Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 2050–2054, March 1993
Genetics

Evaluation of potential models for imprinted and nonimprinted components of human chromosome 15q11–q13 syndromes by fine-structure homology mapping in the mouse

(chromosomal deletions/pink-eyed dilution locus/genomic imprinting/contiguous gene syndromes/γ-aminobutyric acid type A receptors)

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ABSTRACT **Prader–Willi and Angelman syndromes are complex neurobehavioral contiguous gene syndromes whose expression depends on the unmasking of genomic imprinting for different genetic loci in human chromosome 15q11–q13. The homologous chromosomal region in the mouse genome has been fine-mapped by using interspecific (*Mus spretus*) crosses and overlapping, radiation-induced deletions to evaluate potential animal models for both imprinted and nonimprinted components of these syndromes. Four evolutionarily conserved sequences from human 15q11–q13, including two cDNAs from fetal brain (DN10, *D15S12h*; DN34, *D15S9h-1*), a microdissected clone (MN7; *D15F37S1h*) expressed in mouse brain, and the gene for the β3 subunit of the γ-aminobutyric acid type A receptor (*Gabrb3*), were mapped in mouse chromosome 7 by analysis of deletions at the pink-eyed dilution (*p*) locus. Three of these loci are deleted in pre- and postnatally lethal *p*-locus mutations, which extend up to 5.5 ± 1.7 centimorgans (cM) proximal to *p*; *D15S9h-1*, which maps 1.1 ± 0.8 cM distal to *p* and is the mouse homolog of the human gene *D15S9* (which shows a DNA methylation imprint), is not deleted in any of the *p*-locus deletion series. A transcript from the *Gabrb3* gene, but not the transcript detected by MN7 at the *D15F37S1h* locus, is expressed in mice homozygous for the p^{6H} deletion, which have an abnormal neurological phenotype. Furthermore, the *Gabrb3* transcript is expressed equally well from the maternal or paternal chromosome 7 and, therefore, its expression is not imprinted in mouse brain. Deletions at the mouse *p* locus should serve as intermediate genetic reagents and models with which to analyze the genetics and etiology of individual components of human 15q11–q13 disorders.**

Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are clinically distinct disorders with complex developmental and neurological phenotypes often associated with cytogenetically visible deletions of human chromosome 15q11–q13, which are indistinguishable in extent (reviewed in ref. 1). PWS infants display hypotonia and a failure to thrive, which is then followed, 2–4 years later, by hyperphagia with consequent obesity, behavioral disorders, mild to moderate mental retardation with learning disabilities, hypogonadism, short stature, and mild facial dysmorphism (2). AS patients manifest severe mental retardation, absent speech, inappropriate laughter, puppet-like ataxic movements, microbrachycephaly, seizures, an abnormal electroencephalogram, mild hypotonia, and prognathism with tongue protrusion (3). Genomic imprinting plays a major role in the manifestation of PWS and AS (1). About 60–70% of PWS or AS patients carry a 15q11–q13 deletion that is of paternal or maternal origin, respectively, but the deletion is not cytogenetically different in the two cases. Likewise, most chromosomally normal PWS patients are characterized by uniparental maternal disomy for chromosome 15, and a few chromosomally normal AS patients have uniparental paternal disomy (1). In contrast, the remaining cytogenetically normal (biparental) AS patients may be mutant for a gene in the maternally derived 15q11–q13 (4, 5), and AS has been found in a family segregating for a submicroscopic chromosome 15 deletion that includes the gene for the β3 subunit of the γ-aminobutyric acid (GABA) type A receptor (*GABRB3*). No cases of PWS occurred in this family, suggesting that the critical region within 15q11–q13 is different for AS and PWS (6).

Because both PWS and AS are often associated with cytogenetically detectable deletions, they are good examples of contiguous gene syndromes (7). The number and function of genes responsible for each of the distinct phenotypes often exhibited by AS or PWS patients as well as the pattern of expression in different cell types of such genes are all unknown. Indeed, it is not yet completely understood whether all of the major phenotypes observed in PWS and AS are the result of an extremely pleiotropic mutation at a single, imprinted locus or are due to the combined effects of deletion or misexpression of both imprinted and nonimprinted 15q11–q13 loci. Thus, a full understanding of the genetic mechanisms and pathogenesis during development of PWS and AS, as well as of the role(s) of genomic imprinting, would be greatly aided by animal models, of which the mouse is highly suitable. Low-resolution mapping analyses using recombinant inbred strains have demonstrated that DNA clones derived from human chromosome 15q11–q13 identify loci in the mouse that are linked to the region containing the pink-eyed dilution (*p*) locus in chromosome 7 (8–12). Here, we present a higher-resolution map of the *p* region, based on interspecific backcross (IB) analysis and deletion mapping, that refines the homology map of human chromosome 15q11–q13 and the mouse *p* region. This refined map should be useful for relating genes and phenotypes identified in the mouse directly to the task of dissecting the components of PWS, AS, and possibly other disorders associated with alterations in human 15q11–q13.

MATERIALS AND METHODS

Mice. DNA from the inbred strain DBA/2J was purchased from The Jackson Laboratory. p^d/p^{6H} and *Mus spretus* mice

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Abbreviations: AS, Angelman syndrome; GABA, γ-aminobutyric acid; IB, interspecific backcross; PWS, Prader–Willi syndrome; RFLV, restriction fragment length variant; cM, centimorgan(s).

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were imported to Oak Ridge National Laboratory from The Jackson Laboratory and from V. Chapman (Roswell Park Memorial Institute, Buffalo, NY), respectively. All other mice originated and are maintained at Oak Ridge National Laboratory. Viable and lethal mutations at the chromosome 7 pink-eyed dilution (*p*) locus were recovered in the progeny of mutagen-exposed animals crossed to a tester stock marked with seven recessive mutations, including the original *p* mutation of the mouse fancy (see ref. 13 for review). Recessive-lethal *p* mutations (p^l) are maintained by outcrossing heterozygotes (+ c^{ch}/p^l +) to chinchilla (+ $c^{ch}/+$ c^{ch}) animals of the noninbred stock 2A; p^l heterozygotes in the next generation are identified by a testcross to either *p* c^{ch}/p c^{ch} or *ru-2* *p*/*ru-2* *p* animals. Alternatively, some p^l mutations (specifically, $p^{4THO-II}$, $p^{7FR60Lb}$, and p^{6H} in this study) are maintained as p^{7R75M}/p^l heterozygotes. The p^{7R75M} mutation is an intermediate allele of *p*, and p^{7R75M}/p^{7R75M} animals are darker in color than p^l/p^{7R75M} animals.

The generation of segregants from an interspecific backcross between laboratory mice (129/Rl-*p* c^{ch}/p c^{ch}) and *M. spretus* as well as the progeny-testing protocol used to place p^l mutations opposite *M. spretus* chromosomes 7 have been described (14). Fetuses, neonates, or juvenile mice homozygous for $p^{4THO-II}$, $p^{7FR60Lb}$, or p^{6H}, or compound heterozygotes involving p^{6H} and either the prenatally lethal $p^{46DFiOD}$ mutation or the neonatally lethal $p^{4THO-II}$ mutation, were identified in the progeny of a cross of appropriate heterozygotes by their pink eyes and/or dilute fur color. To obtain animals that had inherited the $p^{4THO-II}$ deletion from either the dam or sire, reciprocal crosses were made between 129/Rl-*p* c^{ch}/p c^{ch} and p^{7R75M} +/$p^{4THO-II}$ + mice. Pink-eyed offspring (*p* $c^{ch}/p^{4THO-II}$ + or $p^{4THO-II}$ +/*p* c^{ch}) from each cross were selected for analysis.

DNA Probes. Two human cDNAs (DN10 and DN34) isolated from a human fetal brain library (8, 15, 16) and a microdissected clone (MN7) (17) from chromosome 15q11–q13, as well as a human cDNA to *MYOD1* (14) and mouse cDNAs to *Igf1r* (18) and 34-1-111 (see below), were used in these studies. A *GABRB3* probe (designated 218/219) was prepared by PCR with primers for rat genomic DNA (19).

Molecular Analyses. DNA was prepared from spleen, liver, or fetuses by standard methods (20). Genomic DNA (3–10 μg) was digested with restriction enzymes, and DNA fragments were separated by agarose gel electrophoresis, transferred to nylon membranes, prehybridized, and hybridized as described elsewhere (15, 20). Membranes were washed at 56°C for mouse DNA probes or 42°C for human DNA probes in 0.1× SSC (0.015 M NaCl/0.0015 M sodium citrate)/0.1% SDS. Procedures for preparation of RNA and Northern blots have been described elsewhere (15, 20).

cDNA Cloning. Poly(A)$^+$ mRNA was prepared from the cerebellum, cortex, hypothalamus, and hippocampus of adult female CD-1 mice (Charles River Breeding Laboratories), and cDNA was synthesized following priming with random hexamers according to standard procedures (20). cDNA was made blunt-ended with T4 DNA polymerase, internal *Eco*RI sites were protected by methylation with *Eco*RI methylase, and phosphorylated *Eco*RI linkers were added. After digestion with *Eco*RI, the cDNA was ligated into *Eco*RI-digested, phosphatased arms of Lambda ZAP II (Stratagene), packaged, plated on XL1-Blue bacteria, and screened by plaque hybridization with radiolabeled DN34. Inserts from purified, positive phage were subcloned by direct excision from Lambda ZAP II, according to the manufacturer.

RESULTS

Isolation of a Mouse cDNA to a Candidate Imprinted Human Gene *D15S9* and Its Tight Linkage to *p* in Mouse Chromosome 7. The human DN34 cDNA clone, which detects a DNA methylation imprint at the *D15S9* locus in humans (16), recognizes several related loci in the mouse genome (9, 11). Using analysis of recombinant inbred strains and flow-sorted chromosomes, one DN34-related locus was mapped to chromosome 5, near *Bcd-1*, and one was mapped to chromosome 7 (9), while a mouse genomic clone (*D7Hms1*), isolated by hybridization to DN34, was mapped to chromosome 7 closely linked to the 15q11–q13 locus *D15S12h* (formerly designated *D7Nic1*) (11). To determine the map position of this chromosome 7 locus more precisely, we used DN34 to isolate a 1.2-kb mouse cDNA, designated 34-1-111, from an adult brain library (see *Materials and Methods*). Fig. 1 *A* and *B* shows that while the human cDNA DN34 recognizes several fragments in restriction enzyme-digested mouse DNA, the mouse cDNA 34-1-111 detects a single fragment. In each case, this single fragment is the most intensely hybridizing fragment detected by DN34.

To determine whether the 34-1-111 cDNA recognizes the homolog of the human 15q11–q13 locus *D15S9*, we tested whether a restriction fragment length variant (RFLV) detected by 34-1-111 would segregate with other *p*-region loci in an IB. We had previously shown complete linkage of *p* and *D15S12h* in 182 segregants of an IB (15). The locus detected by 34-1-111 was analyzed on this same panel by determining the segregation pattern of a 2.7-kb *Eco*RI fragment, present in 129/Rl DNA, and a 4.6-kb fragment, present in *M. spretus* DNA. The segregation of *p*/*D15S12h* and these RFLVs

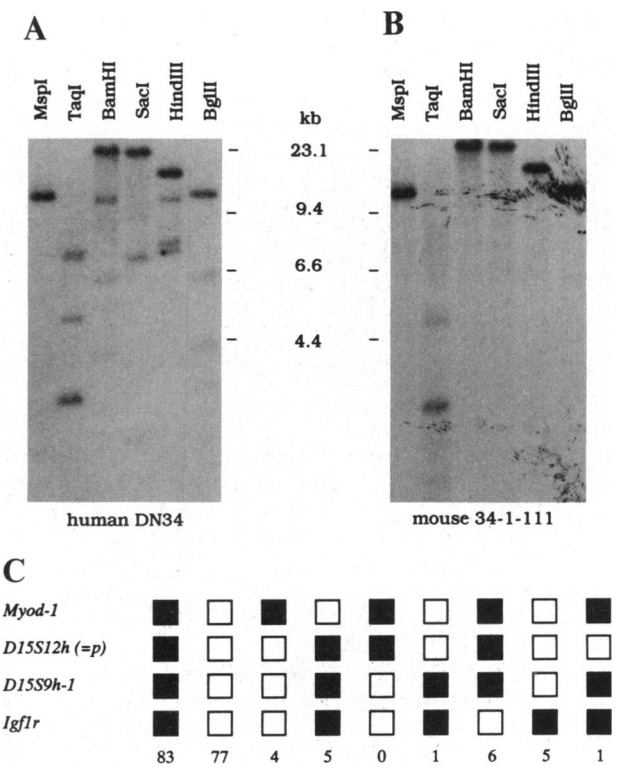

FIG. 1. Isolation of a mouse cDNA homologous to the human *D15S9* locus and its mapping to the *Myod-1*–*Igf1r* region by IB. Southern blot, prepared with DNA from DBA/2J mice digested with the enzymes listed, was hybridized with DN34 (*A*) or 34-1-111 (*B*). Sizes (kb) of *Hin*dIII fragments of phage λ are shown. (*C*) Segregation of RFLVs detected by 34-1-111 (*D15S9h-1*) in 182 segregants of an IB (14, 15) is presented along with segregation at three other chromosome 7 loci [*Myod-1*, *D15S12h* (=*p*), and *Igf1r*]. Solid and open squares represent 129/Rl and *M. spretus* alleles, respectively. Number of segregants in each class is indicated below each column. Recombination frequencies (in cM) and genetic order derived from these data are *Myod-1*–(5.5 ± 1.7)–*p*/*D15S12h*–(1.1 ± 0.8)–*D15S9h-1*–(6.0 ± 1.8)–*Igf1r*.

(which we have designated the *D15S9h-1* locus, replacing the symbol *D7Nic3*; ref. 9), along with alleles of *Myod-1* and *Igf1r*, is presented in Fig. 1*C*. Two recombinants were found between *p*/*D15S12h* and *D15S9h-1*, placing *D15S9h-1* distal to *p* at a distance of 1.1 ± 0.8 centimorgans (cM). Therefore, the 34-1-111 cDNA recognizes the *D15S9/DN34* homolog in mouse DNA. Based on these data, we suggest that the *D15S9h-1* (34-1-111) and *D7Hms1* (ref. 11) loci are identical.

Deletion of the *D15F37S1h* and *Gabrb3* Loci in Lethal *p* Locus Mutations. The close linkage of *D15S9h-1* and *D15S12h* to *p* in the IB, along with the findings (9, 11, 12) that the *D15F37S1h* (formerly designated *D7Nic2* or *D15F32S1h*) and *Gabrb3* loci were closely linked to *D15S12h* by analysis of recombinant inbred strains, suggested that these human 15q11–q13-related loci might be deleted in pre- or neonatally lethal, radiation-induced *p*-locus mutations. We had previously shown that *D15S12h* (DN10) is deleted in a number of radiation-induced *p* mutations (15). Thus, we tested for deletion of the *D15F37S1h* (probe MN7), *Gabrb3* (218/219), and *D15S9h-1* (34-1-111) loci in DNA derived from mice carrying several lethal *p*-locus mutations balanced opposite a *M. spretus* chromosome 7. Fig. 2*A* shows the analysis of a *Taq* I RFLV (2.5 kb in *Mus musculus* DNA and 2.7 kb in *M. spretus* DNA) at the *D15F37S1h* locus, a *Pst* I RFLV (3.6 kb in *M. musculus* DNA and 1.8 kb in *M. spretus* DNA) at the *Gabrb3* locus, and the *Eco*RI RFLV (see above) at the *D15S9h-1* locus. The *D15F37S1h* locus, but not the *Gabrb3* and *D15S9h-1* loci, is deleted in the prenatally lethal p^{3R30M} and $p^{46DFiOD}$ deletions. The $p^{46DFiOD}$ mutation also deletes the entire *p* (*D15S12h*; DN10) transcription unit (15) and the proximal marker *Myod-1* (14), whereas the p^{3R30M} mutation does not delete *Myod-1* (E.M.R. and L.B.R., unpublished data). The *D15S9h-1* locus is not deleted in any of these mutations, and of these three tested loci, only *Gabrb3* is deleted in $p^{4THO-II}$. Fig. 2*B* shows that the $p^{4THO-II}$ deletion interrupts the *p* (*D15S12h*) transcription unit, because in DNA obtained from $p^{4THO-II}/p^{4THO-II}$ homozygotes, which die perinatally (E.M.R. and L.B.R., unpublished data), the DN10 cDNA clone detects only a subset of the fragments present in wild-type DNA. Fig. 2*C* shows the results of sequential rehybridization of MN7, 218/219, and 34-1-111, respectively, to Southern blots of DNA from mice homozygous for two other mutations, $p^{7FR60Lb}$, which die perinatally (E.M.R. and L.B.R., unpublished data) and p^{6H}, which often live to juvenile stages and beyond, and are characterized by small size, nervous behavior, and male sterility (21). *D15F37S1h* is deleted in both p^{6H} and $p^{7FR60Lb}$; the *Gabrb3* probe 218/219 is deleted in $p^{7FR60Lb}$ but not in p^{6H}; and neither mutation deletes the *D15S9h-1* sequence recognized by 34-1-111.

Fig. 3 presents a map of the *Myod-1–Igf1r* region of chromosome 7 based on the deletion-mapping data. The orientation of the map is based on the facts that (*i*) IB analysis places *D15S9h-1* distal to *p*, (*ii*) the $p^{46DFiOD}$ deletion includes *Myod-1* (14) but not the segment of the *Gabrb3* locus defined by the 218/219 probe, and (*iii*) $p^{4THO-II}$ breaks within the *D15S12h* (*p*) transcription unit and includes *Gabrb3* but not *D15F37S1h*. The map in Fig. 3 also shows a locus (*pl*) representing one of a number of genes probably required for prenatal development that are defined by prenatally lethal *p* deletions (ref. 22; L.B.R. and E.M.R., unpublished data).

The *Gabrb3* Gene Is Not Involved in the Neurological Phenotype Observed in p^{6H} Homozygotes. As noted previously, mice homozygous for the p^{6H} mutation are runty, male sterile, and typically manifest a nervous, quivering behavior (21). The results presented in Fig. 2*C* demonstrate that the p^{6H} deletion does not affect the sequence represented by the 218/219 probe at the *Gabrb3* locus. However, because this probe is only a small part of the total *Gabrb3* cDNA, it was still possible that the p^{6H} deletion disrupts expression of the *Gabrb3* gene. This possibility was tested by hybridizing the 218/219 probe to Northern blots of poly(A)$^+$ RNA prepared from brains of p^{6H} homozygotes and heterozygotes. Fig. 4 demonstrates that the predominant 5.5-kb *Gabrb3* transcript is expressed in p^{6H}/p^{6H} mice. As expected from the data in Fig. 2*C*, the ≈14-kb transcript detected by the MN7 probe at the *D15F37S1h* locus (17) is not expressed in the brains of p^{6H}/p^{6H} mice.

The *Gabrb3* Locus Is Not Parentally Imprinted in Mouse Brain. Deletion of at least a segment of the *Gabrb3* coding region in the $p^{4THO-II}$ mutation (Fig. 2*A*) made it possible to test directly whether the expression of this gene is parentally imprinted. Because *GABRB3* in humans maps to the AS critical region (*ANCR*; refs. 6 and 19), a determination of

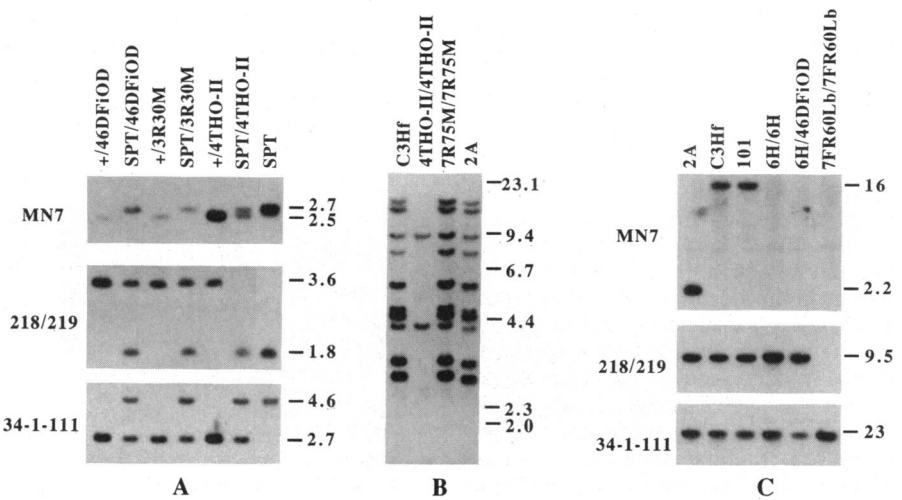

FIG. 2. Mapping of human 15q11–q13-related loci with lethal *p* deletions. (*A*) Spleen DNAs from the indicated genotypes were digested with *Taq* I (MN7; *D15F37S1h*), *Pst* I (218/219; *Gabrb3*), or *Eco*RI (34-1-111; *D15S9h-1*); electrophoresed; blotted to nylon; and hybridized with the probes indicated on the left. Each designation is an abbreviated form of the complete genotype—e.g., 46DFiOD = $p^{46DFiOD}$ and SPT denotes *M. spretus*. Sizes (kb) of RFLVs followed in this analysis are given on the right. (*B*) *Bam*HI-digested splenic or fetal DNA derived from the indicated strains and genotypes was hybridized with DN10 (*D15S12h*) (15). DNA from 101/Rl mice, the other parental control, gave a pattern identical to that observed in C3Hf/Rl (data not shown). (*C*) Southern blot, prepared from *Bam*HI-digested splenic or fetal DNA derived from the indicated strains and genotypes, was hybridized with radiolabeled probes indicated on the left. A *Bam*HI restriction fragment length polymorphism between 2A and C3H/101 DNA observed with MN7 is irrelevant to this analysis.

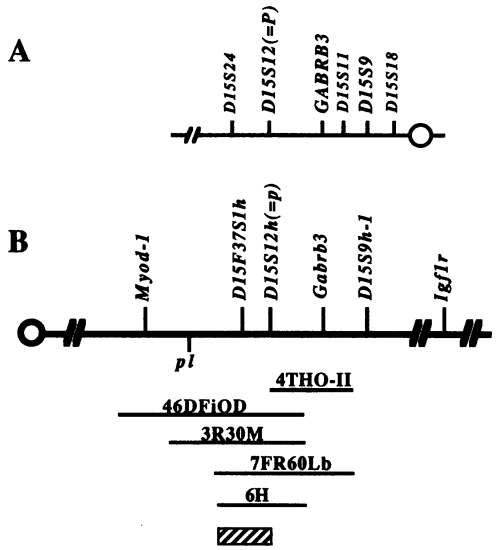

FIG. 3. Map of human 15q11–q13-related region in mouse chromosome 7. Mouse chromosome 7 is indicated in *B*, and the centromere is represented on the left by a circle. See Fig. 1 legend for genetic distances. The *pl* (prenatal lethal) marker below the map represents one of probably a number of genes defined by lethal *p* mutations that are required for normal prenatal development. Extent of deletions with respect to markers on the genetic map is indicated below the map, and the name of each deletion is indicated. Hatched box represents region associated with the p^{6H} neurologic/sterility syndrome (designated R/JG/S in ref. 22). Corresponding region of human chromosome 15q is indicated above the mouse map in *A*. Again, the centromere is indicated by a circle. MN7 clone, which detects the *D15F37S1h* locus in mouse DNA, detects a number of sequences in human DNA; some of these (*D15F37*) map to 15q11–q13, but their order with respect to other loci is not yet known (17).

whether the expression of this gene is imprinted in mouse might provide supporting evidence for any role of *GABRB3* in AS. Consequently, the 218/219 probe was hybridized to Northern blots of total RNA prepared from the brains of $p^{4THO-II}/p$ mice that had inherited the deletion from either the dam or the sire. Fig. 5 demonstrates that the *Gabrb3* gene is expressed equally well from either the maternal or paternal chromosome (i.e., from the chromosome derived from the 129/Rl parent in either case), a result consistent with the fact that the $p^{4THO-II}$ deletion can be passed through either parent without any phenotypic consequence (E.M.R. and L.B.R., unpublished data).

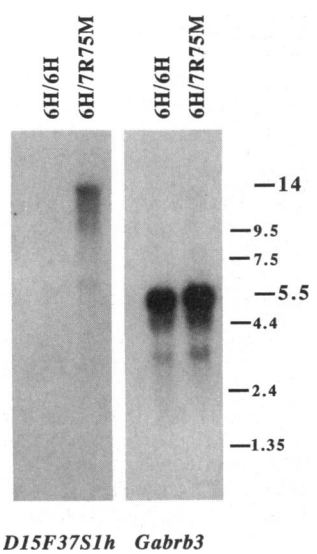

FIG. 4. Analysis of RNA transcripts from the *D15F37S1h* and *Gabrb3* loci in mice heterozygous or homozygous for the p^{6H} deletion. Northern blot, prepared from ≈3 μg of poly(A)$^+$ RNA from adult brains of mice of the indicated genotypes, was hybridized with MN7 (*D15F37S1h*) or 218/219 (*Gabrb3*). Sizes (kb) of RNA ladder standards (BRL) are listed on the right in smaller type; sizes of predominant transcripts detected for each probe are listed in larger type.

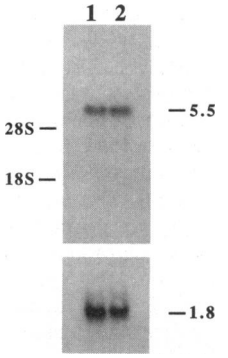

FIG. 5. Analysis of RNA transcripts from *Gabrb3* locus in mice inheriting the $p^{4THO-II}$ deletion either maternally or paternally. Northern blot, prepared from 20 μg of total RNA from adult brains of $p^{4THO-II}/p$ (lane 1) or $p/p^{4THO-II}$ (lane 2) mice, was hybridized with the 218/219 probe. *Gabrb3* transcript in lane 1 derives from the paternal chromosome (the $p^{4THO-II}$ deletion was inherited from the dam) and transcript in lane 2 derives from the maternal chromosome (where the deletion was inherited from the sire). Positions of rRNAs are indicated on the left, and sizes (kb) of transcripts are indicated on the right. (*Lower*) The 1.8-kb transcript detected on this blot by a chicken tubulin control probe.

DISCUSSION

Mapping of four evolutionarily conserved human loci in lethal *p*-locus mutations in mice has demonstrated conservation of fine-structure synteny between human chromosome 15q11–q13 and the *p* region of mouse chromosome 7. This conservation of both gene content and gene order suggests that mutations in the mouse *p* region may serve well as experimental reagents with which to dissect the complex phenotypes of the contiguous gene syndromes PWS and AS. For example, the only significant clinical difference between PWS and AS patients who carry a cytogenetically detectable deletion versus those that do not is that hypopigmentation of the skin, hair, and eyes is often observed in the patients with the deletion (23, 24). The suggestion that the *D15S12* locus is the human homolog of the *p* gene in mice and is likely associated with the hypopigmentation sometimes observed in PWS/AS patients who carry cytogenetically detectable deletions (15, 25), as well as the finding that both copies of *D15S12* are mutated in at least one patient with tyrosinase-positive oculocutaneous albinism (15), provides an illustrative example of the use of mouse mutations for identifying individual components of human contiguous gene syndromes.

Any major gene(s) that contributes to phenotypes unique to either AS or PWS must be imprinted (1). Deletion of the β3 subunit of the GABA receptor (*GABRB3*) in a family segregating AS (6) has made this gene a candidate for the primary defect in AS (6, 19). GABA is an inhibitory neurotransmitter in the mammalian adult central nervous system, although a role in excitatory transmission of neonatal hippocampal neurons has recently been suggested (26). However, it is not yet known whether *GABRB3* is imprinted in humans. We have shown that *Gabrb3* is not parentally imprinted in mouse brain and that mice hemizygous for the gene are phenotypically normal. Others have made similar observations in mice with maternal uniparental disomy of this segment of mouse chromosome 7 (31). If imprinting of specific genes is conserved throughout evolution, as is the case for the *H19* gene in mouse and human (27), one might conclude that *GABRB3* is not a good candidate for AS; however, it remains to be proved whether evolutionary conservation of imprinting at the individual gene level is a general phenomenon. Cogent in this context are the data of Kuwano *et al.* (28), which suggest that the currently defined Angelman critical region (*ANCR*),

which does contain the *GABRB3* gene, is >650 kb and has the potential to encode many other genes.

One gene from human 15q11–q13 that may be imprinted is the proximally mapping locus *D15S9*, identified by the DN34 cDNA. This gene demonstrates a differential DNA methylation imprint between parental chromosomes in humans (16), but further work is required to show whether the gene is functionally imprinted. The homologous locus in the mouse, *D15S9h-1*, identified by the mouse 34-1-111 cDNA clone, maps distal to *p*, between *Gabrb3* and *Igf1r* (Fig. 3), and is not deleted in any *p* deletion reported here. In fact, we have evidence (E.M.R., unpublished data) that this locus is not deleted in any of 31 *p* deletions tested to date, an unexpected result considering that *D15S9h-1* is only 1.1 ± 0.8 cM from *p*. Because the majority of these deletions were derived from mutagenized paternal genomes (E.M.R. and L.B.R., unpublished data), it is tempting to speculate that *D15S9h-1* or a closely linked locus may be functionally imprinted in the mouse and that deletion of this region may be a dominant-lethal event.

Defects at the *Gabrb3* locus cannot be the cause of the abnormal neurological phenotype (21) exhibited by mice homozygous for the p^{6H} deletion since brains of these animals express the *Gabrb3* transcript at normal levels. In this context, it is intriguing that p^{6H}/p^{6H} mice are deleted for the *D15F37S1h* locus and, therefore, do not express the high-molecular-weight transcript in brain that is identified by the MN7 microdissection clone (Fig. 4; ref. 17). $p^{6H}/p^{4THO-II}$ compound heterozygotes are normal in viability, behavior, and fertility, which demonstrates that the p^{6H} neurological/reproductive syndrome maps proximal to *p*, within the region anchored by the expressed *D15F37S1h* locus (Fig. 3). If phenotypes observed in the p^{6H} syndrome are homologous to any nonimprinted components of PWS, AS, or expanded syndromes observed in patients with deletions extending distally to *D15S12* (29, 30), loci included in p^{6H}, or in deletions extending more proximally, could possibly be correlated with components of the corresponding human syndome. Because *Myod-1* maps to human 11p15 (14), it will be important to determine the proximal extent of the human 15q11–q13 homology within the mouse *Myod-1–D15F37S1h* interval. Thus, any new phenotypes or expressed loci identified by proximally extending *p* deletions could then be correlated with the appropriate human genomic region (15q11–q13, 11p15, or others), with subsequent evaluation of such phenotypes and/or loci as models for disorders mapped to that region.

When considering the degree of mutational pleiotropy often observed in human genetic syndromes associated with chromosomal deletions, it will be necessary to distinguish between true pleiotropy, caused by defects at a single locus, and apparent pleiotropy, which might be defined as the manifestation of alterations at a number of loci, as can happen in contiguous gene syndromes. Use of the mouse as an intermediate reagent for studying a multigenic disorder, such as in the analysis of the components of PWS, AS, or other human 15q11–q13 disorders, as reported here, should prove to be one tractable strategy for addressing this question.

Note Added in Proof. The imprinted gene *Snrpn* has recently been closely linked to the *Gabrb3* locus (31, 32).

We would like to thank R. Neve for the DN10 and DN34 cDNA clones; K. Avidano, T. Markland, A. Garcia, C. Culiat, K. Houser, L. Taylor, P. Hunsicker, and C. Montgomery for technical assistance; L. Stubbs, R. Woychik, and D. Johnson for comments on the manuscript; and L. Flaherty for her hospitality during E.M.R.'s sabbatical leave. This work was sponsored by Basil O'Connor Starter Scholar Research Award 5-802 from the March of Dimes–Birth Defects Foundation (R.D.N.); the American Heart Association, Florida Affiliate (R.D.N.); the Deutsche Forschungsgemeinschaft (B.H.); and the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. (E.M.R. and L.B.R.). R.D.N. is a Pew Scholar in the Biomedical Sciences.

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