesting gene mapped to chromosome 16. It can be selected for in somatic cell hybrids by the addition of alanosine and adenine to the tissue culture medium. Using this property of the *APRT* gene a hybrid clone panel containing various translocation products of chromosome 16 in mouse A9 cells has been constructed.³ This panel divides chromosome 16 into six segments and can be used to regionally map genes, either by looking for an expressed human gene product or by Southern analysis. In situ hybridisation studies using the fragile sites can further divide three of these segments and the same technique using other translocations subdivides the 16q22 area even further (figure).

During the construction of the hybrid panel it was found that *APRT* did not appear to be in 16q22 as had been accepted, but in 16q24. Southern analysis of the hybrid panel and in situ hybridisation studies confirmed this location.⁴

TAT

TAT, a deficiency of which results in tyrosinaemia II (Richner-Hanhart syndrome), has been independently mapped to chromosome 16 by two groups^{5,6} using Southern analysis of rodent/human cell panels and by in situ hybridisation to 16q22→q24. A patient with a deletion 16q22:1→q22:3 has been shown to have lost both the haptoglobin (*HP*) and *TAT* genes. The *TAT* gene would thus appear to be firmly mapped to this region.

METALLOTHIONEIN

The *MT* gene cluster was mapped to chromosome 16 by Karin *et al.*⁷ This cluster contains at least 14 genes and pseudogenes with common sequences separated into two groups, *MT1* and *MT2*. This cluster is split

by chromosome rearrangements in acute myelomonocytic leukaemia (AMMoL).⁸ In situ hybridisation studies with a probe to the *MT* cluster and with unique sequence probes to the *MT1B* and *MT2A* genes show that this cluster is proximal to the fragile sites (*FRA16B* and *FRA16C*) in band 16q22:1. This maps the *MT* cluster to the distal end of band 16q21 since the fragile sites are at the interface of bands 16q21 and 16q22. Two new *TaqI* RFLPs have

FIGURE Chromosome 16 showing location of fragile sites, breakpoints of translocations in somatic cell hybrids,³ CY2, 3, etc, and in translocations (TR1, 2, 3) which can subdivide band q22 for gene localisation by in situ hybridisation. Genes and cloned DNA sequences mapped using this approach are shown.

been described for a probe to the 5' flanking region of the *MT2A* gene.¹⁰

α GLOBIN CLUSTER

This cluster contains the α globin genes, which when mutated can give rise to the α thalassaemias,¹¹ and a gene or genes for the rare haemoglobin H/mental retardation syndrome. The cluster contains two functional α globin genes (*HBA1* and *HBA2*), their pseudogenes, the ζ globin gene and its pseudogene, and two hypervariable regions, the ζ intron HVR and the *HBA1*-3'-HVR. Using various probes to this region it has been shown that the cluster is distal to *FRA16A* at 16p12-3¹² and to the breakpoints in two translocations t(1;16)(q44;p13-11) and t(16;22)(p13-11;q11-21). Furthermore, it has been similarly shown to be proximal to an inversion breakpoint mapped at 16p13-12¹³ which would map the locus to 16p13-1. This is at variance with the results of Breuning *et al.*¹⁴ who map the locus to 16p13-3. 3'HVR and *FRA16A* are not closely linked and in situ hybridisation suggests that the locus is more likely to be in 16p13-3 than p13-1.¹² The finding of considerable recombination between two loci which map physically close to each other lends support to the genetic map of this chromosome proposed by Cook *et al.*,¹⁵ based on observed chiasmata, showing the short arm to be relatively long genetically.

HAEMOGLOBIN H DISEASE AND MENTAL RETARDATION

Weatherall *et al.*¹⁶ described three patients with haemoglobin H disease, resulting from an inherited defect of α globin production, associated with mental retardation. A number of further cases have been described.¹⁷⁻²¹ Of nine cases reviewed by Nicholls,²² five had deletion of the α globin genes whereas four had Hb H disease without deletion of α globin. None of the cases had cytogenetically detected deletions, although one was found to have dup(16)(p13-1 $\rightarrow$ pter). The mental retardation seen in these patients varies from mild to severe, and the associated somatic abnormalities are also variable, so that it is unlikely that the non-haematological effects result from mutations at a second single locus.

ADULT POLYCYSTIC KIDNEY DISEASE (*PKDI*)

The locus for adult type autosomal dominant polycystic kidney disease (*PKDI*) has been localised to the short arm of chromosome 16 by the demonstration of genetic linkage to the 3'hypervariable region of the α globin locus.²³ The sum of the published lod scores is 72.01 at a recombination fraction of 0.048 (recombination fraction for males

is 0.076, and for females 0.033).^{24 25} Linkage has also been established between *PKDI* and *PGP* (θ max = 0.0, Z max = 14.4) and between *PGP* and α globin (θ max = 0.04, Z max = 19.19)²⁶ (S T Reenders, unpublished data). The gene order has not been determined by means of genetic linkage, since recombination between *PGP* and *PKDI* has not been observed. However, a probable gene order has been suggested by Breuning *et al.*¹⁴ They studied a fetus with an unbalanced karyotype 46,XX,-16,+(16qter $\rightarrow$ 16p13.3::4q31.1 $\rightarrow$ 4qter) who was found to be hemizygous for 3'HVR and heterozygous for *PGP*. This places the α globin locus distal to *PGP*. Since the gene order *PKDI*-*HBA*-*PGP* is at least 10³ times less likely than the other two possible gene orders (S T Reenders, unpublished data), the likely order for the three linked loci with respect to the chromosome is: cen-*PGP*/*PKDI*-*HBA*-pter. The orientation of the α globin cluster with respect to the centromere has not yet been determined.

No evidence for heterogeneity in the genetic linkage relationships of *PKDI* has been found.²⁵ Preliminary studies of families manifesting atypical features of *PKDI* have also shown genetic linkage to α globin.^{27 28} The 3'HVR probe has been used to carry out prenatal diagnosis of autosomal dominant polycystic kidney disease,²⁹ and has led to the observation of the presence of cysts in fetal kidney during the ninth week of gestation. The polymorphism information content (PIC) of the 3'HVR is 0.96^{2 25} making it a very powerful marker for prenatal detection. Another nine polymorphisms within the α globin cluster have been described³⁰ although these have not been shown to increase the PIC obtained from the 3'HVR.

CHYMOTRYPSINOGEN B

A chymotrypsinogen B (*CTRB*) cDNA clone has been isolated by Bell *et al.*³¹ from the rat. This was used to localise the human *CTRB* gene to chromosome 16.³² *CTRB* has been further localised to 16q11 $\rightarrow$ q22.³³ The rat cDNA clone can also be used to detect a frequent DNA polymorphism within the human *CTRB* gene. This polymorphism has been attributed to the rearrangement of a fragment of at least 4 kb within the genomic sequence of *CTRB*.

PGP, *HAGH*, AND *GPT*

PGP and *HAGH* enzymes are expressed in somatic cell hybrids and were regionally localised to 16p13-11 $\rightarrow$ pter³⁴ using the hybrid cell panel of Callen.³ Red cell *GPT*, which has been inconsistently mapped to 16p and 9q, was excluded from 16pter by the finding of heterozygosity for the

corresponding enzyme in a patient with a ring chromosome 16.³⁵

HAPTOGLOBIN (HP)

This gene had been mapped to 16q22 by in situ hybridisation.³⁶ Further such studies³⁷ have shown the gene to be distal to fra(16)(q22) and linkage studies with this fragile site have shown HP to be 9 cM from it.³⁸

PROTEIN KINASE C β SEQUENCE (PKCB)

Protein kinase C comprises a family of proteins, indistinguishable by conventional protein chemistry, which mediate cellular response to external stimuli. *PCKB* is one of a family of protein kinase C related genes. Genes for the different sequences are on different chromosomes and *PCKB* has been mapped by Southern analysis of somatic cell hybrids to 16pter→q22 and by in situ hybridisation to 16p12→q11.1.³⁹ However, since the gene is unlikely to be in the centric heterochromatin it is probably in the region p12→p11.2.

Cloned DNA fragments

There are several anonymous cloned DNA fragments mapped to chromosome 16. Some of these have come from sorted chromosome libraries.⁴⁰⁻⁴⁴ Some detect RFLPs and others do not (table 2). A number of other segments have been isolated from hybrid cell line DNA libraries and are currently being regionally mapped (Fratini *et al*, Hyland *et al*, unpublished data).

RFLPs

A number of RFLPs shown by anonymous cloned sequences (table 2) or by cloned gene sequences (table 3) are known. Perhaps the most useful of these is the 3'HVR probe which is highly polymorphic and linked to *PGP* and *PKD1*. A cosmid clone has been described which detects a highly polymorphic locus with at least 11 alleles in the 16q22→q24 region.⁴²

TABLE 2 Anonymous cloned DNA segments mapped to chromosome 16.

Probe name	D number	Location	Polymorphism	Reference
L1-36	D16S1	16	BglII	2
ACH 92	D16S3	16p13	-	41
ACH202	D16S14	16q22→q24	XbaI	41
ACH207	D16S4	16q13→q22	TaqI, MspI	41
ACH224	D16S5	16q22→q24	RsaI	41
ACH127	D16S13	16cen→q13	-	41
ACH208	D16S15	16q22→q24	-	41
ACHV1	D16S16	16cen→q13	-	Hyland <i>et al</i> (unpublished data)
ACHV2	D16S17	16q22→q24	-	"
ACHV3	D16S18	16q22→q24	-	"
ACHV4	D16S19	16cen→q13-11	-	"
16B11	D16S6	16q13→16q22	-	Reeders <i>et al</i> (unpublished data)
ACHF1	D16S8	16p13	PvuII	Fratini <i>et al</i> (unpublished data)
ACHF2	D16S9	16p13	-	"
ACHF3	D16S10	16q13→q22	TaqI	"
ACHF4	D16S11	16q13→q22	-	"
ACHF5	D16S12	16q22→q24	-	"
p79-2-23	D16S7	16q22→q24	TaqI	42, 43

TABLE 3 RFLPs on chromosome 16 shown by cloned gene sequences.

Gene	Probe name	Polymorphism	Location	Reference
HBA1*	p 3'HVR	PvuII	16p13	2
APRT	Huap15	TaqI	16q24	2
APRT	48.aprt	TaqI	16q24	2
HP	hapto6	EcoRI	16q22	2
	hp2alpha	HindIII, XbaI	16q22	2
HPR	hapto6	PstI	16q22	2
MT2A	MT2A	TaqI	16q21	10
CTRB	pCXP33	EcoRI, BglII	16q11→q22	33

*Nine other RFLPs within the α globin cluster have been described.³⁰

Gene order

Genes and cloned DNA sequences regionally mapped to chromosome 16 are shown in the figure. The order of all these is not known; however, there is information on order for some from unpublished work from the authors' laboratories.

SHORT ARM

Linkage studies using the disease locus *PKD1* and the fragile site at 16p12.3 (*FRA16A*), and linkage and somatic cell studies using the enzyme polymorphism *PGP* and the RFLP of the *HBA1*-3'HVR, give a probable gene order: cen-*FRA16A*-*PGP*,*PKD1*-*HBA*-pter.

LONG ARM

Similar somatic cell, linkage studies, and in situ hybridisation studies using the fragile site at 16q22 (*FRA16B*), RFLPs associated with the *MT*, *HP*, and *APRT* genes and probe ACHF3, give the order: cen-*MT*,*ACHF3*-*FRA16B*-*D16S4*-*HP*-*FRA16D*-*APRT*-qter.

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