Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6032–6036, August 1988
Genetics

Comparative mapping of DNA markers from the familial Alzheimer disease and Down syndrome regions of human chromosome 21 to mouse chromosomes 16 and 17

(restriction fragment length polymorphism/genetic linkage analysis/recombinant inbred strains/interspecific backcross)

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Communicated by Elizabeth S. Russell, April 18, 1988

ABSTRACT **Mouse trisomy 16 has been proposed as an animal model of Down syndrome (DS), since this chromosome contains homologues of several loci from the q22 band of human chromosome 21. The recent mapping of the defect causing familial Alzheimer disease (FAD) and the locus encoding the Alzheimer amyloid β precursor protein (*APP*) to human chromosome 21 has prompted a more detailed examination of the extent of conservation of this linkage group between the two species. Using anonymous DNA probes and cloned genes from human chromosome 21 in a combination of recombinant inbred and interspecific mouse backcross analyses, we have established that the linkage group shared by mouse chromosome 16 includes not only the critical DS region of human chromosome 21 but also the *APP* gene and FAD-linked markers. Extending from the anonymous DNA locus *D21S52* to *ETS2*, the linkage map of six loci spans 39% recombination in man but only 6.4% recombination in the mouse. A break in synteny occurs distal to *ETS2*, with the homologue of the human marker *D21S56* mapping to mouse chromosome 17. Conservation of the linkage relationships of markers in the FAD region suggests that the murine homologue of the FAD locus probably maps to chromosome 16 and that detailed comparison of the corresponding region in both species could facilitate identification of the primary defect in this disorder. The break in synteny between the terminal portion of human chromosome 21 and mouse chromosome 16 indicates, however, that mouse trisomy 16 may not represent a complete model of DS.**

Human chromosome 21, the smallest autosome comprising 1.9% of the genome, has been extensively characterized by cytogenetic approaches and molecular techniques, including the development of physical and genetic maps (1). A primary impetus for this effort is the role of chromosome 21 in Down syndrome (DS), one of the most common causes of mental retardation (2). Karyotypic analyses of cases of partial trisomy 21 have indicated that only part of the chromosome, band 21q22, is required for full manifestation of the DS phenotype (3–8). Though this region probably contains a few hundred genes that could contribute to the disorder, an unequivocal causal role has not yet been established for any individual locus. However, since the syntenic relationship of several candidate genes such as superoxide dismutase (*SOD1*), the *ets-2* protooncogene (*ETS2*), phosphoribosylglycinamide synthetase (*PRGS*), and the interferon receptors (*IFNAR* and *IFNBR*) has apparently been conserved in the mouse genome, mouse trisomy 16 has been used as an animal model of DS (9, 10).

Interest in human chromosome 21 has increased with the recent localizations of the defect causing familial Alzheimer disease (FAD) and the gene (*APP*) encoding the precursor for amyloid β protein to the proximal half of 21q (11, 12). FAD is the autosomal dominantly inherited form of the common late-onset neurodegenerative disorder that results in the gradual and devastating impairment of memory and cognition. Amyloid β protein is a major component of the neuritic plaques seen in sporadic and inherited forms of Alzheimer disease (AD) and in DS. To assess the potential utility of mouse chromosome 16 in the investigation of AD and DS, we have compared the genetic linkage relationships of the murine homologues of a number of loci spanning 21q, including the genes *APP*, *SOD1*, and *ETS2* and several cross-hybridizing anonymous DNA loci.

METHODS

Mice. Progenitor inbred mouse strains AKR/J, C57BL/6J, C3H/HEJ, C57L/J, and DBA/2J and the recombinant inbred (RI) sets AKXD, AKXL, BXD, and BXH were purchased from The Jackson Laboratory. Recombination estimates and 95% confidence intervals (CIs) for the RI analysis were determined from Silver (13).

Interspecific Backcross. C57BL/6J – *Re Tr*/+ + females crossed to *Mus spretus* [Spain] males and F_1 hybrid females were backcrossed to C57BL/6J males. Genomic DNAs from each backcross progeny were prepared from samples of spleen and liver as described (14). Approximate 95% CIs on the estimated recombination frequency were calculated as described (15).

DNA Probes and Southern Blot Hybridization. The human chromosome 21 probes tested for cross-hybridization were pGSE9 (*D21S16*), 511-1H/511-2P (*D21S52*), pPW228C (*D21S1*), pPW245D (*D21S8*), FB68L and HL124 (*APP*), pSG1-10 (*SOD1*), 524-5P (*D21S58*), H33 (*ETS2*), 520-10R (*D21S56*), pGSE8 (*D21S15*), pPW231C (*D21S3*), and pPW242B (*D21S7*) (12, 16–21). DNA probes were prepared and labeled for hybridization to Southern blots of genomic DNA digested with 10–35 restriction enzymes as described (22, 23).

***Glo* Typing.** The BXD RI strains were characterized with respect to quantitative variation in erythrocyte glyoxylase

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Abbreviations: DS, Down syndrome; FAD, familial Alzheimer disease; RFLP, restriction fragment length polymorphism; SDP, strain distribution pattern; CI, confidence interval; RI, recombinant inbred.

activity, which results from a cis-acting element that maps to the *Glo-1* structural gene locus (24). To facilitate classification, each BXD strain was outcrossed to the SWR/J strain, which bears the *Glo-1*^{b} electrophoretic variant. The relative contribution of the *Glo-1*^{a} allele from the BXD strain to the *Glo-1*^{a}/*Glo-1*^{b} allozyme pattern was compared to (SWR/J × C57BL/6J)F_1 and (SWR/J × DBA/2J)F_1 controls. RI strains with relatively little GLO-1A homodimer were judged to carry the C57BL/6J allele and strains with relatively more of the GLO-1A homodimer were judged to carry the DBA/2J allele.

RESULTS

To define the murine counterparts for the DS and FAD chromosomal regions, probes for human chromosome 21 DNA loci spanning 21q were employed for restriction fragment length polymorphism (RFLP) linkage analysis in the mouse. The murine homologues for *SOD1* and *ETS2*, *Sod-1* and *Ets-2*, respectively, have previously been localized to the distal portion of mouse chromosome 16. These human loci from 21q22 are considered markers for the region of chromosome 21 associated with manifestation of the DS phenotype (9, 10).

The linkage relationships of the murine homologues detected by the human chromosome 21 probes were initially assessed by using four sets of RI strains (25), each derived by inbreeding the progeny of a cross between two parental inbred strains. Comparison of the strain distribution patterns (SDPs) of the genotypes for any two loci with differing parental genotypes provides a measure of their linkage relationship, thereby allowing the rapid chromosomal localization of new loci relative to those previously typed in the same RI sets.

Each human DNA probe was checked by Southern blot analysis for its degree of cross-hybridization to DNA from the progenitor inbred mouse strains. The potential for using anonymous human DNA RFLP markers for interspecies mapping depends on the extent of divergence in the homologous DNA sequences between the two species. In general, coding sequences would be expected to show less divergence than noncoding sequences. Therefore, anonymous DNA markers that show cross-hybridization are more likely to contain coding sequences. Only seven of the chromosome 12 DNA markers tested (see *Methods*) showed reproducible cross-hybridization to mouse DNA: the genes *SOD1*, *ETS2*, and *APP* and the anonymous DNA sequences *D21S16*, *D21S52*, *D21S56*, and *D21S58*.

Subsequently, the cross-hybridizing markers were screened for RFLPs by hybridizing under nonstringent conditions to Southern blots containing up to 35 restriction enzyme digests of genomic DNA from a series of inbred mouse strains: A/J, AKR/J, C57BL/6J, C3H/HeJ, C57L/J, DBA/2J, and SJL/J. With the exception of *ETS2* and *D21S58*, the markers revealed RFLPs (Table 1) in the progenitor inbred strains making them useful for analysis of some RI lines with representatives of the DS (*SOD1*, *APP*, *D21S56*) and FAD regions (*D21S16*, *D21S52*). An example of the polymorphic cross-hybridizing Southern blot signal detected by a human DNA probe is illustrated in Fig. 1*A*, where a cDNA probe for *APP* revealed a *Msp* I RFLP between the C57BL/6J and the DBA/2J progenitor mouse strains.

For each marker displaying a RFLP in the mouse, genotypes of individual RI strains from the appropriate RI sets (AKXD, AKXL, BXD, and BXH) were determined. The SDPs for these markers are presented in Table 2. Comparison of these to the SDPs for a bank of 400 previously mapped loci was performed to assign chromosomal locations for the DNA markers. For the anonymous human DNA markers, we have used the human locus symbol to identify the homologous murine locus. For the homologue of the *APP* locus, we propose the symbol *App*.

The results of the RI analysis in the BXD and AKXD sets revealed that *App* and *D21S16* are closely linked with six differences among 49 lines, corresponding to a genetic separation of 3.7% recombination (CI = 1.6–9.9). However, these markers each displayed eight differences with the SDP for *Sod-1* in 26 strains of the BXD set of RI strains, though their human homologues display relatively close linkage. It was not possible to exclude these loci from chromosome 16 because of the paucity of previously assigned markers for this chromosome. Furthermore, *App*, *D21S16*, and *D21S52* gave negative linkage scores for markers on all other chromosomes, making it impossible to assign their chromosomal location.

Table 1. RFLPs used in RI mouse strains and interspecific backcross linkage analysis

Inbred strains					Interspecific backcross			
Locus	Restriction enzyme	RI strain progenitors*	Polymorphic fragments, kb	Constant fragments, kb	Locus	Restriction enzyme	*M. spretus* fragments, kb	C57BL/6J fragments, kb
D21S16	*Bgl* II	A, B	4.6	—	*D21S16*	*Xba* I	8.2	3.7
		D	3.3	—	*App*	*Xba* I	7.8	3.8
	*Bam*HI	A, B	18.0	—	*D21S52*	*Xba* I	10.1	4.4
		D	7.3	—	*Sod-1*	*Pvu* II	4.3	8.6, 7.0, 6.4
App	*Msp* I	D, H	4.0	3.9, 2.7, 2.0, 0.6, 0.4	*D21S58*	*Taq* I	3.8, 1.8	2.3, 1.2
		A, B	2.4	3.9, 2.7, 2.0, 0.6, 0.4	*Ets-2*	*Bam*HI	3.2	8.0
D21S52	*Taq* I	A	3.5	—				
		L	2.3	—				
	Pvu II	L	6.3	—				
		A	3.2	—				
Sod-1	*Pst* I	B, L	15.0	5.6, 5.8				
		A, D	9.5	5.6, 5.8				
	*Bam*HI	B, L	10.5	19.0				
		A, D	8.0	19.0				
D21S56	*Xmn* I	A, D, H	9.4	18.0				
		B, L	8.0	18.0				

kb, Kilobases.
*A, AKR/J; B, C57BL/6J; D, DBA/2J; H, C3H/HEJ; L, C57L/J.

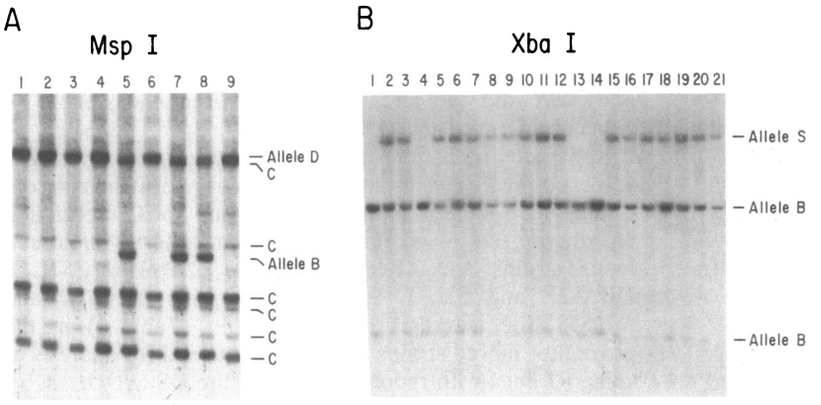

FIG. 1. Segregation of RFLPs in the recombinant inbred strains and the interspecific mouse backcross. Representative autoradiograms are shown for *Msp* I (*A*) and *Xba* I (*B*) digests of mouse DNA hybridized to the human APP cDNA probes HL124 and FB68L, respectively. (*A*) Lanes 1–9, mouse DNAs of a subset of the BXD RI strains. (*B*) Lanes 1–21, DNAs from some of the backcross progeny of the interspecific cross. Alleles for the progenitor strains C57BL/J, DBA/2J, and *M. spretus* [Spain] are shown as generic symbols B, D, and S, respectively. C represents a constant DNA band.

The RI analysis provided a chromosome assignment for *D21S56*, which showed significant linkage with loci in the proximal region of chromosome 17. *D21S56* is tightly linked to the quantitative variant of the glyoxylase-1 (*Glo-1*) locus, with no recombinants in 26 informative RI strains. Similarly, *D21S56* maps 5.4% recombination (CI = 2.6–12.7) distal to *Hba-4ps*, the hemoglobin α-chain pseudogene locus (nine differences in 55 RI strains), 0.6% recombination (CI = 0.02–3.8) from *Pim-1*, the preferred integration site for mink cell focus-forming viruses (one difference in 43 strains), 1.0% recombination (CI = 0.03–7.0) from *Crya-1*, the lens α-crystallin locus (one difference in 26 strains), and 2.6% recombination (CI = 1.1–7.0) proximal to *H-2*, the major histocompatibility locus (five differences in 56 strains) (26–31). The data are most consistent with the gene order *Hba-4ps-(Glo-1, D21S56)-Pim-1-Crya-1-H-2*. However, since the placement of *Glo-1* and *D21S56* proximal to *Pim-1* is based on a single crossover strain, alternative arrangements are possible.

Although RI analysis is a rapid method for detecting genetic linkage, classical backcross analysis provides greater sensitivity for larger recombination fractions and a more reliable means of determining the relative order of the various markers. Therefore, we used an interspecific backcross between C57BL/6J and *M. spretus* [Spain] to firmly establish that all of the cross-hybridizing loci except *D21S56* reside on chromosome 16 and to determine their map order. The use of an interspecific backcross, which in this case involved breeding C57BL/6J males with the F_1 female offspring of an interspecific cross between C57BL/6J and *M. spretus* [Spain], is more efficient than one involving only inbred strains due to the increased likelihood of finding segregating RFLPs for each marker locus (32). RFLPs distinguishing the parental mouse strains for each of the cross-hybridizing probes were identified by screening with up to 10 restriction enzymes (Table 1) and were subsequently used for linkage analysis using a combination of 63 progeny of the backcross (Fig. 1*B* and Table 3).

Significant linkage was detected among all six loci signifying the conservation of an extended syntenic group on mouse chromosome 16 and human chromosome 21. One crossover separated *Ets-2* from all the other loci. Three additional crossovers split the loci into two groups: (*Ets-2, Sod-1, D21S58*) and (*App, D21S16, D21S52*). Taken together, these data suggest the order (*App, D21S16, D21S52*)-(*D21S58, Sod-1*)-*Ets-2* (Table 3). This linkage group spans 6.4% recombination (CI = 2.0–15.0) with *Ets-2* being 1.6% recombination (CI = 0.01–8.0) from the (*D21S58, Sod-1*) cluster.

The 4.8% recombination observed between *Sod-1* and *App* in the backcross contrasts with the inability to prove linkage of these markers by the less-sensitive RI analysis. At this genetic distance, we would have expected 4 of 26 RI strains to differ between the two loci, corresponding to a recombination frequency of 5.0%, with 95% confidence limits of 1.2% to 18.3%. The observed eight differences in the SDPs predict a recombination frequency of 14.3%, which is well within these confidence limits. However, although the RI data are consistent with the backcross analysis, it is not by itself sufficient to prove linkage. Similarly, the estimate of 3.7% recombination between *App* and *D21S16* from the RI analysis appears to be at odds with the failure to detect any crossovers in 63 backcross progeny. However, the probability of such an occurrence is about 9% if the recombination estimate is accurate and as high as 50% if the lower confidence limit of this estimate is the true value.

Table 2. RI SDPs for mouse homologues of human chromosome 21 loci

AKXD strain	1	2	3	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	26	27	28				
D21S16	D	D	D	D	D	A	D	A	A	D	D	A	A	D	A	A	D	A	A	D	D	D	A				
App	D	D	A	D	D	A	D	A	A	D	A	A	A	D	A	A	D	A	A	D	D	D	A				
AKXL strain	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38									
Sod-1	A	A	A	L	L	A	L	A	A	A	A	L	L	A	—	L	—	A									
D21S52	A	L	L	L	L	A	L	L	A	L	A	L	L	A	A	A	A	A									
D21S56	L	A	L	A	L	L	A	L	L	L	L	A	A	L	L	L	L	A									
BXD strain	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32	
D21S16	B	D	B	B	D	B	D	D	B	D	D	D	B	D	D	B	B	D	D	D	D	B	D	B	B	D	
App	B	D	D	B	B	B	D	B	B	D	D	D	B	D	D	D	B	D	D	D	D	B	D	B	B	D	
Sod-1	B	B	B	B	B	D	D	B	B	D	D	B	B	D	D	B	B	B	D	D	D	D	D	B	B	B	
D21S56	D	B	D	D	B	D	D	B	B	B	B	D	D	B	B	D	D	B	D	D	B	D	B	D	D	D	
Glo-1	D	B	D	D	B	D	D	B	B	B	B	D	D	B	B	D	D	B	D	D	B	D	B	D	D	D	
BXH strain	2	3	4	6	7	8	9	10	11	12	14	19															
App	B	B	B	B	H	H	B	B	H	H	B	H															
D21S56	H	H	B	H	H	B	B	B	B	H	H	B															

For each RI strain, the symbol shown indicates the presence of an allele characteristic of one or the other of the progenitors from which the strains were derived (A, AKR/J; B, C57BL/6J; D, DBA/2J; H, C3H/HEJ; L, C57L/J).

Table 3. Interspecific backcross linkage: allelic combinations inherited from the F_1 parent

Allelic combination	Loci					n
	D21S16 *D21S52* *App*		*Sod-1* *D21S58**		*Ets-2*	
Parental	B		B		B	23
	S		S		S	36
Recombinant	B		B	×	S	0
	S		S	×	B	1
	B	×	S		S	2
	S	×	B		B	1
Total						63

*Due to lack of DNA, *D21S58* was typed in 58 of the F_1 progeny. The five mice not typed included four showing no recombination between any of the other markers and one of the "B S S" recombinant type.

A comparison of the interspecific mouse linkage map with the human female linkage map (refs. 17, 33; J.F.G., unpublished data) shows the extent of the syntenic region shared by the two species (Fig. 2). The order of the loci in the mouse is consistent with the established order of the human loci extending from the 21q11 band through 21q22.3: *(D21S52, D21S16)-APP-SOD1-D21S58-ETS2*, thereby demonstrating linkage as well as synteny conservation. In man, however, the linkage group spans a much larger genetic distance of ≈39% recombination. The relative compression of the syntenic group in the mouse is due to a lack of recombination between *D21S16* and *App*, whose homologues display 16% recombination in the human female, and to much tighter linkage of *Sod-1* and *Ets-2* than their human homologues. By contrast, the linkage distance between the amyloid precursor protein gene and the superoxide dismutase locus is relatively similar in both species. Results of the RI analysis giving a genetic distance of 3.7% recombination (CI = 1.6–9.9) between *App* and *D21S16* suggest that apparent compression

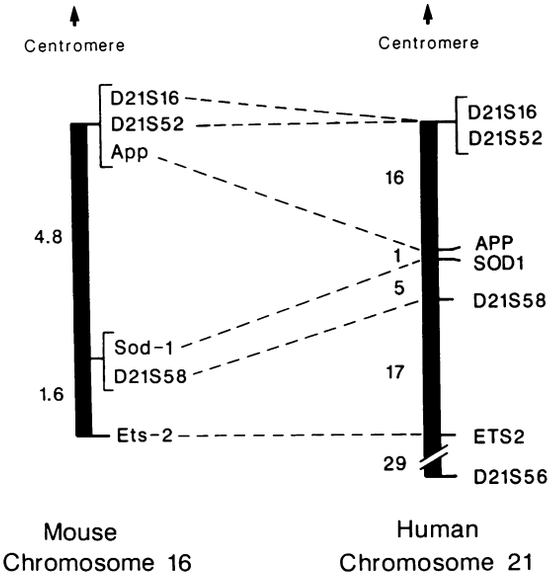

FIG. 2. Comparison of the syntenic linkage maps of human chromosome 21 and mouse chromosome 16. The illustrated mouse genetic linkage map of chromosome 16 was constructed by using the interspecific backcross data for comparison with the previously established linkage map of human chromosome 21 (17, 33). Genetic distance is given as recombination frequency. Since sex-specific differences in recombination frequency have been observed on human chromosome 21, the map displays the recombination frequency for human female meioses (all F_1 animals in the backcross were female). The relative order of loci within brackets is not known.

of the interspecific linkage map in this particular region could be due in some degree to the interspecific nature of the backcross.

In the human, the frequency of recombination on 21q increases dramatically toward the telomere (17, 33), resulting in an estimated 29% recombination between *ETS2* and *D21S56*, both located in the terminal 21q22.3 subband. Within this region there is a break in synteny with the mouse, since *D21S56* maps unequivocally to mouse chromosome 17.

DISCUSSION

Mapping of the human and mouse genomes has revealed the existence of conserved linkage relationships for many genes, with syntenic stretches averaging about 8.1 ± 1.6 centimorgans in the mouse (34, 35). Consequently, the murine homologues of human genes associated with specific genetic disorders may often be present in a conserved linkage group of significant size, creating the potential for generating new insights into the human disorder by detailed comparative analysis of the homologous mouse region. For disorders involving gene dosage, such as DS, the development of an accurate mouse model also becomes a possibility. Previous studies have established that mouse chromosome 16 contains some genes whose human homologues reside in 21q22, the critical DS region of chromosome 21, and have led to directed attempts to produce mice with trisomy 16. These do not survive as live-born animals but do display some phenotypic features reminiscent of the human disorder (9).

Recent molecular genetic investigations have also implicated chromosome 21 in AD, a common late-onset neurodegenerative disorder producing progressive dementia. The defect causing the inherited form of AD has been assigned by linkage analysis to chromosome 21 (11). In addition, the gene encoding the precursor of the amyloid β protein observed in the senile plaques of AD has been cloned and mapped to this autosome (12). The fact that aged DS patients also develop AD-like neuropathology suggests that AD may involve overexpression of a gene in the proximal portion of 21q.

To explore the potential for mouse trisomy 16 to act as a model of DS and AD, we have expanded and constructed a detailed linkage map for the region of synteny with human chromosome 21. Overall, loci spanning at least 39% recombination of the long arm of human chromosome 21 are present on mouse chromosome 16. The three identified genes included in the analysis, *APP*, *SOD1*, and *ETS2*, all lie in the region of chromosome 21 associated with manifestation of the DS phenotype and are present in the same relative order on mouse chromosome 16. Considering the previously mapped biochemical markers *PRGS*, *IFNAR*, and *IFNBR*, six genes have now been mapped to these homologous chromosome regions.

The inclusion of anonymous DNA sequences in the linkage analysis has revealed that the region of homology extends to *D21S16* and *D21S52*, markers linked to FAD, raising the possibility that mouse chromosome 16 contains a normal homologue of the FAD locus. Identification of coding sequences in genomic DNA is often facilitated by assessing the relative degree of conservation of particular sequences between man and mouse (36). Detailed molecular comparison of corresponding mouse and human regions surrounding the disease gene might therefore hasten identification of the primary defect in FAD.

In the mouse, the shared loci retain the same order as in the human but span just 6.4% recombination. Even allowing for general differences in recombination frequency in the two species, this number is still surprisingly low. A similar apparent condensation of the genetic map has been reported by Reeves *et al.* (37), who used a cross between the inbred *Mus musculus domesticus* (BALB) and the wild-derived

subspecies *Mus musculus musculus* (Czech II) to estimate the genetic distance between *Sod-1* and *Ets-2*. These investigators observed only one crossover in 86 chances when (Czech II × BALB/cPt)F_1 females were backcrossed with Czech II males. When the same distance was estimated from a backcross involving two inbred strains [(BALB/cBy × CBA/J) × BALB/cBy], five recombinations were detected in 61 events (37). The presence of a small inversion involving one of the flanking markers could have the effect of suppressing recombination without obviously changing the gene order (14, 38). An alternative explanation is that the region between *D21S16* and *APP* in the human contains additional DNA sequences not present on mouse chromosome 16, thereby increasing the relative genetic distance between the two loci. This would have profound significance for the use of trisomy 16 as a model of DS, since some of this additional material on chromosome 21 might contribute to the DS phenotype.

The conserved linkage group does not involve the entire 21q arm, as there is a break in synteny distal to *ETS2*, with the homologue of *D21S56* mapping to mouse chromosome 17. Recently, two other loci from the terminal region of 21q22, *CRYA*, encoding lens crystallin α A (29, 39, 40), and *CBS*, encoding cystathionine β-synthetase (41), have been reported to have mouse homologues in the proximal portion of mouse chromosome 17, placing them close to *D21S56*. This suggests that some genes associated with the DS phenotype may not have homologues on mouse chromosome 16. Trisomy for this mouse chromosome may not, therefore, provide a complete model for the human disorder, and the effect of increased dosage for genes from the region of chromosome 17 homologous to human 21q22 warrants investigation.

We thank Karen Griffin for assistance in the typing of this paper and Gordon Stewart and Robert Hallewell for DNA probes. This work was funded by National Institutes of Health Grants NS20012, GM32461, GM39414, and AG06865. S.V.C. received a fellowship from the National Huntington's Disease Association. J.F.G. is a Searle Scholar of the Chicago Community Trust.

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