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Concordance between isolated cleft palate in mice and alterations within a region including the gene encoding the β_3 subunit of the type A γ-aminobutyric acid receptor

(deletion mapping/*p*-locus mutations/complementation analysis/facial development/complex syndromes)

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ABSTRACT **Genetic and molecular analyses of a number of radiation-induced deletion mutations of the pink-eyed dilution (*p*) locus in mouse chromosome 7 have identified a specific interval on the genetic map associated with a neonatally lethal mutation that results in cleft palate. This interval, closely linked and distal to *p*, and bracketed by the genes encoding the α_5 and β_3 subunits of the type A γ-aminobutyric acid receptor (*Gabra5* and *Gabrb3*, respectively), contains a gene(s) (*cpl*; cleft palate 1) necessary for normal palate development. The *cpl* interval extends from the distal breakpoint of the prenatally lethal p^{83FBFo} deletion to the *Gabrb3* locus. Among 20 *p* deletions tested, there was complete concordance between alterations at the *Gabrb3* transcription unit and inability to complement the cleft-palate defect. These mapping data, along with previously described *in vivo* and *in vitro* teratological effects of γ-aminobutyric acid or its agonists on palate development, suggest the possibility that a particular type A γ-aminobutyric acid receptor that includes the β_3 subunit may be necessary for normal palate development. The placement of the *cpl* gene within a defined segment of the larger *D15S12h* (*p*)–*D15S9h-1* interval in the mouse suggests that the highly homologous region of the human genome, 15q11–q13, be evaluated for a role(s) in human fetal facial development.**

Abnormalities in facial development are among the most common and emotionally wrenching birth defects seen in the general population (1). Dissection of the genetic component of particular facial development defects—for example, cleft lip with or without cleft palate (CL/P) in humans—has proved complex, and many hypotheses have been advanced as to the mode of inheritance (2). Although most cases of nonsyndromic CL/P are best accounted for by a multifactorial model, one or a few major loci have been implicated in its etiology (2–5), and there have been conflicting reports of linkage in humans between the transforming growth factor α gene (*TGFA*) and a major CL/P locus (4–6). Despite the many remaining current uncertainties about the mode of inheritance of clefting defects, it is known that CL/P is genetically and developmentally distinct from isolated cleft palate (CP) (7) and that a genetic locus in the X chromosome in humans is implicated in clefting defects (ref. 8 and the references therein).

The mouse has proved to be an exceptional model system for studying the effects of teratogenic and/or intrinsic genetic factors in palate development. Problems in palate-shelf elevation and fusion are believed to be among the primary reasons for clefting of the secondary palate (9). Clefting defects can be induced in mice by a wide range of compounds administered to pregnant females on days 13–14 of gestation (for review, see ref. 10) and are usually associated with a delay in the reorientation of palatal shelves from a vertical to a horizontal position, which allows ossification to occur before the shelves meet and fuse. Recently, a major locus associated with susceptibility to glucocorticoid-induced CP has been localized to mouse chromosome 8 (11).

A number of single-gene mutations in the mouse also result in facial defects, including CP, as components of their phenotype (e.g., *Dc*, dancer; *Tw*, twirler; *oel*, open eyelids with CP; *pc*, phocomelic; *sho*, shorthead; *ur*, urogenital; see ref. 12 for original references), and unbalanced segregants of certain translocations display CP in addition to other abnormalities (N. L. A. Cacheiro, personal communication). Phillips (14) described a recessive, radiation-induced, neonatally lethal mutation (p^{11}, renamed p^{cp}) at the pink-eyed dilution (*p*) locus in mouse chromosome 7, that exhibits isolated CP, suggesting that a locus involved in secondary palate formation is closely linked to the *p* locus.

Here we describe the identification of another neonatally lethal mutation of *p* that manifests isolated CP and present both genetic- and molecular-mapping data that define a specific genetic region, closely linked and distal to *p*, that contains a gene or genes involved in normal palate development. Both complementation and RNA expression data suggest a model in which deficiency of a particular subtype of the type-A γ-aminobutyric acid ($GABA_A$) receptor may contribute to the clefting defects observed in mice homozygous for this mutation, consistent with earlier observations that GABA or its agonist diazepam interfere with normal palate development in mice (15).

MATERIALS AND METHODS

Mice. All mice were bred at the Oak Ridge National Laboratory. The origin of additional *p* mutations is described elsewhere (16). Initially, complementation analyses involving $p^{4THO-II}$ and other radiation-induced *p* mutations (*p**) were accomplished by making reciprocal crosses of $p^{4THO-II}$ $+/+$ $c^{ch} \times p^{*}$ $+/+$ c^{ch} mice and scoring for pink-eyed-dilute progeny at birth and beyond. To collect late-gestation fetuses for gross analysis of the palate and for preparation of RNA and DNA, $p^{4THO-II}/p^{7R75M}$ females were crossed to various p^{*}/p^{7R75M} or p^{*} $+/+$ c^{ch} males. p^{7R75M} is a radiation-induced mild allele of *p* in that it is darker in color when homozygous than when heterozygous with most alleles of *p*; thus, recessive-lethal *p**s can be maintained easily in p^{*}/p^{7R75M} heterozygotes. $p^{4THO-II}/p^{*}$ fetuses at 17.5 or 18.5 days of ges-

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Abbreviations: CP, cleft palate; CL(P) cleft lip with or without cleft palate; GABA, γ-aminobutyric acid; E*n*, embryonic day *n*.

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tation [embryonic day (E) 17.5 or E18.5] (where the morning of finding a vaginal plug was defined as day 0.5) could be classified by their pink eye color.

DNA Probes and Southern/RNA Analyses. The derivation of probe 218/219, a PCR-amplified cDNA fragment of the rat *GABRB3* gene, is described elsewhere (16, 17). A probe (designated 300/301) for the rat *GABRA5* gene was prepared by PCR of rat brain cDNA with primers 5′-GCCTTGGAAGCAGCTAAAATC and 5′-CTCAGAAGTCTTCTCCTCAGA. This PCR amplifies a 183-bp fragment extending from nt 1194–1377 in the cDNA sequence (18). [This cDNA sequence was originally designated *GABRA4* in ref. 18, but it is most often designated *GABRA5* in the literature (e.g., ref. 19); thus, we shall also designate this particular gene as *GABRA5*.] Procedures for the preparation of DNA and RNA and for performing Southern- and RNA-blot analysis are described elsewhere (16). Poly(A)$^+$ RNA was isolated by affinity chromatography with Mini-Ribosep oligo(dT) columns (Collaborative Biomedical Products, New Bedford, MA).

RESULTS

CP in Mice Homozygous for a Radiation-Induced, Neonatally Lethal *p*-Locus Mutation. Intercrosses of mice heterozygous for $p^{4THO-II}$, a mutation at the pink-eyed dilution (*p*) locus that was recovered in the progeny of males exposed to tritiated water, produce pink-eyed homozygotes that usually die shortly after birth. Because neonatal lethality, accompanied by clefting of the palate, had previously been observed in mice homozygous for the presumed-radiation-induced p^{cp} mutation (14), fetuses obtained from intercrosses of $p^{7R75M}/p^{4THO-II}$ mice were analyzed at E18.5 for the presence of CP. Fig. 1 (right) shows that $p^{4THO-II}/p^{4THO-II}$ homozygotes do, indeed, have a CP, compared to a wild-type littermate that exhibits a normal palate (Fig. 1, left). Because animals that are homozygously deleted for DNA at, or tightly linked to, the *p* locus do not necessarily exhibit CP (16, 22), the CP phenotype is likely to be caused by a mutation(s) tightly linked to *p* that is disrupted by the $p^{4THO-II}$ (and presumably p^{cp}) mutation. We have designated this locus *cp1* (CP 1; designated as CP in ref. 22).

Manifestation of the CP phenotype in $p^{4THO-II}/p^{4THO-II}$ homozygotes, like that observed in p^{cp}/p^{cp} homozygotes (14, 23), is not completely penetrant. We did observe four exceptional pink-eyed-dilute offspring ($p^{4THO-II}/p^{4THO-II}$) in 350 progeny of a $p^{7R75M}/p^{4THO-II}$ intercross, which suggested ≈95% penetrance of CP. None of the four exceptional mice had CP, although all were runty and nervous; two died at ≈2.5 weeks of age, whereas the other two lived longer than 9 mo, and at least one female was fertile. Tail DNA from three of these animals failed to hybridize by Southern blot analysis to the 218/219 *GABRB3* probe, which is known to be deleted in $p^{4THO-II}$ (16), thereby confirming them as $p^{4THO-II}/p^{4THO-II}$ homozygotes (data not shown). Thus, the phenotypes of these exceptional, surviving mice suggest that it is the CP disorder that is responsible for the recessive neonatal lethality seen in homozygotes.

Deletion Mapping of the *cp1* Gene. We have been able to refine the genetic interval containing *cp1* by determining whether other radiation-induced, lethal *p* deletions can complement the $p^{4THO-II}$ CP phenotype (Table 1). [More detailed results of a much larger complementation analysis of *p*-locus mutations will be reported elsewhere (L.B.R., C.S.M. & E.M.R., unpublished work).] Seven mutations (p^{6H}, $p^{46DFiOD}$, p^{83FBFo}, p^{80K}, p^{25DVT}, p^{4R250H}, $p^{12R30Lb}$) were able to complement the CP defect in compound heterozygotes carrying $p^{4THO-II}$. However, 12 other mutations (p^{8R250M}, p^{47DTD}, p^{2HATh}, p^{45DTD}, p^{116G}, p^{132G}, p^{30PUb}, $p^{23DFiOD}$, p^{55PB}, $p^{7FR60Lb}$, p^{8FDFoD}, $p^{226THO-I}$) could not complement the CP phenotype.

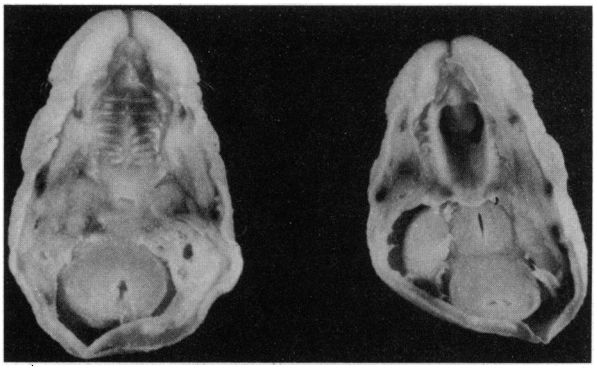

FIG. 1. CP in *p*-deletion homozygotes. Ventral aspect of heads of two fixed E18.5 fetuses (with jaw removed). CP is clearly visible in the $p^{4THO-II}/p^{4THO-II}$ fetus (right); a wild-type littermate (left) illustrates a normal palate.

The $p^{46DFiOD}$ mutation is deleted for the *D15S12h (p)* locus as well as for the proximal marker *Myod1* but not for a probe for the *Gabrb3* locus, whereas the $p^{4THO-II}$ deletion breaks within *D15S12h (p)* and is deleted for a probe from the *Gabrb3* locus (16). Consequently, *cp1* must map distal to *D15S12h (p)*, within the limits of the $p^{4THO-II}$ deletion but distal to the distal breakpoint of the $p^{46DFiOD}$ deletion.

Because $p^{4THO-II}$ is deleted for the *GABRB3* probe, each mutation tested in the complementation analysis could be tested directly for deletion of the *Gabrb3* locus in the corresponding $p^*/p^{4THO-II}$ compound heterozygote. Fig. 2 *A* and *B* and Table 1 show that, except for the p^{8FDFoD} and $p^{226THO-I}$ mutations (see below), deletion of a 9.0-kb *Pvu* II fragment detected by the 218/219 *GABRB3* probe completely corre-

Table 1. Genetic and molecular characteristics of radiation-induced *p*-locus mutations used to define the *cp1* locus

Mutation (p^l)*	$p^l/p^{4THO-II}$		Deletion		Gabrb3 mRNA§
	Lethality†	CP	*Gabra5*‡	*Gabrb3*‡	
$p^{4THO-II}$	NL	+	−	−	−
$p^{7FR60Lb}$	NL	+	−	−	ND
p^{6H}	V	−	+	+	+
$p^{46DFiOD}$	V	−	+	+	+
p^{83FBFo}	V	−	−	+	+
p^{80K}	V	−	+	+	+
p^{25DVT}	V	−	+	+	ND
p^{4R250H}	V	−	+	+	ND
$p^{12R30Lb}$	V	−	+	+	ND
p^{8R250M}	NL	+	−	−	ND
p^{47DTD}	NL	+	−	−	ND
p^{2HATh}	NL	+	−	−	ND
p^{45DTD}	NL	+	−	−	ND
p^{116G}	NL	+	−	−	ND
p^{132G}	NL	+	−	−	ND
p^{30PUb}	NL	+	−	−	ND
$p^{23DFiOD}$	NL	+	−	−	ND
p^{55PB}	NL	+	−	−	ND
p^{8FDFoD}	NL	+	−	+	AB
$p^{226THO-I}$	NL	+	−	+	−

*Mice homozygous for $p^{4THO-II}$ and $p^{7FR60Lb}$ die neonatally; p^{6H} homozygotes die at variable times during juvenile life; mice homozygous for all other mutations die before birth.

†NL, neonatally lethal; V, viable.

‡Determined by Southern blot analysis with the 300/301 probe (*GABRA5*) or the 218/219 probe (*GABRB3*). −, Deletion of probe sequence; +, no deletion.

§Determined by RNA blot analysis of $p^l/p^{4THO-II}$ fetal head RNA. −, No transcript detected; +, normal amount and size of transcript detected; AB, aberrantly sized transcript detected; ND, not done.

lates with the inability of a mutation to complement $p^{4THO-II}$ for the CP phenotype.

Neither p^{8FDFoD} nor $p^{226THO-I}$ could complement $p^{4THO-II}$, but neither was deleted for the *GABRB3* probe used (Fig. 2*B* and Table 1). We therefore tested whether a wild-type Gabrb3 transcript could be detected in these exceptional combinations. Fig. 3*A* shows an RNA blot of poly(A)$^+$ RNA prepared from heads of late-gestation pink-eyed fetuses (i.e., $p^{8FDFoD}/p^{4THO-II}$ and $p^{226THO-I}/p^{4THO-II}$) that exhibited CP. Hybridization of this blot with the 218/219 GABRB3 probe detected a size-altered, slightly larger Gabrb3 transcript (≈6 kb) (lane b; see arrow) in $p^{8FDFoD}/p^{4THO-II}$ RNA, and no transcript at all in $p^{226THO-I}/p^{4THO-II}$ RNA (lane e), as compared to the 5.5-kb transcript detected in RNA from wild-type littermate controls (e.g., lane c). The size-altered transcript from p^{8FDFoD} was also notably reduced in intensity when compared to the wild-type transcript found in p^{7R75M}/p^{8FDFoD} heterozygotes (Fig. 3*A*, lane a). Thus, normal transcription from the *Gabrb3* locus is affected by these two mutations, even though neither deletes the 3′ GABRB3 probe 218/219. Four of the mutations (p^{83FBFo}, p^{6H}, $p^{46DFiOD}$, and p^{80K}) that are not deleted for the GABRB3 probe and that are able to complement the *cp1* defect expressed the wild-type 5.5-kb Gabrb3 transcript (Fig. 3*A*; lanes f–i). Thus, among the 20 mutations that were analyzed, there is complete concordance between genomic and/or transcriptional alterations at the *Gabrb3* locus and the inability to complement for the CP phenotype.

Because of the complete concordance between alterations at the *Gabrb3* locus and the CP phenotype, it was of interest to test whether the expression of *Gabrb3* is consistent with a potential role in the development of fetal palate. Hybridization of the 218/219 probe to a Northern blot of total RNAs derived from E14.5, E15.5, or E16.5 whole fetuses, or from E16.5 fetal heads, showed that the 5.5-kb Gabrb3 transcript is expressed at these stages (Fig. 3*A*; lanes j–m).

Deletion Mapping of *Gabra5* Excludes Its Candidacy for *cp1*. The gene (*Gabra5*) for the α_5 subunit of the $GABA_A$ receptor is known to be linked to the *GABRB3* locus in human 15q11–q13 (24). We therefore tested whether a probe (designated 300/301), derived from the segment of the published GABRA5 cDNA sequence showing the least homology with other α subunits (18), was likewise linked to *Gabrb3* in the mouse. Fig. 2*C* demonstrates that probe 300/301 hybridizes to a 4.0-kb *Pvu* II fragment in wild-type DNAs that is deleted in $p^{4THO-II}/p^{4THO-II}$ DNA. All of the DNAs mutant at *Gabrb3* (13 of 13, including p^{8FDFoD} and $p^{226THO-I}$) are deleted for *Gabra5* (not all data are shown, but are summarized in Table 1). Of the seven mutations that complement *cp1* and are not mutant at *Gabrb3*, only p^{83FBFo} is deleted for the 300/301 GABRA5 probe (Fig. 2*C* and Table 1). These data suggest that *Gabra5* maps between *D15S12h (p)* and *Gabrb3*, and that the p^{83FBFo} distal breakpoint maps between *Gabra5* and *Gabrb3*.

Integration of these complementation and molecular data thus defines a specific genetic interval containing the *cp1* locus (Fig. 4). This interval is defined on the proximal side by the distal breakpoint of p^{83FBFo}, the deletion that includes *Gabra5* but complements $p^{4THO-II}$ for CP. The *cp1* interval can extend distally only to the *Gabrb3* locus itself because p^{8FDFoD} and $p^{226THO-I}$ both fail to complement the CP abnormality but do not remove the entire *Gabrb3* locus.

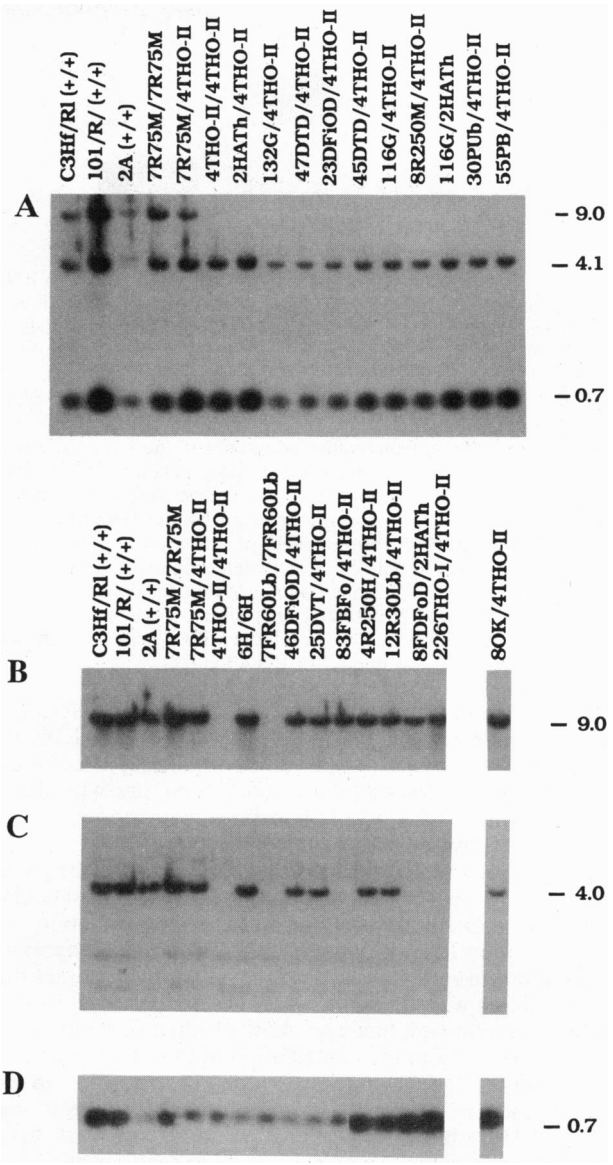

FIG. 2. Mapping of *cp1*, *Gabrb3*, and *Gabra5* with lethal *p* deletions. (*A*) A Southern blot of 10 μg of *Pvu* II-digested DNA, prepared from animals of the indicated genotypes, was hybridized to probe 218/219 from the *Gabrb3* locus (the 9.0-kb fragment), along with probe 34-1-111, from the *D15S9h-1* locus (16) as a control probe (the 4.1- and 0.7-kb fragments). (*B–D*) Southern blots of 10 μg of *Pvu* II-digested DNA, prepared from animals of the indicated genotypes, was sequentially hybridized to *GABRB3* probe 218/219 (*B*), to *GABRA5* probe 300/301 (*C*), and to control probe 34-1-111 (*D*). The faintly hybridizing fragments observed in *C* are probably due to cross hybridization with related genes encoding other α subunits. Both 4.1-kb and 0.7-kb *Pvu* II fragments recognized by control probe in *D* were present, but only the 0.7-kb fragment is shown.

DISCUSSION

The results of complementation analyses among a number of lethal *p*-locus mutations, combined with molecular-mapping data, have suggested that a locus (*cp1*), responsible for recessive-lethal CP in the mouse, maps distal to *p* within a specific region bracketed proximally by the p^{83FBFo} distal breakpoint and distally by the *Gabrb3* locus. Of the *p* mutations tested, 13 were mutant at *cp1*, and all 13 had defects in the *Gabrb3* gene: 11 of these were deleted for a *Gabrb3* cDNA probe, and 2 showed anomalies in the size and/or level of Gabrb3 transcript. The 7 mutations that complemented *cp1* were not deleted for the *Gabrb3* probe, and normal Gabrb3 transcripts could be detected from at least four of these mutant chromosomes (including p^{83FBFo}, which is the complementing deletion whose breakpoint maps closest to the *Gabrb3* gene).

The complete concordance of the CP phenotype with alterations at the *Gabrb3* locus is provocative. It is known that the GABA agonist diazepam is teratogenic in mice, with

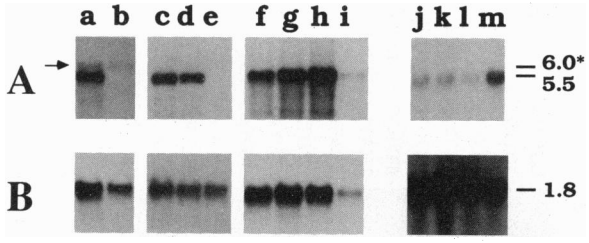

FIG. 3. Expression analysis of the Gabrb3 transcript. (*A*) RNA blots of poly(A)$^+$ RNA from fetal tissue were hybridized with *GABRB3* probe 218/219. Lanes: a–i, E17.5 or E18.5 fetal heads: a, p^{7R75M}/p^{8FDFoD}; b, $p^{8FDFoD}/p^{4THO-II}$; c, $+/p^{7R75M}$ or $+/p^{4THO-II}$; d, $p^{7R75M}/p^{226THO-I}$; e, $p^{226THO-I}/p^{4THO-II}$; f, $p^{83FBFo}/p^{4THO-II}$; g, $p^{6H}/p^{4THO-II}$; h, $p^{46DFiOD}/p^{4THO-II}$; i, $p^{80K}/p^{4THO-II}$; j, E14.5 whole fetus; k, E15.5 whole fetus; l, E16.5 whole fetus; m, E16.5 head. Lanes j–m contain RNA isolated from wild-type fetuses of the C3Hf/Rl inbred strain. The sizes of the transcripts were determined by comparison to RNA ladder (Life Technologies). The asterisk and arrow identify the 6.0-kb size-altered transcript detected by the *GABRB3* probe from the mutant p^{8FDFoD} chromosome. In lane c, the genotype of the wild-type fetuses could not be precisely determined (they were either $+/p^{7R75M}$ or $+/p^{4THO-II}$). Lane i was grossly underloaded, and the transcript can be readily detected only after a 1-week exposure of film. (*B*) Hybridization of the same blots with a probe for chicken tubulin, as a control for amount of RNA loaded.

CP being one of the abnormalities observed in offspring of pregnant mice treated with this drug at around E14, the critical period for palatal-shelf reorientation (25). Likewise, treatment of developing fetal palates in culture with diazepam, or with GABA itself, interferes with normal palate development by inhibiting palate-shelf reorientation (15). GABA has also been reported (26) to be present in the palate in E13–E15 fetuses, consistent with the expression of GABA receptors during the critical period for palate formation. We have shown by RNA-blot analysis that *Gabrb3* is expressed in whole fetuses and in fetal heads at development times that are coincident with the development of the palate. It has not yet been determined, however, how much of this expression is due exclusively to the contribution of the developing fetal brain. Laurie *et al.* (19) reported "very weak or no" *in situ* hybridization of a *Gabrb3*-specific antisense probe to sections of E14 rat brains; however, they did report weak signal from this probe when it was hybridized to sections of E17 rat brains. Thus, it will be necessary to assay by sensitive techniques whether *Gabrb3* expression in mouse brain at E14.5 accounts for some or all of the hybridization observed in our analysis of entire E14.5 fetus. *In situ* hybridization of *Gabrb3*-specific probes to sections of palate shelves should help resolve this question. Nonetheless, several lines of evidence (genetic mapping, expression during critical developmental times, and previous pharmacological/toxicological studies) suggest that *Gabrb3* itself may be a candidate for *cp1*, and that the lack of a specific $GABA_A$ receptor subtype, in which β_3 comprises one of the subunits, may be responsible for the defects in palate formation seen in $p^{4THO-II}/p^{4THO-II}$ homozygotes.

The $GABA_A$ receptors belong to a heterogeneous group of ligand-gated ion (chloride) channels that are composed of a number of subunits (e.g., α_1–α_6, β_1–β_3, γ_1–γ_3, δ, ε) (27). The genes encoding each subunit are expressed in different regions of the nervous system and during different stages of development (19). Structurally and functionally different receptors may be formed by certain combinations of these subunits; for example, at least five $GABA_A$ receptor subtypes, each composed of a different combination of subunits and each expressed in different regions of the nervous system, have been observed by immunofluorescence (28). Moreover, it is known that $GABA_A$ receptor RNA is expressed in tissues outside the central nervous system (19).

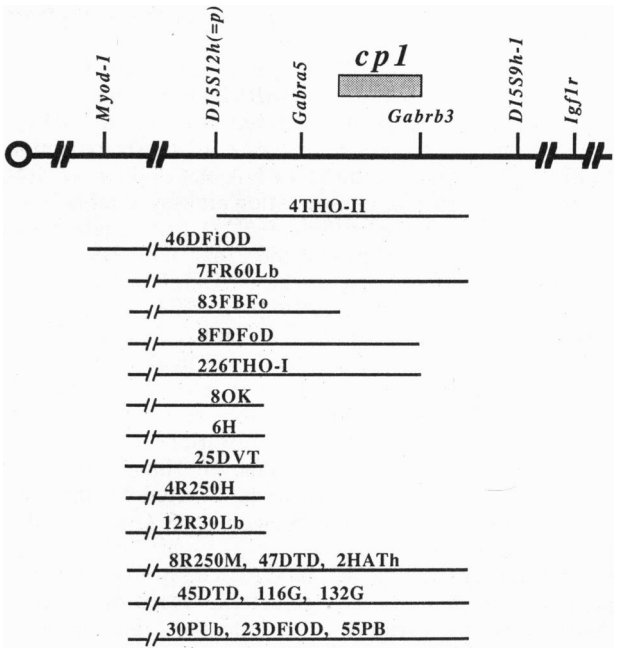

FIG. 4. A refined genetic map surrounding the *Gabrb3* locus, indicating localization of the *cp1* gene. A map of the *Myod1–Igf1r* region of chromosome 7 (16), modified to incorporate our data; the centromere is indicated by the circle at left. Extents of *p* deletions are indicated by lines below map, and the name of each deletion is indicated. The endpoints of independently isolated deletions that cannot yet be discriminated by molecular probes are shown as terminating at the same place. The interval containing *cp1* is indicated by the shaded box and is bounded on the distal side by the *Gabrb3* locus and on the proximal side by the p^{83FBFo} distal breakpoint. No correlation with any physical distance is implied by spacing of loci. Recombination frequencies (16) are as follows: *Myod1*–5.5 ± 1.7–*D15S12h(p)*–1.1 ± 0.8–*D15S9h-1*–6.0 ± 1.8–*Igf1r*.

Thus, perhaps one of the many possible $GABA_A$ receptor subtypes might function in normal palate development, another question that may be resolved by *in situ* hybridization experiments that use probes specific for each of the $GABA_A$ receptor subunits.

It has been proposed that GABA may function *in vivo* by acting as a normal inhibitor of palate-shelf movement (15). However, if the β_3 subunit of a $GABA_A$ receptor is, indeed, necessary for normal palate development, it presently is difficult to explain why the phenotype observed when a receptor subunit is deleted, as in the *p*-deletion homozygotes or compound heterozygotes reported here, is similar to that observed when an inhibitory GABAergic pathway is stimulated (15). Perhaps any alteration, either positive or negative, in a GABAergic pathway in the palate could disrupt its normal development. In addition, if the lack of a specific $GABA_A$ subtype is indeed causally associated with defects in palate development, that subtype cannot include the α_5 subunit, because the p^{83FBFo} mutation, which deletes at least a portion of the *Gabra5* gene, can complement the *cp1* defect.

The deletion of *GABRB3* in a family segregating Angelman syndrome has evoked the proposal that this gene may be involved in the pathogenesis of Angelman syndrome (29). However, this human 15q11–q13 deletion is described as being >650 kb in size with a large coding potential (13), and recently a probe for *GABRB3* was shown to map distal to the Angelman syndrome critical region (20). Indeed, the strong correlation between the *cp1* defect and alterations at the *Gabrb3* locus reported here, along with the finding that *Gabrb3* is not parentally imprinted in mouse brain and that mice hemizygous for this gene are phenotypically normal (16, 21), suggest that alternative hypotheses be considered for the

role of the β_3 subunit of the $GABA_A$ receptor in human development. Among these hypotheses would be one in which the β_3 subunit is involved in aspects of facial development in the fetus, which can be tested rigorously in the mouse by gene-transfer/mutant-phenotype-rescue experiments. However, it is necessary to point out that, at present, the region between the distal breakpoint of p^{83FBFo} and *Gabrb3* (i.e., the current, most accurate definition of the *cpl* region) is still of unknown physical size. Thus, it is possible that the *cpl* gene maps within this region, proximal to *Gabrb3*. Nonetheless, regardless of the actual identity of the *cpl* gene itself, the strong conservation of fine-structure synteny (16) between the *D15S12h (p)–D15S9h-1* region in mice and the 15q11–q13 region in humans suggests that 15q11–q13 might be evaluated in the context of the genetics of facial dysmorphisms in human populations.

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