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The Gene for Cystathionine β-Synthase (*CBS*) Maps to the Subtelomeric Region on Human Chromosome 21q and to Proximal Mouse Chromosome 17

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Summary

The human gene for cystathionine β-synthase (*CBS*), the enzyme deficient in classical homocystinuria, has been assigned to the subtelomeric region of band 21q22.3 by in situ hybridization of a rat cDNA probe to structurally rearranged chromosomes 21. The homologous locus in the mouse (*Cbs*) was mapped to the proximal half of mouse chromosome 17 by Southern analysis of Chinese hamster × mouse somatic cell hybrid DNA. Thus, *CBS/Cbs* and the gene for αA-crystalline (*CRYA1/Crya-1* or *Acry-1*) form a conserved linkage group on human (HSA) chromosome region 21q22.3 and mouse (MMU) chromosome 17 region A-C. Features of Down syndrome (DS) caused by three copies of these genes should not be present in mice trisomic for MMU 16 that have been proposed as animal models for DS. Mice partially trisomic for MMU 16 or MMU 17 should allow gene-specific dissection of the trisomy 21 phenotype.

Introduction

Homocystinuria, an inborn error of the transsulfuration pathway, is in most cases due to deficient activity of the enzyme cystathionine β-synthase (CBS). Biochemically, this autosomal recessive disease is characterized by the presence of homocysteine and also by elevated methionine and decreased cysteine concentrations in plasma and urine. The clinical spectrum of homocystinuria comprises mental retardation, optic lens dislocation, osteoporosis, skeletal abnormalities, an increased incidence of thromboembolic events, and a predisposition to psychiatric illness (Mudd et al. 1985; Abbott et al. 1987). The pathophysiological mechanism by which the enzyme defect produces the clinical manifestations is not well understood. However, animal studies have demonstrated that infusion of homocysteine alone can cause arteriosclerotic lesions similar to those seen in patients with homocystinuria (Harker et al. 1976). Results of two recent studies in humans have demonstrated a correlation between mild homocyst(e)inemia and coronary artery disease (Boers et al. 1985; Kang et al. 1986) and suggest that homocystinuria carriers may be at increased risk for arteriosclerotic disease.

The gene locus for CBS was originally assigned to human chromosome 21 by measuring activities of the enzyme in rodent × human somatic cell hybrids (Skovby et al. 1984). When a rat cDNA for CBS was cloned, the chromosome assignment was confirmed and further refined to band 21q22 (Kraus et al. 1986). This band contains the region of 21 that has been implicated in Down syndrome (DS) (Niebuhr 1974). On the basis of phenotypic comparisons, Lejeune (1975) had previously considered homocystinuria as "contretype" to DS and had predicted that the gene for homocystinuria would be found on chromosome 21. This hypothesis further implies that increased CBS activity present in cells from individuals with trisomy 21 somehow contributes to the DS phenotype.

Chromosomal gene mapping has identified several other gene loci—including superoxide dismutase-1 (*SOD1*), phosphoribosylglycinamide synthetase

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Table 1

Correlation of CBS-specific Human Restriction Fragment with Human Chromosomes in Chinese Hamster × Human Somatic Cell Hybrids

Hybrid	CBS	Human Chromosome																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
XII-2D aza	+	−	−	+	−	−	+	−	−	L	−	−	+	+	+	+	+	−	−	−	−	+	−	−	−
XII-2D-1d aza	+	−	−	+	−	−	−	−	−	−	−	−	−	+	+	+	P	−	−	−	−	+	−	P	−
XII-4A	−	−	−	+	−	−	−	−	+	−	−	+	−	+	P	−	+	−	+	−	+	−	−	+	+
XIII-7A HAT	−	−	−	−	−	L	−	L	+	L	−	−	−	+	P	+	−	−	+	−	+	−	+	P	−
XVIII-54A aza	−	−	+	+	+	+	+	−	−	+	−	+	+	+	+	−	+	−	+	+	+	−	−	L	−
XVIII-7B-3a aza	+	+	P	P	+	+	+	−	+	P	−	+	+	+	+	−	+	−	+	+	+	+	+	P	−
XXI-51B	−	−	−	+	+	+	−	+	+	+	+	+	−	−	+	P	+	+	+	+	+	−	+	P	+
29-1F-3a aza	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Discordant (no.)		3	4	4	5	5	3	5	6	4	5	6	3	4	3	3	4	5	7	5	7	0	5	3	6

Note.—P = partial chromosomes; and L = low (<0.1/cell) copy numbers.

(*PRGS*), phosphofructokinase, liver-type (*PFKL*), the interferon α and β receptors (*IFNAR* and *IFNBR*) (Raziuddin et al. 1984) and the oncogene *ETS2* (Watson et al. 1986)—that map to the region known to generate DS-like features when present in triplicate. In the mouse, homologous loci for some of these genes have been mapped to the distal half of chromosome 16 (MMU 16) (Cox and Epstein 1985). The proximal half of MMU 16 is homologous to human chromosome 22q (Francke et al. 1982). Therefore, a mouse partially trisomic for the distal region of chromosome 16 was considered a suitable animal model for human DS (Epstein et al. 1985; Reeves et al. 1986). Since, on clinical grounds, the extra copy of *CBS* may be involved in generating the DS phenotype, the mapping of the CBS gene in the mouse becomes important in validating trisomy of MMU 16 as a model for DS.

Here we report the assignment of the mouse CBS gene (*Cbs*) to a region of mouse chromosome 17 by Southern analysis of Chinese hamster × mouse somatic cell hybrid DNA. Furthermore, we have used in situ hybridization to rearranged human chromosomes 21 to more precisely delineate the region of homology between human (HSA) chromosome 21 and MMU 17.

Material and Methods

Somatic Cell Hybrids

Chinese hamster × human somatic cell hybrids used for mapping the human gene for cystathionine β-synthase (*CBS*) locus included series XII and XIII (Francke et al. 1976), series XVIII (Francke 1984), series XXI (Francke and Francke 1981), and series 29 (Francke et al. 1985). Chinese hamster × mouse somatic cell hybrids for the primary chromosomal assignment of *Cbs* in mouse were derived from series I (Francke and Taggart 1979) and from the EBS, EAS, NZS series (Francke et al. 1977). The rat × mouse hybrid RTM9 HAT contains a mouse Rb(11;13) chromosome (Cox et al. 1982) and is included in our mouse mapping panel because MMU 11 is consistently absent from Chinese hamster × mouse hybrids. The chromosomal content of each hybrid cell line is listed in tables 1 (human mapping panel) and 2 (mouse mapping panel).

For regional mapping of *Cbs* on MMU 17, fibroblasts from a mouse homozygous for the reciprocal translocation *T(10;17)11Rl* (Cacheiro and Russell 1975) were fused with the HPRT-deficient Chinese hamster cell line V79/E36 and selected in HAT medium (D. Röhme and H. Lehrach, personal communication). Mouse parental fibroblasts and hybrid lines hMTA 9 and hMTA 9:19 were obtained from Lisa Stubbs and Hans Lehrach (European Molecular Biology Laboratory, Heidelberg). Chromosome analysis after trypsin-Giemsa banding, carried out in our laboratory, revealed the mouse fibroblast strain to be pseudotetraploid, with few chromosome rearrangements and four pairs of translocation chromosomes present; the breakpoint on MMU 17 was interpreted to be in band 17C (for band nomenclature see Nesbitt and Francke 1973). At the time of DNA

Table 2

Correlation of CBS-specific Mouse Fragments with Mouse Chromosomes in Chinese Hamster × Mouse and in Rat × Mouse Somatic Cell Hybrids

Hybrid	CBS	Mouse Chromosome																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
RTM9 HAT	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−
EAS5-2a	+	+	+	+	−	+	+	P	+	−	−	−	−	−	+	+	+	+	−	+	+
EAS5-4a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	+
EAS5-5a	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−
EAS5-5c	+	+	+	−	−	−	−	+	−	−	−	−	−	−	+	−	−	+	−	−	+
EAS5-7c	+	−	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	−	+	+
EAS5-17	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
EBS4	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	+
EBS10	+	L	+	+	−	−	L	+	−	L	+	−	+	L	+	+	+	−	+	−	+
EBS11	−	+	−	−	−	−	−	+	−	−	+	−	+	−	−	+	+	−	−	−	+
EBS18	+	−	+	+	−	−	L	+	L	−	L	−	L	−	−	+	−	+	−	−	−
EBS51	+	−	+	+	−	−	+	L	+	+	−	−	+	+	−	+	−	+	+	+	+
EZS25	+	−	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	L	+	+
I-10C HAT	+	+	+	+	+	−	+	+	+	L	+	L	+	−	+	+	+	+	+	+	+
I-13A-la aza	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	P
I-13A-2a aza	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	−
I-18A-2a aza	−	+	+	−	L	+	L	−	−	−	+	−	+	−	−	−	−	−	+	+	−
I-18A HAT	+	+	+	−	+	−	+	−	−	−	L	−	+	−	−	−	+	+	+	+	+
Discordant (no.)		7	3	4	6	10	3	4	4	8	10	12	6	8	5	4	8	1	6	5	4
Informative (no.)		17	18	18	17	18	15	16	17	16	16	17	17	17	18	18	18	18	17	18	17

Note.—P = partial chromosomes; and L = low (<0.1/cell) copy number.

extraction, hybrid hMTA 9 had retained the 17^T chromosome in 100% of cells and had lost the 10^T derivative, while hybrid hMTA 9:19 had retained 10^T in 50% of cells and 17^T in <10% (U. Francke and B. Foellmer, unpublished data).

Patients with Rearranged Chromosomes 21

Regional assignment of *CBS* on HSA 21 was carried out by in situ hybridization to normal and structurally rearranged human chromosomes, as described in detail in the accompanying paper (Münke et al. 1988). On the basis of cytogenetic and molecular hybridization data, the rearrangements were interpreted as follows: del(21), an interstitial deletion of chromosome 21, del(21)(pter→q22.11::q22.13→qter); r(21), a ring chromosome: r(21)(p12→q22.3); and t(21;22), a balanced reciprocal translocation between the long arms of chromosomes 21 and 22: t(21;22)(q22.3;q11.2). Cytologically, the breakpoints in subband 21q22.3 could not be distinguished, but in situ hybridization with several DNA probes provided evidence for the translocation breakpoint being more proximal than the ring breakpoint (Münke et al. 1988).

Hybridization Probe

Probe p610 is a 1.7-kb cDNA in pBR322 that codes for CBS and was derived from a rat liver cDNA library (Kraus et al. 1986). It was employed for filter and in situ hybridization experiments.

Southern Analysis

DNA of Chinese hamster × human somatic cell hybrids and control cell lines was extracted, digested with *Eco*RI, size fractionated by agarose-gel electrophoresis, blotted to nitrocellulose filters, and hybridized to the p610 probe labeled by nick-translation to specific activities of 2–4 × 10^8 cpm/μg, following standard procedures. Hybridization and washes were similar to those described in the accompanying paper (Münke et al. 1988), except that the conditions were less stringent. Hybridization was at 40 C, and washes were at 50 C in 1 × SSC, 1% SDS for two 30-min periods.

For mapping of *Cbs,* Chinese hamster (V79/380-6 cells) and mouse (3T3 cells) DNAs were digested with 14 different restriction enzymes: *Bam*HI, *Ban*II, *Bgl*II, *Bst*XI, *Dra*I, *Eco*RI, *Hin*dIII, *Msp*I, *Nsi*I, *Pst*I, *Pvu*II, *Ras*I, *Taq*I, and *Xmn*I. Optimal resolution be-

tween Chinese hamster and mouse CBS-specific DNA fragments was obtained with *Rsa*I. The Chinese hamster × mouse hybrid DNAs were digested with *Rsa*I and analyzed by Southern blotting as described above.

In Situ Hybridization

By means of the method of Harper and Saunders (1981), probe p610 was nick-translated with three tritium-labeled nucleotides (^{3}H-dATP, ^{3}H-dCTP, and ^{3}H-dTTP) to specific activities of 1–4 × 10^7 cpm/μg. Denatured probe at concentrations of 25–50 μg/ml was hybridized overnight at 37 C, and washes were done at 39 C. Slides were exposed for 12–24 days. Chromosomes were stained with quinacrine and subsequently with Wright stain according to a method described elsewhere (Münke et al. 1984).

Results

Assignment of the Human CBS Locus to Chromosome 21 by Southern Analysis

Filters containing *Eco*RI-digested DNA from Chinese hamster × human somatic cell hybrids and from Chinese hamster and human controls were hybridized with the CBS-specific rat cDNA probe p610 (fig. 1). The human 12-kb fragment was only observed in hybrids containing human chromosome 21. Hybrid cell line 29-1F-3aza has retained chromosome 21 as the only human chromosome and was positive for the human-specific CBS fragment. Every other human chromosome could be excluded by three or more discordant hybrids (table 1).

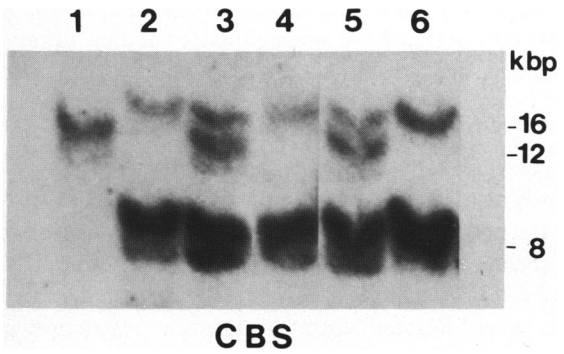

Figure 1 Southern hybridization of *Eco*RI-digested DNA with rat CBS cDNA clone p610. Lane 1, human; lane 2, Chinese hamster V79/380-6; lanes 3–6, Chinese hamster × human hybrids (lane 3, 29-IF-3a aza; lane 4, XXI-51B; lane 5, XVIII-7B-3a aza; and lane 6, XVIII-54A aza). Hybrids in lanes 3 and 5 are positive for the 12-kb human fragment.

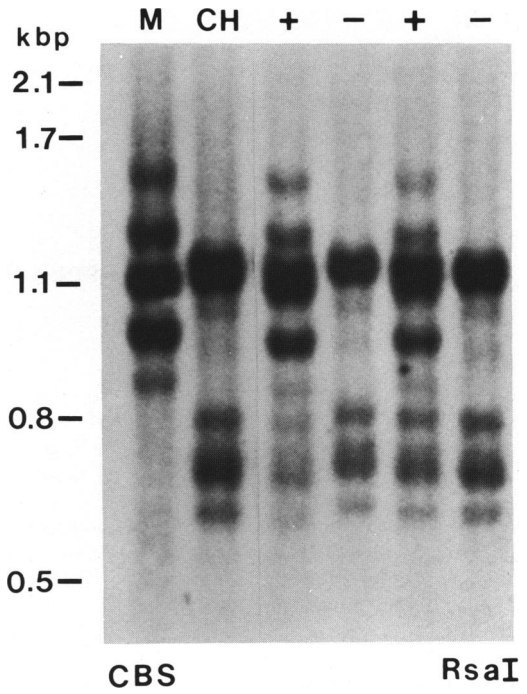

Figure 2 Mapping of *Cbs* in mouse. Filter hybridization of *Rsa*I-digested DNA with probe p610. Lane M, mouse 3T3 cells; and lane CH, Chinese hamster V79/380-6 cell line. Chinese hamster × mouse hybrid cell lines in lanes designated with a plus sign (+) are positive; and those designated with a minus sign (−) are negative for the mouse-specific CBS fragments.

Assignment of Cbs Locus to Mouse Chromosome 17

Hybridization of probe p610 to filters with *Rsa*I-digested DNA from Chinese hamster × mouse somatic cell hybrids and controls resulted in five distinct fragments for each species (fig. 2). In 17 of 18 hybrid cell lines, all five mouse-specific fragments were concordant with MMU 17. The discordant hybrid EBS10 was positive for all five fragments and may contain part of a rearranged MMU 17 that was not detectable by cytogenetic analysis. MMU 16 was excluded as a possible site for *Cbs* by eight discordant hybrids. Every other mouse chromosome was excluded by three or more discordancies (table 2).

Regional Localization of Cbs on MMU 17

The five mouse-specific *Rsa*I fragments were present in DNA from hybrid hMTA 9 and absent in DNA from hybrid hMTA 9:19. This result places *Cbs* into region A-C of MMU 17 (Nesbitt and

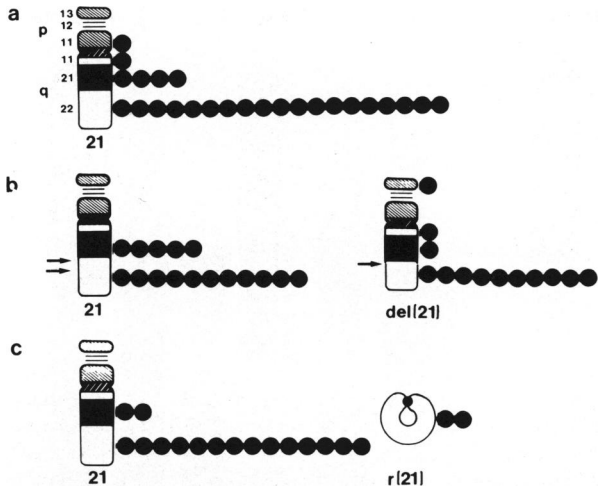

Figure 3 Distribution of autoradiographic silver grains after in situ hybridization with ^{3}H-labeled probe p610 over (*a*) normal chromosome 21, (*b*) del(21) and normal homologue, and (*c*) ring(21) and normal homologue.

Francke 1973), proximal to the breakpoint in the T(10;17)11Rl translocation.

Regional Localization of Human CBS *by In Situ Hybridization*

Since *CBS* had been unequivocally assigned to HSA 21 by Southern analysis of somatic cell hybrids, silver grains generated by in situ hybridization with probe p610 were recorded on chromosome 21 only. In 82 metaphase cells from normal controls, 279 grains were associated with chromosomes (3.4 grains/cell). Of these, 25 (9%) were over chromosome 21, with a peak at the distal end of band q22 (fig. 3*a*). Identical grain distribution patterns were observed over normal chromosomes 21 in cells that also had a structurally abnormal 21 (fig. 3*b*, 3*c*).

The regional assignment was further refined by use of cells with rearranged chromosomes 21 (Münke et al. 1988). The number and distribution of grains were similar over the del(21) chromosome and the normal homologue. This result excludes *CBS* from the deleted region 21q22.11→q22.13 (figs. 3*b*, 4).

Analysis of cells with the t(21;22)(q22.3;q11.2) translocation revealed a greater number of grains over the der(22) translocation chromosome containing only part of the distal subband (21q22.3→qter) than over the der(21) chromosome (data not shown). This indicates that *CBS* is located distal to the break in 21q22.3 (for illustration, see fig. 6 of the preceding paper by Münke et al. [1988]).

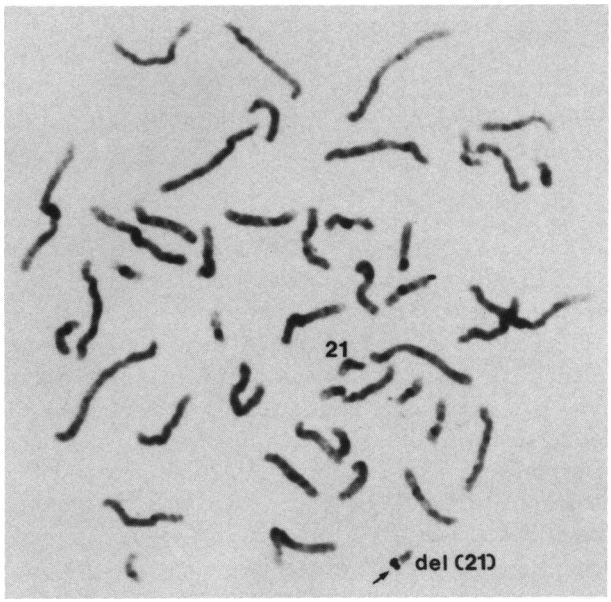

Figure 4 Metaphase spread with interstitial deletion of chromosome 21. Arrow points to CBS-specific silver grains at the distal end of the del(21) chromosome.

After hybridization of the CBS-specific probe to cells with the ring chromosome 21, 14 of 61 cells analyzed had grains over band 21q22 of the normal homologue. Nine of these grains were touching the chromosome at the telomeric region. Only two of the 61 cells had a grain over the r(21) (fig. 3*c*); these were considered background.

Thus, the *CBS* locus can be excluded from most of 21q and assigned to a region within subband 21q22.3 distal to the breakpoints in both the t(21;22) translocation and the r(21) chromosome. This is the most telomeric of the subregions defined in our studies and is shared with *D21S25* (Münke et al. 1988).

Search for RFLP with CBS Probe

More precise mapping can be done by means of family studies. In an attempt to place the *CBS* locus on the genetic linkage map of HSA 21, we carried out an extensive search for RFLPs by using the rat cDNA probe. DNA samples from 8–10 unrelated individuals were digested with 21 different restriction endonucleases: *Bam*HI, *Ban*II, *Bcl*I, *Bgl*II, *Bst*XI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Msp*I, *Nsi*I, *Pst*I, *Pvu*II, *Rsa*I, *Sac*I, *Sal*I, *Stu*I, *Taq*I, *Xba*I, and *Xmn*I. No RFLPs were detected. We plan to continue the search with flanking human genomic sequences as soon as they become available. On the basis of the

sizes of genomic restriction fragments that hybridized with the complete cDNA probe (e.g., *TaqI*, 2.5 kb and 2.9 kb; *SalI*, 4.6 kb; *PstI*, 2.1 kb and 2.7 kb; and *PvuII*, 1.8 kb and 5.3 kb), we estimate the size of the gene to be in the 4–6-kb range.

Discussion

A rat cDNA clone for CBS was used to (1) confirm the assignment of the *CBS* locus to HSA 21 and (2) regionally map *CBS* to the subtelomeric part of band 21q22.3. The homologous locus in the mouse (i.e., *Cbs*) has been assigned to the proximal half of chromosome 17 (MMU 17).

Our regional localization of human *CBS* is inconsistent with one previous report. Chadefaux et al. (1985) have measured CBS levels in fibroblasts of three patients with partial deletions and in two patients with partial duplications of the long arm of chromosome 21. Their results suggested a *CBS* localization at the interface between bands q21 and q22.1. Other investigators, however, have observed a wide range of CBS activity in fibroblasts of normal controls (Freeman et al. 1975; Fowler et al. 1978) and have been unable to demonstrate a gene-dosage effect with the expected 3:2 ratio when comparing CBS activities in trisomic fibroblasts to those in disomic cells (Skovby et al. 1984). Furthermore, no decrease in CBS activity below normal values was observed by Chadefaux et al. (1985) in their three cases with partial deletions that, together, covered the entire chromosome 21. The problems associated with dosage studies of constitutively expressed enzyme genes in trisomy 21 have been reviewed (Francke 1981). Several gene loci that are now known to be located elsewhere had erroneously been assigned to chromosome 21 by measuring enzyme activities in cells of patients with DS. Particularly relevant is the report by Chadefaux et al. (1984), which assigned the locus for liver-type 6-phosphofructokinase (*PFKL*) to 21q21→pter by measuring red cell phosphofructokinase (PFK) activities. In contrast, *PFKL* recently has been assigned to subband 21q22.3 by using somatic cell hybrids and a monoclonal anti–liver PFK subunit-specific antibody (Van Keuren et al. 1986).

Because of grain scatter, in situ hybridization to normal chromosomes 21 does not provide high-resolution gene mapping. We have made use of structurally rearranged chromosomes 21 with two different breakpoints within subband q22.3 (Münke et al. 1988). The results of our in situ hybridizations exclude *CBS* from 21q22.1 and place it into the distal part of subband 21q22.3 (see Münke et al. 1988, fig. 6).

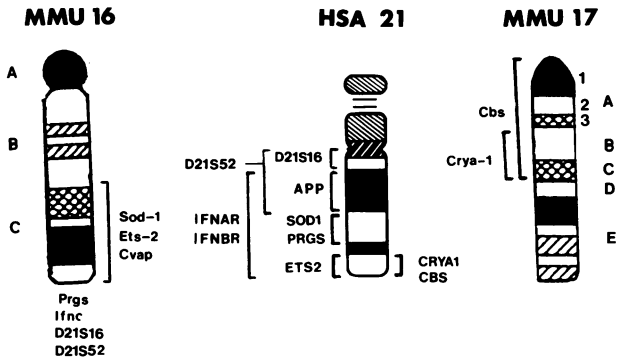

Figure 5 Comparative mapping of genes and anonymous DNA sequences on human chromosome (HSA) 21 and mouse chromosomes (MMU) 16 and 17. Ideograms are ISCN standard for HSA 21 and from Nesbitt and Francke (1973) for the mouse chromosomes. For gene symbols and references see Discussion.

This subregional assignment is also supported by human/mouse comparative mapping information (fig. 5). In addition to *CBS*, one other gene locus is known to reside on HSA 21 and MMU 17: αA-crystalline (*CRYA1*), a structural protein found in the lens of the mammalian eye (Quax-Jeuken et al. 1985; Skow and Donner, 1985). Recently, the human *CRYA1* locus was assigned to subband 21q22.3 by Southern analysis of somatic cell hybrids (Hawkins et al. 1987). The homologous locus in mouse (*Crya-1* or *Acry-1*) is closely linked to the major histocompatibility complex (MHC) on proximal MMU 17 and maps between *H-2K* and the glyoxylase-1 locus (*Glo-1*) (Skow and Donner 1985). In the rat, *Acry-1* is also linked to the MHC (Skow et al. 1985). Our assignment of *Cbs* to the proximal (A-C) region of MMU 17 is consistent with *Cbs* being close to *Acry-1*. It is noteworthy that the homologous loci in the human, *GLO1* and the *MHC*, map to the short arm of chromosome 6 (Francke and Pellegrino 1977; McBreen et al. 1977). The region containing *Acry-1* and *Cbs* may have been inserted between *Glo-1* and *H-2K* in rodents—or been removed from this site and placed near *SOD1* in primates—after the divergence of primates and rodents from a common ancestor. Comparative mapping of these genes in other mammalian species will be needed to trace the steps of this evolutionary rearrangement.

Another group of genes on HSA 21 have homolo-

gous loci on MMU 16: *SOD1* and *PRGS*, both assigned to band 21q22.1 and the distal region of MMU 16 (Cox and Epstein 1985); the receptor for interferons α and β on HSA 21q21→qter (Raziuddin et al. 1984) and on MMU 16 (Cox et al. 1980); the protooncogene *ETS2* on HSA 21q22→qter (Watson et al. 1986) and distal to *Sod-1* on MMU 16 (Reeves et al. 1987); and the β amyloid precursor protein gene (*APP*) on HSA 21q11.2→q22.2 (Tanzi et al. 1987) and on the distal region of MMU 16 (*Cvap*) (Philip et al. 1987; Reeves et al., in press). In addition to known genes, anonymous DNA markers *D21S16* and *D21S52* on HSA 21q11 and 21q11→q21, respectively, have been linked to genes on MMU 16 (Cheng et al. 1987). Several genes on HSA 21 have not yet been mapped in the mouse. Those with a proximal assignment in 21q11.2→q22.2, such as glycinamide ribonucleotide transformylase (*PGFT*) (Hards et al. 1986), are likely to belong to the conserved syntenic group on MMU 16, while distally located genes such as *PFKL* (Van Keuren et al. 1986) may well be on MMU 17.

Since it is likely that CBS (inasmuch as DS has features opposite to homocystinuria) and ACRY (possibly related to presenile cataracts present in DS) play a role in generating the DS phenotype when present in three gene copies, a mouse model with partial trisomy of MMU 16 alone cannot be expected to manifest the full spectrum of human DS, although several pertinent features—such as intrauterine growth retardation, facial dysmorphy, retardation of brain development, congenital heart disease (aortic arch anomalies and endocardial cushion defects), and immunologic and hematologic defects—have been reported (Epstein et al. 1985).

Familial Alzheimer disease has been linked to the β amyloid precursor protein locus (*APP*) on the proximal region 21q11→q21 (St. George-Hyslop et al. 1987). Since an Alzheimer-like dementia is a feature of DS, it seems likely that APP plays a role. Therefore, the notion that a very small "pathogenetic" segment of 21q is responsible for the DS phenotype has to be reconsidered. Clinical cytogenetic observations have provided evidence that triplication of band 21q22 alone is sufficient to produce the more classical DS features (Aula et al. 1973; Mikkelsen 1974; Williams et al. 1975; Raoul et al. 1976; Wahrman et al. 1976; Pfeiffer et al. 1977; Emberger et al. 1980; Summitt 1981; Kirkilionis and Sergovich 1986), whereas triplication of the proximal region (21pter→q21) is not sufficient to do so (Williams et al. 1975; Raoul et al. 1976; Hagemeijer and Smit 1977; Daniel 1979; Leschot et al. 1981; Kitsiou-Tzeli et al. 1984).

Subband localization of the critical region for DS has been attempted but is controversial. Assignments have been made to 21q22.1 (Sinet et al. 1976; Casalone et al. 1979; Huret et al. 1987), 21q22.1→q22.2 (Poissonnier et al. 1976), 21q22.2→qter (Cervenka et al. 1977; Habedank and Rodewald 1982; Taysi et al. 1982), or 21q22.3 (Mattei et al. 1981). Inverted dicentric tandem translocations (with presumed loss of distal 21q material) present in addition to a normal 21 have been described in individuals with DS (Niebuhr 1974; Berg et al. 1980). Some case reports clearly indicate that triplication of only a part of 21q22 results in a DS phenotype with certain clinical features missing (Niebuhr 1974; Mattei et al. 1981; Habedank and Rodewald 1982; Huret et al. 1987). Thus, trisomy for most of 21q22.1→q22.3 may be necessary to produce the full clinical spectrum of this syndrome, and localization of the critical region for DS to one of the three subbands may not be possible. With the precise delineation of 21q homologous regions in mice, the contribution of individual genes to the DS phenotype can be studied in mice trisomic for the corresponding regions on MMU 16 and MMU 17.

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