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# Transchromosomal mouse embryonic stem cell lines and chimeric mice that contain freely segregating segments of human chromosome 21

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**At least 8% of all human conceptions have major chromosome abnormalities and the frequency of chromosomal syndromes in newborns is >0.5%. Despite these disorders making a large contribution to human morbidity and mortality, we have little understanding of their aetiology and little molecular data on the importance of gene dosage to mammalian cells. Trisomy 21, which results in Down syndrome (DS), is the most frequent aneuploidy in humans (1 in 600 live births, up to 1 in 150 pregnancies world-wide) and is the most common known genetic cause of mental retardation. To investigate the molecular genetics of DS, we report here the creation of mice that carry different human chromosome 21 (Hsa21) fragments as a freely segregating extra chromosome. To produce these 'transchromosomal' animals, we placed a selectable marker into Hsa21 and transferred the chromosome from a human somatic cell line into mouse embryonic stem (ES) cells using irradiation microcell-mediated chromosome transfer (XMMCT). 'Transchromosomal' ES cells containing different Hsa21 regions ranging in size from ~50 to ~0.2 Mb have been used to create chimeric mice. These mice maintain Hsa21 sequences and express Hsa21 genes in multiple tissues. This novel use of the XMMCT protocol is applicable to investigations requiring the transfer of large chromosomal regions into ES or other cells and, in particular, the modelling of DS and other human aneuploidy syndromes.**

## INTRODUCTION

The human aneuploidy syndromes, such as Down syndrome (DS), are caused by the abnormal copy number of normal genes, whose function is thought to be dosage sensitive. Such syndromes are very common, highly deleterious and of great clinical interest, but are not tractable to current standard genetic approaches, such as linkage analysis, for determining which genes underlie the different features of these phenotypes (1–4). Some progress has been made in mapping the dosage-sensitive genes that give rise to DS, by correlating phenotype and genotype in rare individuals who are partially trisomic for Hsa21 and, for example, there is broad agreement that major effect gene(s) for mental retardation lie within a few megabases of the marker *D21S55* (5–7). However, as patient phenotypes are so variable, other phenotype–genotype correlations are difficult to find and can be controversial.

The mouse provides us with an experimental system that we can manipulate to resemble human trisomy 21 and in which we can map dosage-sensitive genes. One approach to capitalize on the genetic tractability of the mouse for understanding aneuploidy has been the creation of an '*in vivo* library'—a panel of yeast artificial chromosome (YAC) transgenic mice that contain a total of ~2 Mb of contiguous Hsa21 sequence (8). By assaying the mice for a specific cognitive deficit and correlating this with the presence or absence of Hsa21 YACs, one region, containing the *Minibrain* gene, has been implicated in causing neurological/behavioural deficits when it is present in three copies (9). By extending the concept of the *in vivo* library and placing large regions of a freely segregating Hsa21 into a mouse, we can make phenotype–genotype correlations without *a priori* assumptions about, for example, where dosage-sensitive genes map, whether such genes are likely to fit into current cloning vectors or which regulatory sequences are necessary to mimic the subtle 3:2 dosage of genes on trisomic compared with disomic chromosomes. A chromosomal *in vivo* library also gives us a model system in which to test the candidacy of such genes, and to study the interactions of individual genes with the rest of the genome. In addition, a mouse model provides us with a resource for analysing tissues at all developmental stages, and for testing therapies in the whole animal. Mice are not human, and we do not believe any mouse model will mimic human DS exactly, but they do provide us with a realistic and current route to understanding the molecular genetics and pathophysiology of DS and other aneuploidy syndromes.

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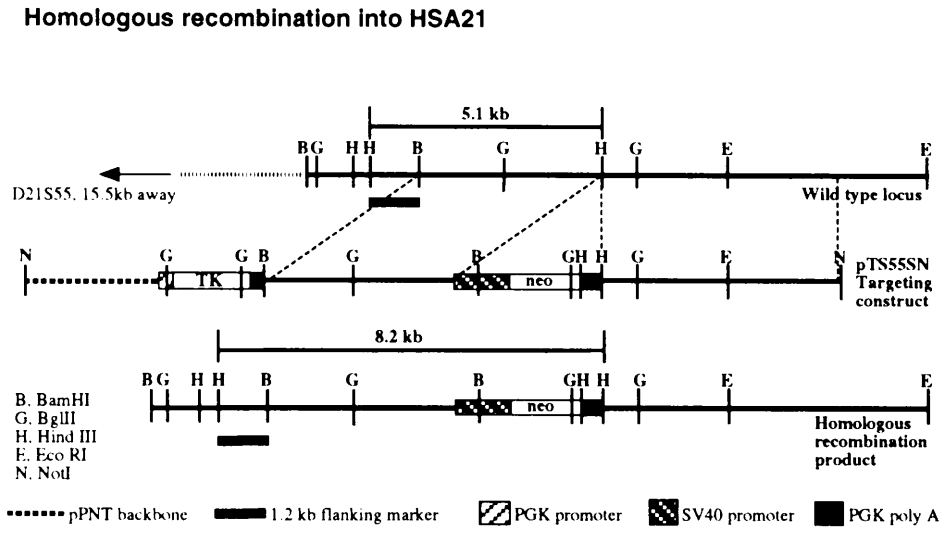


**Figure 1.** Scheme of homologous recombination near the locus *D21S55* with the pTS55SN targeting construct. pTS55SN contains the *neo* and *TK* genes to allow positive and negative selection (46) and has 9.3 kb of isogenic DNA from a site 15.5 kb from *D21S55*. The genomic sequence of this region is known, and we have inserted the *neo* gene into intron 2 of the *GIRK2* gene, whose expression appears unaffected (Fig. 5). Correctly targeted HT1080 cell lines were identified by the presence of an 8.2 kb *Hin*dIII fragment rather than the wild-type 5.1 kb *Hin*dIII fragment, when hybridized with the 1.2 kb flanking probe (data not shown).

To create 'transchromosomal' mice that carry freely segregating Hsa21 sequences, we have targeted a dominant selectable marker into the chromosome and have developed a protocol for moving large chromosome regions into mouse embryonic stem (ES) cells by a process of irradiation microcell-mediated chromosome transfer (XMMCT). The transchromosomal ES cells contain freely segregating human fragments and have three doses (two mouse, one human) of the genes on the trisomic chromosome. We have determined the Hsa21 content of 21 different transchromosomal ES cell lines and, using standard blastocyst injection methods, have created chimeric mice from four of the cell lines; these mice express human genes. Our panel of transchromosomal cell lines and chimeric mice are the first step towards creating an '*in vivo* library' for Hsa21 regions that span megabases. To our knowledge, this is the first time that XMMCT has been used in association with ES cell technologies to create a mouse model of aneuploidy.

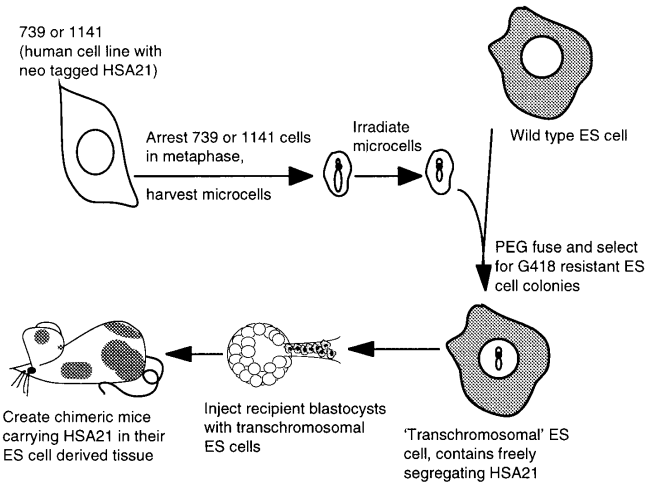


**Figure 2.** XMMCT protocol, as described in Materials and Methods, for placing Hsa21 sequences in mice.

## RESULTS

### Dominant selectable marker on Hsa21

To create transchromosomal ES cell lines, we needed a human donor line containing Hsa21 tagged with a dominant selectable marker. We created two new cell lines, 739 and 1141, by inserting a neomycin resistance gene (*neo*) near the locus *D21S55* by homologous recombination in the human cell line HT1080 (10) (Fig. 1). The *D21S55* locus was chosen for targeting because it lies within a region thought to contain gene(s) involved in the major aspects of DS, in particular mental retardation (5,6). The HT1080 cell line was chosen because it is one of very few human transformed cell lines known to be a successful donor in MMCTs (11). Both 739 and 1141 were used in our subsequent experiments.

### XMMCT and transchromosomal ES cell lines

Having targeted *D21S55*, we proceeded to transfer regions of Hsa21 into mouse ES cells by XMMCT (12,13) (Fig. 2). Briefly, the donor cell lines 739 and 1141 were treated with colcemid to induce microcell formation (14) and then centrifuged in a percoll gradient containing cytochalasin B (15,16). The microcell layer was collected and irradiated to kill any remaining donor human cells and to produce breaks in the human chromosomes (17); a series of experiments was carried out at irradiation levels of 3.5, 10, 20, 30, 40 and 50 krad. Irradiated microcells were fused to D3 ES cells using polyethylene glycol and then colonies were selected in G418 (Fig. 2). Resistant colonies were picked and, from then on, cultured in non-selective medium.

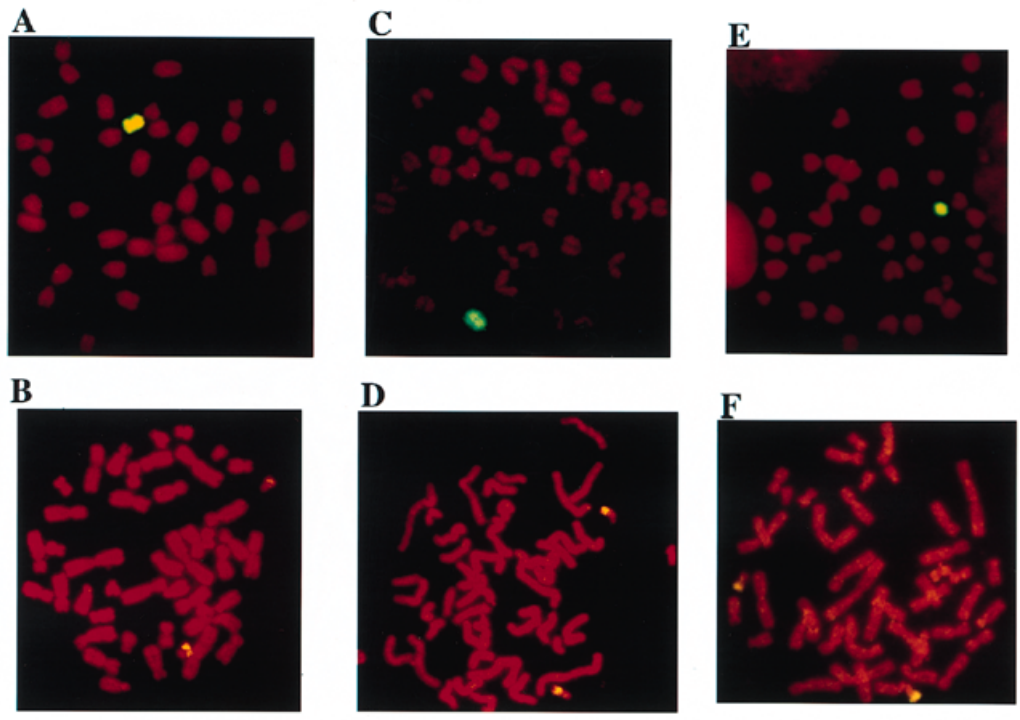


**Figure 3.** FISH (human Cot1 DNA probe hybridized to metaphase spreads of transchromosomal ES cell line) and IRS–FISH (Alu PCR products from each transchromosomal cell line as probe, hybridized to normal human male metaphase spreads) of transchromosomal cell lines containing Hsa21. (**A**) FISH of transchromosomal cell line 43-Q; (**B**) IRS–FISH with a probe derived from transchromosomal cell line 43-Q; (**C**) FISH of transchromosomal cell line 44-2; (**D**) IRS–FISH with a probe derived from transchromosomal cell line 44-2; (**E**) FISH of transchromosomal cell line 47-14; (**F**) IRS–FISH with a probe derived from transchromosomal cell line 47-14.

We carried out 14 XMMCT experiments, in which a total of $4.2 \times 10^9$ microcells were fused to $1.4 \times 10^9$ ES cells. From these, we isolated 49 different ES cell lines that were shown by Southern blot analysis to contain the *neo* gene, Hsa21 sequences adjacent to its site of integration and the *D21S55* locus. These lines have growth characteristics and morphologies identical to the parental D3 cells and are derived from experiments carried out at all six irradiation levels. Fluorescence *in situ* hybridization (FISH) analysis with human Cot1 DNA as the probe was undertaken to determine human chromosome number and whether the donor human DNA had integrated or was freely segregating in the lines; it is known from radiation fusion experiments that centromeres tend to be maintained in cell hybrids after irradiation of donor human chromosomes (18–20). We found three categories of transchromosomal cell line: 27 lines have one freely segregating human chromosome; 20 cell lines have two or more freely segregating human chromosomes; and two cell lines contain freely segregating human chromosomes and a fragment of human DNA that has integrated into the mouse genome (Fig. 3A, C and E, and data not shown). In transchromosomal cell lines cultured for extended periods, we found that human chromosomes are maintained in the absence of selection after at least six passages.

We undertook interspersed repetitive sequence (IRS)-FISH analysis to determine the chromosomal origin of the human DNA in our 27 transchromosomal cell lines that contain one single freely segregating human chromosome (Fig. 3B, D and F, and data not shown). Labelled Alu PCR products from 21 of the lines hybridize to Hsa21 only in human male metaphase spreads; products from six of the 27 lines hybridize to Hsa21 and to Hsa10, 14, 16p (twice), 18 and 19, respectively, indicating that material in addition to Hsa21 was transferred into the ES cells. These six cell lines were derived from XMMCT at 3.5, 30, 40 and 50 krad.

DNAs from the transchromosomal cell lines containing one Hsa21-derived freely segregating chromosome were subjected to PCR and Southern blot analysis with 33 markers spaced, on average, 1–2 Mb apart on Hsa21, to ascertain the integrity and content of the human chromosome (Fig. 4). We found that the transferred Hsa21 chromosomes have different stretches extending distal and proximal from *D21S55*, attached to a human centromere. This centromere is most probably derived from Hsa21 as judged by our results with the probe *D21Z1* (Fig. 4A). The stretches of Hsa21 appear likely to be contiguous, and while more detailed restriction mapping is required to confirm this, we note that other studies find almost no rearrangements of human chromosome fragments after irradiation (13,18) and no evidence of radiation breakage 'hotspots' on Hsa21 (21).

Our sample size from each irradiation level is too small to detect statistically significant correlations between chromosome content and irradiation; however, we do see a trend that those cell lines produced from lower irradiation doses tend to have larger human chromosomes, whereas those from higher doses tend to have smaller human fragments (Fig. 4). A study of a large set of radiation hybrids found that, in general, donor human chromosome fragment size was dependent on irradiation dose, but there was great variability between individual cell lines produced at the same dose (18).

The 21 transchromosomal lines containing one freely segregating human chromosome derived exclusively from Hsa21 were karyotyped to determine mouse chromosome number; 15 cell lines had a euploid modal number of 40 mouse chromosomes plus one

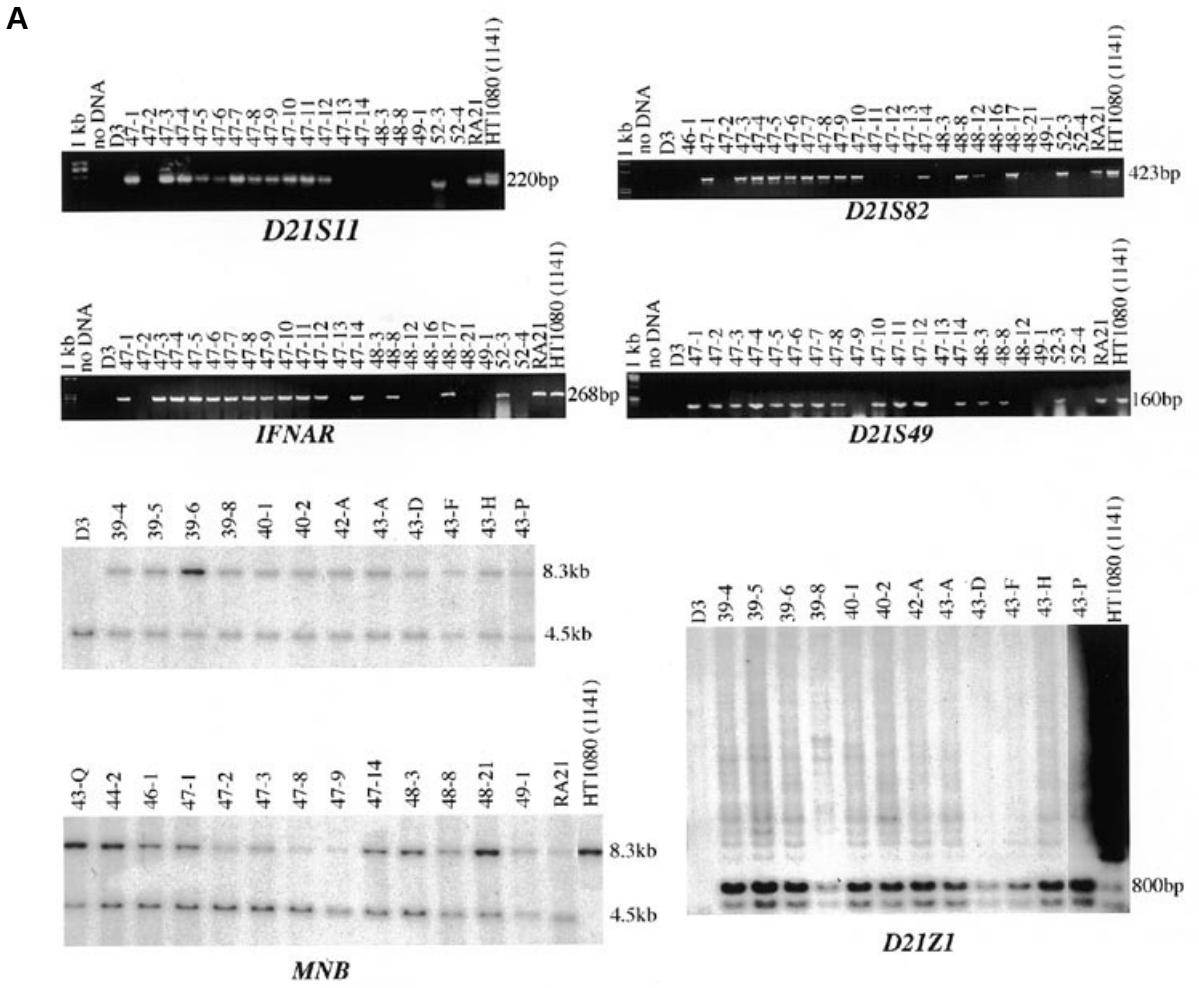


**Figure 4.** Hsa21 content of transchromosomal cell lines containing one freely segregating human chromosome derived exclusively from Hsa21, and chimeric mice produced from cell lines 47-1, 43-O, 49-1 and 46-1. (**A**) PCR and Southern blot analysis with a representative set of Hsa21 markers. The cell line name, marker name and expected size of the fragment are indicated. Transchromosomal cell lines are analysed with D3 wild-type ES cell line, RA21 (a cell hybrid containing Hsa21 only on a mouse background) and HT1080(1141) (targeted human cell line described above) as controls. *D21S11* (GenBank accession no. 188664), *D21S82* (192002), *IFNAR* (185155) and *D21S49* (196282) primer details are as given in the GenBank database. *MNB*: whole human cDNA hybridized to a *Hin*dIII digest of genomic DNAs; the 8.3 kb fragment is human, the 4.5 kb fragment is mouse. *D21Z1*: genomic DNA fragment (GenBank accession no. 166570) hybridized to an *Eco*RI digest of genomic DNAs. (**B**) The cell line names and microcell irradiation dose (in parentheses), and chimeric mice (cell lines 47-1, 43-O, 49-1 and 46-1) are shown above the vertical bars that indicate the Hsa21 regions thought to be present, as judged by the panel of Hsa21 markers shown on the left. Hsa21 loci have been positioned approximately according to the relative distances on the LDB map (22). Note that *D21S5* is a short arm marker, *D21Z1* detects the Hsa21 centromere, and loci *D21S16–COL6A1* run in order from the centromere to the 21q telomere. According to the LDB map, the length of Hsa21 from 21pter to 21qter is 50 Mb and the length of 21q from the centromere to 21qter is 49 Mb. All marker details are given in the GenBank database, except for *D21S55* which is described in (47), *SOD1* described in (48), and *SIM2* and *GIRK2* which are described in the legend to Figure 5.

transchromosomal Hsa21 (>10 metaphases examined); in the remaining six lines, >50% of cells were aneuploid with respect to mouse chromosome number. We have found a similar frequency of aneuploidy in other experiments involving gene targeting of D3 cell lines, and thus the aneuploid lines are most probably derived from the normal D3 background, rather than arising from the XMMCT protocol itself (V.L.J. Tybulewicz, unpublished data).

## Hsa21 in chimeric mice

We injected four transchromosomal cell lines into C57BL/6 blastocysts to create chimeric mice carrying Hsa21 chromosome portions. All the ES cell lines contain the Hsa21 centromere (Fig. 4); cell line 47-1 appears to contain the whole of Hsa21q; 43-O contains a segment of ~10.5 Mb from *D21S394* to *COL6A1*; 49-1 contains a smaller genomic region of ~4 Mb extending from at least *D21S394* to *ETS2*, and 46-1 contains the smallest Hsa21 fragment in our set of transchromosomal ES cell lines, of ~220 kb from *D21S55* to *GIRK2* [Figs 4B and 5A, Table 1; distances between markers are taken from the LDB map (22) except for *SIM2* to *GIRK2* which comes from Ohira *et al.* (23); 220 kb is the distance between *D21S55* and the 5′ end of *GIRK2*]. From injections with all four transchromosomal ES cell lines, we recovered live pups that are chimeric, as judged by coat colour and by flow cytometric analysis for the presence of the D3-specific Ly9.1 antigen on peripheral blood lymphocytes (data not shown).

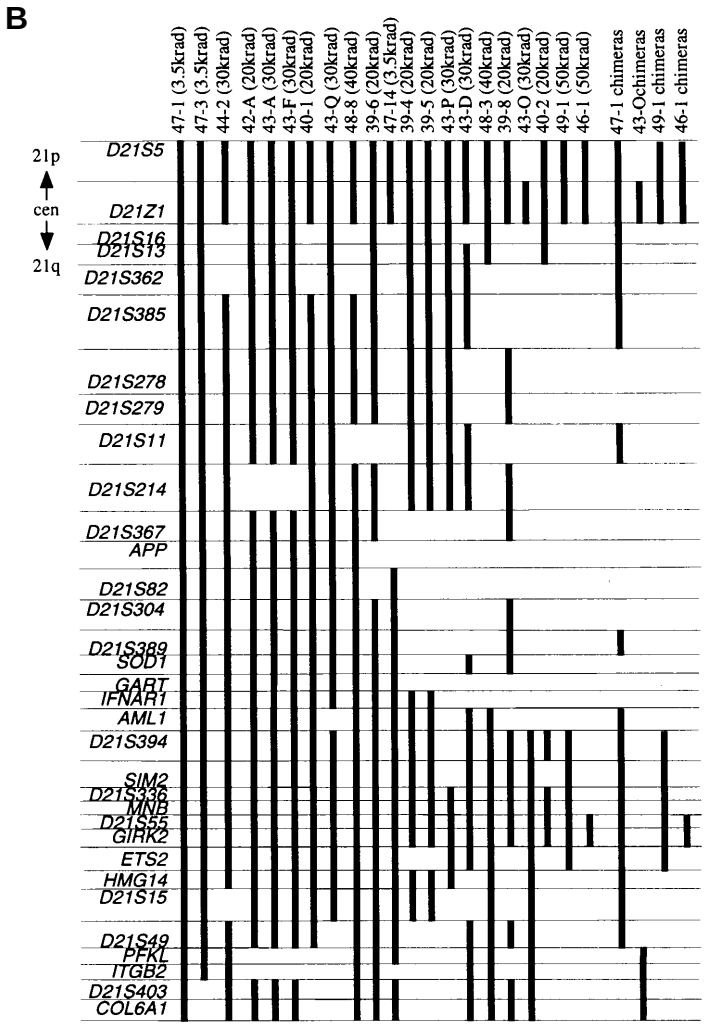


**Figure 4.** *Continued*

**Table 1.** Summary of Hsa21 DNA, gene expression and phenotype data from transchromosomal chimeric mice

| Cell line | Number of chimeras born | Number of chimeras Hsa21 positive/total number tested | Number of chimeric mice positive for expression of human genes in brain or heart/total number of chimeric mice tested (only Hsa21 positive chimeras were analysed | | | | | Number of mice showing kyphosis total number of chimeras |
|---|---|---|---|---|---|---|---|---|
| | | | SIM2 | MNB | GIRK2 | ETS2 | ITGB2 | |
| 47–1 | 6 | 2/6 | 0/2 | 2/2 | 2/2 | 2/2 | N | 0/6 |
| 43-O | 23 | 12/16 | N/A | N/A | N/A | N/A | 2/2 | 0/23 |
| 49–1 | 36 | 15/36 | 4/4 | 4/4 | 4/4 | 4/4 | N/A | 5/36 |
| 46–1 | 10 | 8/10 | N/A | N/A | 2/2 | N/A | N/A | 0/10 |

N/A, not applicable (the gene is not present in the mice).

The process of chromosome transfer into ES cells could alter their ability to form chimeras with adult mouse tissues. PCR analysis of tail biopsy DNAs shows that the chimeras contain human sequences, and thus the XMMCT protocol does not appear to compromise differentiation of ES cells, and also the human chromosome fragment is maintained *in vivo* (Fig. 5A). However, not all chimeras tested had detectable levels of human DNA—this may be because of low level chimerism for the transchromosomal ES cell line or because of human chromosome loss in some mice (Table 1).

Chimeric mice made with cell lines 49-1 and 46-1 contain the expected Hsa21-derived sequences (15 and eight chimeras, respectively); tail biopsy DNAs from ten 43-O chimeric mice contain the expected Hsa21 sequences, except for a region that extends from *D21S394* to *D21S49*, which is present in the original 43-O ES cell line but appears to be deleted in the mice. Similarly,

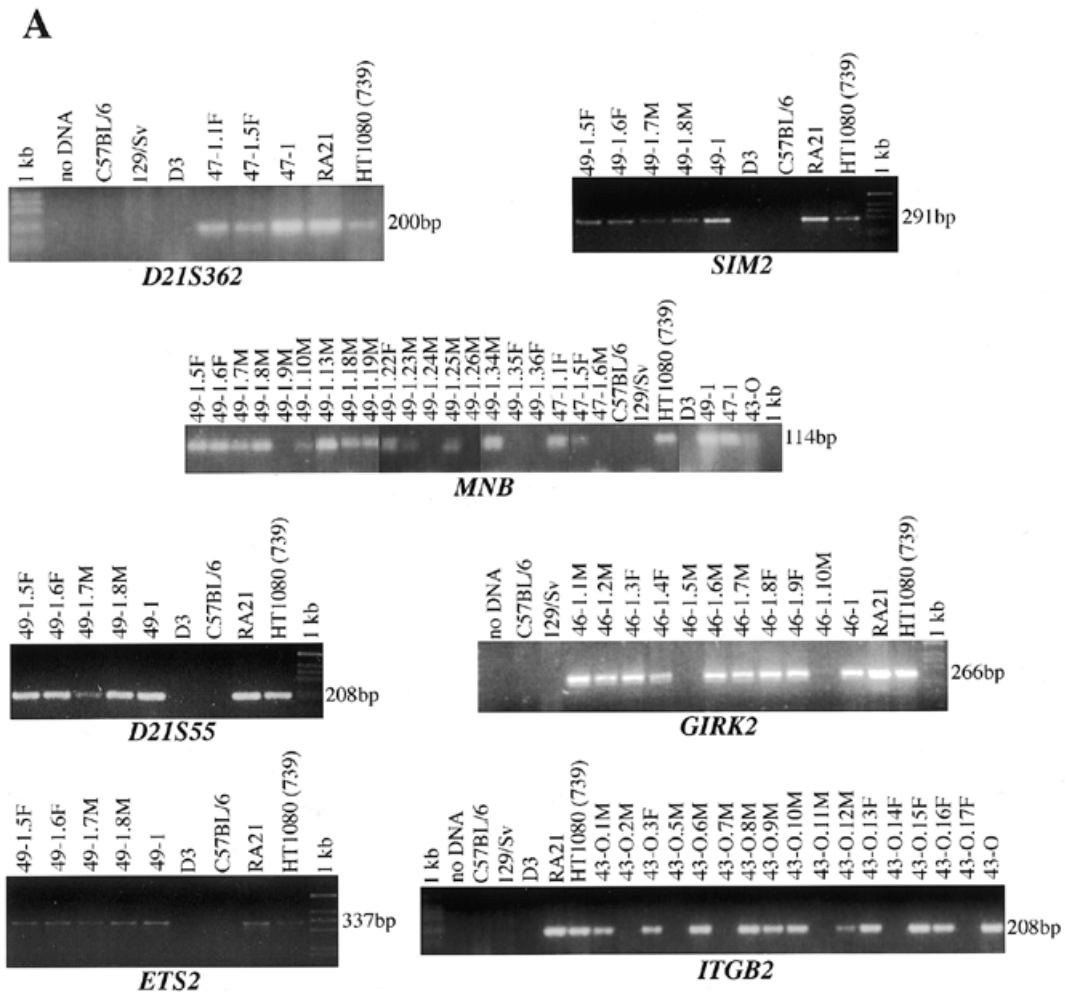


**Figure 5.** Hsa21 content and human gene expression of transchromosomal cell lines and chimeric mice made with these lines. (**A**) PCR analysis of a representative set of markers in genomic DNA from cell lines and tail biopsy DNA of chimeric mice derived from injections of these lines into C57BL/6 blastocysts (M, male; F, female). Also included is PCR analysis of genomic DNA of the parental D3 ES cell line, the donor targeted human cell line HT1080(739), a monochromosomal cell hybrid containing Hsa21 as its only human component on a mouse background (RA21), and tail biopsy DNA from a C57BL/6 mouse and a 129/Sv mouse. Expected fragment sizes of PCR products are shown. *D21S362* (GenBank accession no. 190735); *SIM2* (SIM2F, AAAGCCAACAAACCAAGAC; SIM2R, TTGTAGCAAACACGAGCC); *MNB* (MNBF, GTTGTAAAGGCATATGATCGTGTG; MNBR, GTTCATGAGCTCAAGAAGTCGCAC); *D21S55* (47); *GIRK2* (GIRK2F, CCCAAAATACTACACATCC; GIRK2R, GTTTGTCTTCAGCTCACC); *ETS2* (accession no. SHGC-6939); ITGB2 (accession no. 181564). Some chimeras show no amplification of the markers, possibly because of low levels of chimerism (<10%). (**B**) RT–PCR results of five genes that map within the Hsa21 region present in the chimeric mice (*SIM2*, *MNB*, *GIRK2*, *ETS2* and *ITGB2*). The expected sizes of RT–PCR products are shown; in each case, the primers span an intron which precludes amplification of genomic DNA under these conditions. In addition, RT–PCR analysis of *GdX*, a ubiquitously expressed X-linked mouse gene, was included to control for the presence of RNA in every mouse sample; the *GdX* primers span a small intron (115 bp), the expected sizes of RT–PCR (126 bp) and genomic DNA (241 bp) products are shown. *SIM2* (SIM2RNAF, GATGACCGCTGTCCTCACGGC; SIM2RNAR, CATATACTGCCTGATCTTCAAG), *MNB* (MNBRNAF, CAACCTCTAACTAACCAGAGGCG; MNBRNAR, TCCACACGATCATATGCCTTTAC), *GIRK2* (GIRK2RNAF, TTCATCCCGTTGAACCAGACGG; GIRK2RNAR, CCCATCCTCCAGGGTCAGGAC), *ETS2* (ETS2RNAF, TACTCAGCTCTGAGCAGGAGTTTC; ETS2RNAR, AACGTTCGATGTCATCCAGTGTTA), *ITGB2* (ITGB2F, GAGTGACGCACAGGAACCAG; ITGB2R, ATGGCAGAGGCTGCGGTCTC), *APP* (APPF, CATCCTGCAGTATTGCCAAGAG; APPR, CACAAAGTGGGGATGGGTCTTG), *GdX* (see ref. 5).

DNAs from two 47-1 chimeras also show chromosome fragmentation, with at least four deletions compared with the parental transchromosomal ES cell line (Fig. 4B). Because we are seeing the same deletions in chimeras made from the same transchromosomal ES lines, the most likely explanation is that the cell lines deleted in culture and we have injected subclones of them. Interestingly, the most telomeric breakpoint in both the 47-1 and 43-O mice lies in the ~670 kb that separate *D21S49* and *PFKL*; five of the 21 cell lines analysed in detail also have a breakpoint within this region (Fig. 4B).

It is possible that the XMMCT protocol could abolish human gene expression within the ES cells and/or chimeric mice. To investigate this, we carried out RT–PCR analysis of human genes known to map to the Hsa21 fragments within the injected cell lines (Fig. 5B, Table 1): RNA was made from adult brain and heart from chimeric mice, human adult brain (frontal neo cortex),

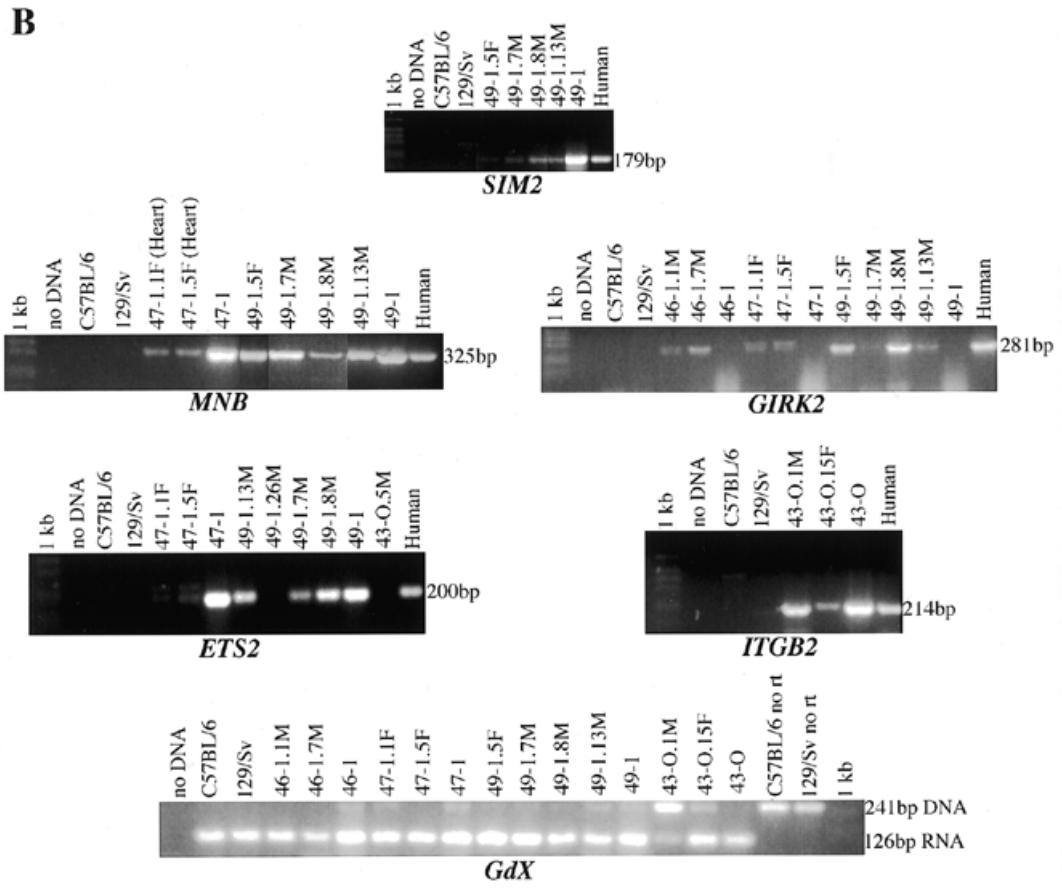


**Figure 5.** *Continued*

and controls, i.e. the parental transchromosomal cell lines C57BL/6 and 129/Sv mice (the D3 ES cells are derived from the 129/Sv strain of mice). The RT–PCR primers are human specific and exonic, but each pair spans one intron, thus ensuring that we detect transcribed products only.

All human genes present in the cell line DNAs were expressed in the corresponding ES cell RNA samples, with the exception of *GIRK2*, which was transcribed in adult chimeric mouse tissues, but was undetectable in the transchromosomal cell lines (Fig. 5B). *Girk2* is not detectable by RT–PCR until E14 of development in the mouse (24), and so would not be expected to be expressed in ES cell lines but would be transcribed in adult brain; thus, our results suggest that appropriate gene regulation is maintained in the transchromosomal cell lines. Human gene expression in the chimeric mice is variable, depending on the cell line, the gene and levels of chimerism in the tissue being assayed (Table 1). Most human genes that are present in chimeric mouse DNAs are detected by RT–PCR in the appropriate mouse tissues; however, for example, we were unable to detect *SIM2* transcription in adult brain or heart from two 47-1 chimeric mice (Table 1).

## Mouse phenotype

To assess the mice phenotypically, we carried out a standard analysis, the primary screen of the SHIRPA protocol (25), on the male chimeras and age- and sex-matched C57BL/6 and 129/Sv control mice. In total, six C57BL/6 mice, three 129/Sv mice and 22 chimeras were scored for a range of morphological and behavioural features. Test scores for most characteristics were within the normal range for all control mice, though we did notice differences between the two strains. In general, C57BL/6 mice were more active in the open test arena and also more aggressive and vocal when handled than 129/Sv mice. C57BL/6 animals had higher scores for transfer arousal and touch escape, and showed higher pelvic elevations. Morphologically, both strains look similar (except for coat colour) and show similar body sizes and weights at the same age.

We noticed marked morphological abnormalities in a subset of the 49-1-derived chimeras. Five high percentage (>50% agouti by coat colour) 49-1 chimeras were smaller than controls and littermates, and had kyphosis, which was combined with scoliosis in two of the mice. These five chimeras had shortened snouts, apparently due to a relative facial shortening seen in particular in the eye to nose length and the interocular distance, with a small degree of antero-posterior skull shortening. Other skeletal abnormalities included short forelimbs.

Twenty chimeric mice (from all four cell lines) were X-rayed, plus four C57BL/6 males and two chimeras consisting of the same C57BL/6 (blastocyst) and D3 (ES cell) combination in which the D3 cells were heterozygous for a *Brca2* gene targeting mutation (26). Examination of the X-rays clearly showed the morphologically visible kyphosis in the five affected 49-1 chimeras. Three of these mice also showed associated scoliosis with apparent loss of intervertebral disc space in the vertebral

bodies at the thoracico-lumbar level. Neither the controls nor 47-1, 43-O or 46-1 chimeras showed any gross malformation.

The finding of skeletal abnormalities was intriguing, because the *ETS2* gene is present in three copies in the 49-1 cell line, and transgenic studies have shown that a subtle increase in *Ets2* expression gives rise to mice with a variety of skeletal abnormalities including shortened snouts, abnormally shaped heads, shorter necks and kyphosis (27). Furthermore, similar skeletal abnormalities are found in trisomy 16 mice (which have three copies of *Ets2*) (28), and human DS individuals tend to have shorter necks and shorter long bones than non-DS individuals (29). However, we cannot rule out the possibility that the phenotype is due to a gain-of-function mutation in the ES cell line, as three out of the five mice that show kyphosis have no detectable human DNA sequences in tail biopsy DNA, and chimeric mice derived from the 47-1 cell line which contains *ETS2* (which is expressed, at least in brain) show no kyphosis.

### Germline transmission

We have bred chimeras extensively; 41 male chimeras from all four transchromosomal cell lines were test bred against (C57BL/6×DBA/2)$F_1$ mice and, of 2230 pups born, all were black and thus derived from the host blastocyst, not the ES cells.

## DISCUSSION

The development of new technology is an important step for creating mouse models of human aneuploidy syndromes. Currently such models fall into three classes, each with limitations: in the first class lie the mouse aneuploidies. Although only partial autosomal aneuploidies survive much beyond birth, these can be helpful for phenotypic studies but have limited use for the fine mapping and isolation of dosage-sensitive genes. Such mice include the Ts65Dn mouse, which is aneuploid and carries an extra small marker chromosome composed of the centromere of chromosome 17 and a large portion of chromosome 16, and the Ts1Cje mouse which has a chromosome rearrangement in which a smaller region of chromosome 16 has translocated onto chromosome 12; thus, the mice are euploid but partially trisomic for chromosome 16 (30–32). Genetic manipulation by Cre-*loxP*-induced recombination (33,34) will increase the range of partial aneuploidies; however, each human chromosome has homologous linkage groups on a number of mouse chromosomes and thus it will be difficult to create mouse trisomies with gene sets that are identical to human trisomies. YAC transgenic mice provide a more tractable model for human aneuploidy syndromes.

In the case of DS, Hsa21 YAC transgenic mice have been assayed for a reproducible cognitive deficit, and then, by creating new transgenics with decreasing stretches of Hsa21 DNA, the *Minibrain* gene has been implicated in neurological aspects of DS (8,9). However, this method of assaying for dosage-sensitive genes is too laborious for scanning a whole chromosome or chromosome arm, and has the disadvantage that large genes [such as the Hsa21 gene *DSCAM* (35)] may not fit onto YAC transgenic constructs and genes with distant regulatory sequences can be interrupted. Lastly, various single gene transgenic mice have been proposed as models for aspects of DS, but these involve just one gene (of the ~800–1000 thought to lie on Hsa21), usually with inappropriate levels and patterns of expression (28). Nevertheless, the YAC and single gene transgenics indicate that most human transgenes are expressed from their own promoters and can give rise to abnormal phenotypes from a subtle increase in gene product dose (9,27).

Recently, a paper by Tomizuka *et al*. was published (36) in which an MMCT protocol was used to create essentially transchromosomal mice carrying independently segregating stretches of the human immunoglobulin gene regions on Hsa2, 14 and 22. That study indicates the value of using MMCT for different biological investigations, and also shows that human chromosome fragments can survive stably in adult mouse tissues, and some can be transmitted through the mouse germline. Our research shows that the novel modification of irradiation MMCT is a successful approach for placing Hsa21 fragments of different sizes into mice and thus potentially modelling DS. In addition, we make one more important point for XMMCT modelling of human aneuploidy syndromes *per se* (rather than just creating transchromosomal mice): it is essential to use a human donor cell line, as we have done, and not a human–mouse cell hybrid as in the studies of Tomizuka *et al*. (36), because, firstly, there is no straightforward method to determine if donor mouse DNA has integrated into the genome of the recipient ES cell and, secondly, complex rearrangements of human and mouse DNA in the extra chromosome would be extremely difficult to detect—and our results suggest that in 22% of cases the extra chromosome consists of DNA fragments not selected for, i.e. from chromosomes other than Hsa21. We used a human somatic cell as a donor, in which we had targeted a dominant selectable marker into Hsa21; in future, it may be possible to tailor the DT40 chicken cell system (37,38) to XMMCT studies of human aneuploidy, by capitalizing on its high rate of homologous recombination, ability to form microcells and the genomic differences between the human/chicken donor and mouse recipient (39).

It is now apparent that germline transmission of an extra chromosome may be unlikely through the mouse male germline. In the Ts65Dn mouse, for example, the extra chromosome is transmitted through females only, and males are infertile, most probably because of arrest in male germline meiosis (30,32). Tomizuka *et al*. have bred transchromosomal female chimeras derived from a 39, X0 ES cell line that contains Hsa2 fragments, and produced 67 pups, of which 22 carried Hsa2 sequences (36). In contrast, four transchromosomal male chimeras derived from an XY ES cell line carrying a small portion of Hsa2 produced 316 pups of which two carried Hsa2 fragments. Thus, transmission of an extra, partially trisomic or human chromosome is possible through the mouse germline; however, transmission appears only practicable through the female germline (as is similarly the case with human trisomy 21). Our best strategy for germline transmission of Hsa21 is to create chimeric mice from transchromosomal female ES cell lines, and we are currently attempting to produce these cell lines.

Transchromosomal mice and cell lines provide us with the flexibility of a model genetic system for assessing the molecular consequences of aneuploidy. For example, for particular traits, dosage-sensitive candidate genes could be mapped and then their effects assayed by further rounds of genetic manipulation to reduce a three dose back to a two dose in mice. We can also start to address an essential feature of DS, the difference in penetrance and severity of most traits, by placing the extra chromosome onto different genetic backgrounds and determining which regions of the genome affect these traits. This is relevant to the non-DS

population, as many of the characteristic features of DS also occur in other individuals, not apparently trisomic for Hsa21.

For a recent review of this and other strategies for creating trisomy in the mouse, see ref. 49.

## MATERIALS AND METHODS

### Targeting HT1080 cells

We initiated a two-step chromosome walk by screening a genomic phage library from the human fibrosarcoma cell line, HT1080 (10) with a 2.5 kb *Eco*RI fragment from the *D21S55* locus. We isolated ~22 kb of *D21S55* flanking DNA from which we built an isogenic construct, pTS55SN, for electroporation into HT1080 cells. pTS55SN contains 9.3 kb of genomic DNA derived from a site 15.5 kb away from *D21S55* and is based on the pPNT targeting vector (40). An SV40-neo cassette was inserted into a *Hin*dIII site in the middle of the 9.3 kb homology region, and a PGK-TK cassette was placed adjacent to this genomic DNA (Fig. 1). The construct was linearized by digestion at the unique *Not*I site of the pPNT vector. A total of $68 \times 10^6$ HT1080 cells were electroporated with the linearized construct as described (41). Cells were then plated at limiting dilutions in 96-well plates, and double selection was applied 24 h later (400 μg/ml G418, 5 μM ganciclovir). A total of 1209 colonies survived and these were picked 10 days after electroporation and expanded into 24-well plates. DNA from the double-selected colonies was analysed by digestion with *Hin*dIII and probed with a 1.2 kb flanking fragment that detects a 5.1 kb fragment in parental HT1080 DNA and an 8.2 kb fragment in homologous recombinants (Fig. 1). From the 1209 colony DNAs, we found two cell lines, 739 and 1141, with a correct targeting event. Further restriction enzyme analysis and hybridization results indicated that both cell lines contain a single integrant and the structure of the targeted locus was as expected (data not shown).

### Production, harvesting and irradiation of microcells

The 739 or 1141 targeted HT1080 cells were micronucleated by prolonged arrest in colcemid (0.04 μg/ml colcemid for 48 h). Twenty-four 175 cm flasks of cells were harvested by trypsinization, resuspended in a mixture of serum-free Dulbecco's modified Eagle's medium (DMEM) and percoll (1:1), loaded into 50 ml polycarbonate Oak Ridge centrifuge tubes (Sorval, Stevenage, UK) containing cytochalasin B (10 μg/ml) and centrifuged (16 000 *g*) for 1 h to separate microcells from cell debris and whole cells. Following centrifugation, the microcells were recovered from the top of the gradients, washed and resuspended in serum-free DMEM. Microcells were filtered though 8 μM then 5 μM polycarbonate filters (Costar, High Wycombe, UK) to enrich for small microcells containing single chromosomes. The filtered microcells were irradiated at room temperature using a Nordion γ Cell Irradiator with a $^{137}$Cs source at a rate of 1000 rad/min; total doses used ranged from 3.5 to 50 krad.

### Fusion of microcells to mouse ES cells

Irradiated microcells were counted using a haemocytometer and mixed with equal numbers of ES cells that had been harvested previously using conventional techniques (typical numbers ranged from $100 \times 10^6$ to $300 \times 10^6$ microcells and $100 \times 10^6$ ES cells). The cell mix was pelleted and resuspended in 10 ml of serum-free DMEM containing 10 μg/ml PHA-P (Difco, West Molesey, UK), and microcells were allowed to agglutinate for 30 min at 37°C. Following agglutination, cells were fused in suspension using 2 ml of PEG 1500 (50%; Boehringer Mannheim, Lewes, UK). The PEG suspension was diluted with 20 ml of serum-free DMEM and cells were allowed to recover for 30 min at room temperature. Following incubation, cells were plated at a density of $2 \times 10^6$ cells/10 cm dish onto feeder layers in standard ES cell medium and cultured overnight. The next day, the medium was replaced with ES cell medium containing G418 (500 μg/ml) and cells were selected in this for 2 weeks. Surviving colonies were then picked and all further culture took place in medium without selection.

### Genotyping transchromosomal ES cell lines

*Karyotyping*. Conventional techniques were used to produce metaphase spreads from the transchromosomal cell lines, and chromosomes were counted after Leishman's staining.

*FISH analysis*. Labelling and hybridization of human Cot1 DNA to transchromosomal metaphase spreads were carried out essentially as described (18,42). Briefly, chromosome slides were treated with RNase for 1 h at 37°C and dehydrated in an ethanol series. The slides were then denatured in formamide at 75°C for 3 min, and immediately dehydrated in a cold ethanol series and hybridized to a similarly denatured biotin-labelled human Cot I DNA probe, under coverslips in a humid chamber at 37°C overnight. After hybridization, slides were washed in 50% formamide at 42°C for 20 min, followed by two 2× SSC washes at 42°C for 10 min. Hybridized human DNA was detected by a sandwich technique using fluorescein isothiocyanate (FITC)–avidin/anti-avidin antibodies/FITC–avidin treatment in series. After staining with propidium iodide/4′,6-diamidino-2-phenylindole (DAPI) antifade solution, the slides were analysed using a Leitz Aristoplan fluorescent microscope.

*IRS–FISH analysis*. This protocol was carried out essentially as in ref. 43, and the TC65 Alu-PCR primer was used to generate the probe from each cell line (44).

*DNA marker analysis*. PCR and Southern blotting/probe hybridization were carried out using standard techniques.

### Production of chimeras

Chimeras were produced using standard methods (45). D3 transchromosomal ES cells were injected into C57BL/6 blastocysts, and (C57BL/6×CBA/Ca)$F_1$ females were used as foster mothers.

### Genotyping transchromosomal chimeric mice

For DNA marker analysis, PCR and Southern blotting/probe hybridization were carried out using standard techniques. DNA was prepared from tail biopsies of chimeric mice.

### RT–PCR of transchromosomal chimeric mouse tissue

RNA was prepared from brain, heart and kidney of control (C57BL/6 and 129/Sv) and chimeric mice, human adult brain

(frontal neo cortex), D3 and transchromosomal ES cell lines, using the Qiagen Rneasy total RNA kit. RNA was then reverse transcribed using the Stratagene (Cambridge, UK) RT–PCR kit and first strand cDNA was used as template for PCR.

### Phenotypic and histological studies

Phenotype testing was performed according to the first stage of the SHIRPA protocol (21) (http://www.mgu.har.mrc.ac.uk/handbook/Mproto.html ). X-rays were taken post-mortem following formalin fixation, using a Faxitron X-ray machine at 30 keV for 1 min; film used was Kodak standard.

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