# Isolated cleft palate in mice with a targeted mutation of the LIM homeobox gene *Lhx8*

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**Formation of the mammalian secondary palate is a highly regulated and complex process whose impairment often results in cleft palate, a common birth defect in both humans and animals. Loss-of-function analysis has linked a growing number of genes to this process. Here we report that *Lhx8*, a recently identified LIM homeobox gene, is expressed in the mesenchyme of the mouse palatal structures throughout their development. To test the function of *Lhx8 in vivo*, we generated a mutant mouse with a targeted deletion of the *Lhx8* gene. Our analysis of the mutant animals revealed a crucial role for *Lhx8* in palatogenesis. In *Lhx8* homozygous mutant embryos, the bilateral primordial palatal shelves formed and elevated normally, but they often failed to make contact and to fuse properly, resulting in a cleft secondary palate. Because development of other craniofacial structures appeared normal, the impaired palatal formation in *Lhx8*-mutant mice was most likely caused by an intrinsic primary defect in the mesenchyme of the palatal shelves. The cleft palate phenotype observed in *Lhx8*-mutant mice suggests that *Lhx8* is a candidate gene for the isolated nonsyndromic form of cleft palate in humans.**

The secondary palate in mammals forms through a complex process characterized by a series of events involving cell proliferation and migration, cell differentiation, production of extracellular matrix, and cell death (1). Recently, studies with targeted mutations in mice have revealed a growing number of genes that play crucial roles in that process. These genes encode a variety of molecules including transcription regulators (2–8), growth factors (9, 10), enzymes for signaling molecule synthesis (11, 12), and receptors (13–15). By association studies, some of these genes have also been identified as candidate genes (16, 17) whose mutations may cause cleft palate in humans, a common birth defect that results from impairments in palatal development.

LIM homeobox genes encode a family of transcription regulators that share common structural features. They all contain two tandemly repeated cysteine-rich double-zinc finger motifs called LIM domains, in addition to a homeodomain. Whereas the homeodomain is a DNA-binding domain, the LIM domains are essential for regulating the activity of these molecules by interacting with other proteins (18–22). Genetic studies in various organisms have shown that members of the LIM homeobox gene family are required for the patterning (23, 24) or the specification and differentiation of different cell types during embryonic development (25–38).

*Lhx8*, also called *L3* (39) and *Lhx7* (40), is a recently identified member of the LIM homeobox gene family. During mouse embryogenesis, *Lhx8* has been shown to be expressed in several regions in the head, including the maxillary and mandibular processes and the ventral forebrain (39, 40). *In situ* chromosomal hybridization has revealed that the *Lhx8* gene is localized on mouse chromosome 3, H3–4 (41). Homologs of genes in that region have been mapped to human chromosome 4, q25–31 (http://www.ncbi.nlm.nih.gov/Omim/Homology), a region that has been linked to craniofacial clefting (42). This raised the possibility that *Lhx8* may be involved in development of the secondary palate.

To test whether *Lhx8* has a role in palatal development, we further examined the expression of *Lhx8* at different stages during palatogenesis and generated a mutant mouse with a targeted deletion of the gene. We show that *Lhx8* is continuously expressed in the mesenchyme of the normal developing palatal structures. Disruption of *Lhx8* gene function causes impairments in palatal shelf contact and fusion that lead to the formation of a cleft secondary palate.

## Materials and Methods

**Generation of *Lhx8* Knockout Mice.** A plasmid containing the entire mouse *Lhx8* gene was obtained by PCR screening of a mouse bacterial artificial chromosome library (GenomeSystems, St. Louis) with primers (sense, 5′-TGTTCCCGCTGTGGCAG-GCACAT-3′; antisense, 5′-ACAGAGGACCTTCTCCTCCAC-CAA-3′) synthesized according to the *Lhx8* cDNA sequence (39). To construct the targeting vector, exons 4–6 of the *Lhx8* gene were replaced with a neomycin resistance gene flanked by 3.0-kb (5′) and 3.4-kb (3′) homologous sequences and by the thymidine kinase gene (see Fig. 2*A*). The vector was linearized and electroporated into the R1 line of embryonic stem (ES) cells (43). Targeted clones were double selected with G418 (350 μg/ml) and gancyclovir (2 mM), and screened by Southern blot analysis with both a 5′ probe and a 3′ probe outside the flanking homologous sequences (see Fig. 2*A*). The ES cells that were heterozygous for the targeted *Lhx8* mutation were microinjected into C57BL/6 blastocysts to produce chimeric mice that carried the mutation into the germ line. These mice were mated with C57BL/6 wild-type mice to generate *Lhx8* heterozygous animals that were subsequently crossed to produce F₂ offspring for analysis.

**Genotyping of *Lhx8* Knockout Mice.** Genotypes of the mice were determined by either Southern blot or PCR analysis of genomic DNA prepared from tail biopsies or yolk sacs of embryos. The probes used for Southern analysis were the same as those used for ES cell screening. For PCR analysis, a pair of primers from exon 5 of the *Lhx8* gene (the same as those used for screening the mouse bacterial artificial chromosome library; see above) was used to amplify an 150-bp fragment from the wild-type allele, and a pair of primers from the neomycin resistance gene (sense, 5′-TCGTCCTGTACGGACCGCAGTTT-3′; antisense, GATCCCCTCAGAAGAACTCGT-3′) was used to amplify a 550-bp fragment from the mutant allele.

**Histological Analysis and Skeletal Staining.** *Lhx8* heterozygous mice were mated, and noon on the day when vaginal plugs were observed was considered as embryonic day 0.5 (E0.5). Embryos were isolated at different stages and fixed in 4% paraformaldehyde at 4°C overnight. The embryos were embedded in paraffin
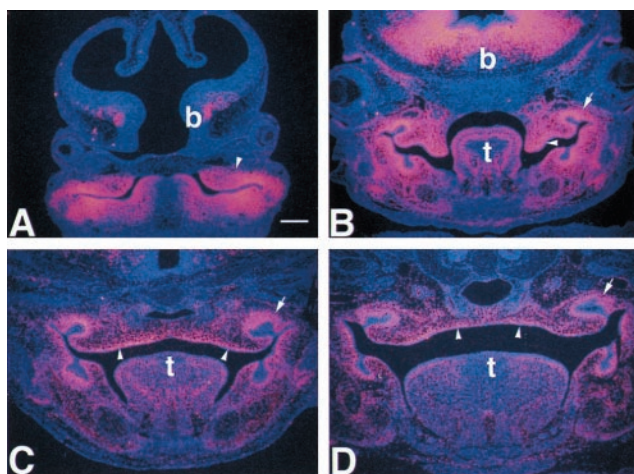
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**Fig. 1.** *In situ* hybridization, showing expression of *Lhx8* mRNA at different stages of palatal development. (*A*) Expression of *Lhx8* in the maxillary processes (arrowhead) of an E11.5 embryo. (*B*) Expression of *Lhx8* in the palatal shelves (arrowhead) of an E13.5 embryo, before shelf elevation. (*C*) Expression of *Lhx8* in the palate shelves (arrowheads) of an E14.5 embryo, after shelf elevation and contact. (*D*) Continuous expression of *Lhx8* in the palate of an E16.5 embryo, after fusion of the palate shelves. Notice that *Lhx8* was also expressed in the tooth bud (arrows), tongue (t), and the ventral forebrain (b). Scale bar in *A* represents 200 μm for all panels.

and sectioned at a thickness of 5 μm. The sections were mounted on silanated slides and stained with hematoxylin/eosin (H&E) or used for *in situ* hybridization. For skeletal staining, skinned and eviscerated newborn mice were fixed overnight in 95% ethanol and stained with alcian blue and alizarin red as previously described (5).

***In Situ* Hybridization.** *In situ* hybridization was performed on paraffin sections by previously published procedures (44). The $^{35}$S-labeled antisense RNA probe used was synthesized by *in vitro* transcription with a linearized pGEM-T Easy vector (Promega) that contained the full-length *Lhx8* coding sequence.

### Results

**Expression of *Lhx8* During Palatal Development.** We examined the expression of *Lhx8* mRNA in the palatal region at different stages during development by *in situ* hybridization. At E11.5, *Lhx8* mRNA was detected in the maxillary processes (Fig. 1*A*). From E13.5 to E16.5, while palatogenesis progressed through palatal shelf outgrowth, elevation, contact, and fusion, *Lhx8* was continuously expressed in the mesenchyme of the palatal shelves (Fig. 1 *B–D*). This pattern of expression suggests that *Lhx8* could play a role in palatal development. Consistent with previous studies (39, 40), we also detected *Lhx8* mRNA in the ventral telencephalon and in other structures derived from the first branchial arch, such as the molar tooth buds and the tongue (Fig. 1 *B–D*).

**Cleft Palate in *Lhx8* Knockout Mice.** To test the function of the *Lhx8* gene in palatal development, we used homologous recombination in ES cells to generate mice with a targeted deletion of exons 4–6 of the *Lhx8* gene (Fig. 2*A*). These exons encode part of the first LIM domain, the entire second LIM domain, and part of the homeodomain of the Lhx8 protein. Correctly targeted ES cells were selected by Southern blot analysis and were used to produce mutant mice. Mice heterozygous for the *Lhx8* mutation appeared normal and fertile. These mice were subsequently crossed to generate offspring that were genotyped by Southern hybridization or PCR (Fig. 2*B*) and used for phenotypic analysis.
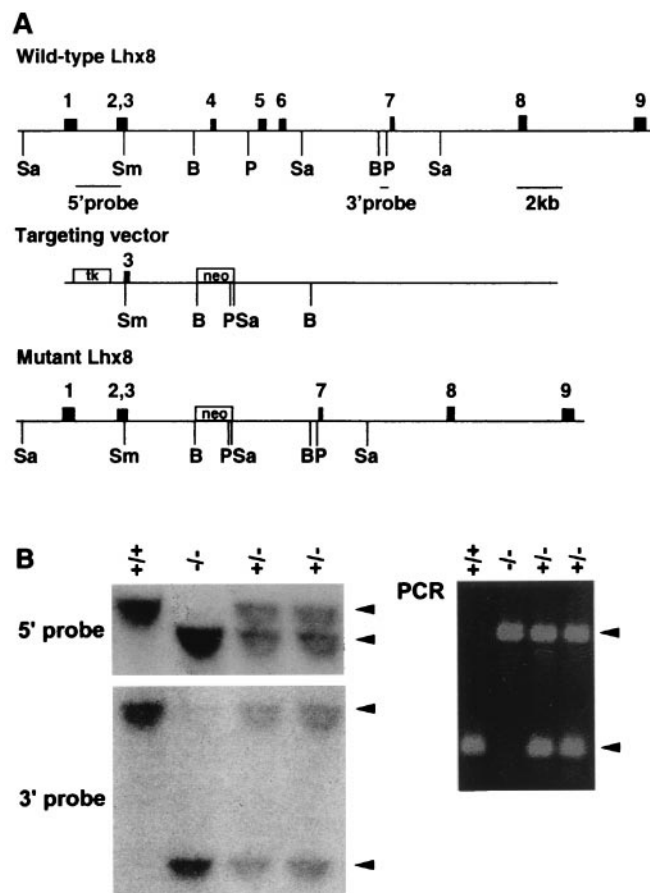


**Fig. 2.** Targeted mutation of the *Lhx8* gene by homologous recombination. (*A*) Schematic representation of the wild-type *Lhx8* gene, the targeting vector, and the mutated *Lhx8*. (*B*) Southern (*Left*) and PCR (*Right*) analyses of genomic DNA derived from offspring of a cross between heterozygous parents. After *Sac*I digestion, the 5′ probe detected an 11-kb band of the wild-type allele and a 9-kb band of the mutant allele (arrowheads). The 3′ probe detected a 6.6-kb band of the wild-type allele and a 4.6-kb band of the mutant allele (arrowheads) after *Pst*I digestion. PCR amplified an 150-bp band from the wild-type allele and a 550-bp band from the mutant allele (arrowheads). B, *Bam*HI; P, *Pst*I; Sa, *Sac*I; Sm, *Sma*I; neo, the neomycin resistance gene; tk, the thymidine kinase gene.

*Lhx8* homozygous mutant mice were born alive and their gross morphology appeared normal (Fig. 3*A*). However, many of them were found dead (9 out of 19 or 47.4%) within 24 h. Genotyping of animals that survived after weaning showed that, of a total of 265 offspring from heterozygous crosses, only 23 (8.7%) were homozygous mutants. Gross examination of the oral cavity of the homozygous mice that died after birth revealed that they all had a complete cleft of the secondary palate (Fig. 3*G*), as compared with wild-type (Fig. 3*F*) or heterozygous controls. The secondary palate in homozygous animals that survived was normal. We also examined embryos at E18.5. Among 10 homozygous mutant embryos, 6 (60%) showed a cleft palate. Thus, disruption of the *Lhx8* gene in mice resulted in a phenotype of cleft palate with incomplete penetrance.

To analyze the defect of bone formation in *Lhx8* mutant mice, skeletal staining of the skull was performed. Unlike those in wild-type controls (Fig. 3*H*), the palatal bones in the homozygous mutant animals with cleft palate failed to extend toward the midline at the base of the skull (Fig. 3*I*). This defect was restricted to the palatal region. Other bones of the skull appeared normal in mutants (Fig. 3 *C* and *E*) as compared with wild-type (Fig. 3 *B* and *D*) animals.
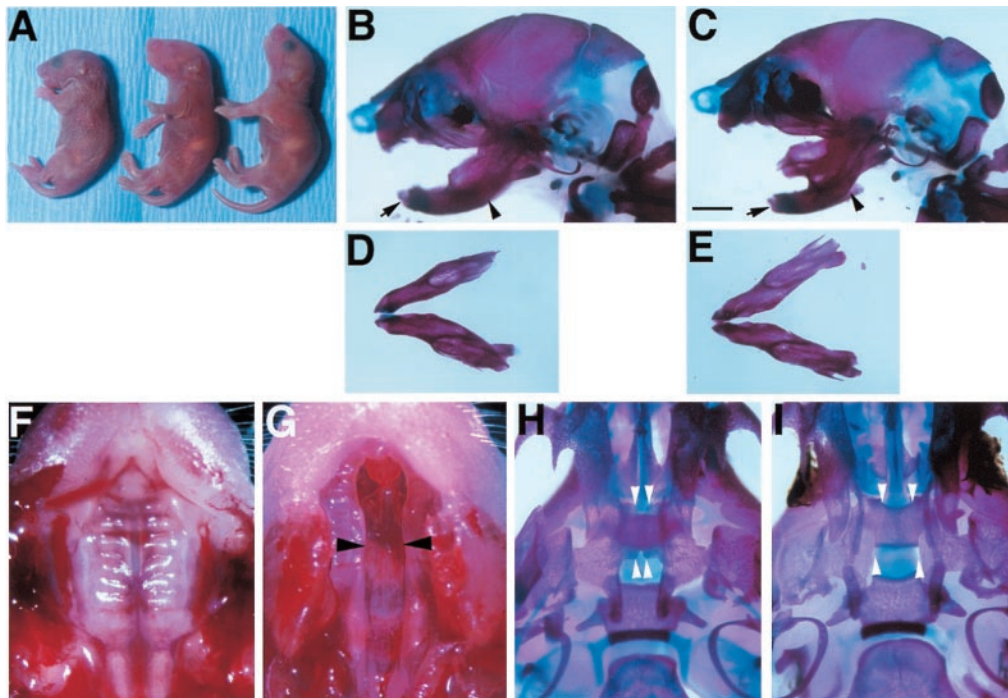
**Fig. 3.** Cleft palate in *Lhx8* knockout mice. (*A*) Two dead newborn *Lhx8* homozygous mice (*Left* and *Center*) are compared with their wild-type littermate (*Right*). (*B* and *C*) Skeletal staining of the head of a newborn *Lhx8* homozygous mutant with cleft palate (*C*) as compared with that of a wild-type control (*B*), viewed from the side. The craniofacial features of the mutant mouse with cleft palate appeared grossly normal. In the mutant, the mandible was lower because the mouth was more widely open when the animal was fixed. Arrows and arrowheads point at the tip and the middle part of the mandibles in both the wild-type (*B*) and the mutant (*C*) animals, respectively. (*D* and *E*) Top view of the mandible of a newborn *Lhx8* mutant animal with cleft palate (*E*) as compared with that of a wild-type control (*D*). The mandible of the mutant appeared normal. (*F* and *G*) Ventral view of the upper jaw of a newborn *Lhx8* homozygous mutant (*G*) as compared with that of a wild-type control (*F*). Notice a cleft of the secondary palate (indicated by arrowheads). (*H* and *I*) Ventral view of the base of skulls of newborn wild-type (*H*) and *Lhx8* homozygous mutant (*I*) mice stained with alcian blue and alizarin red. The palatal bones in the mutant failed to grow toward the midline (indicated by arrowheads). The scale bar in *C* represents 6.6 mm for panel *A* and 1.3 mm for panels *B*–*I*.

**Impaired Palatal Development in *Lhx8* Knockout Mice.** To better define the role of *Lhx8* in palatogenesis, we monitored palatal development in *Lhx8* mutant embryos at different stages. During normal development, palatal shelves were observed in E13.5 embryos as they grew out from the maxillary processes on either side of the tongue (Fig. 4*A*). At E14.5, the palatal shelves on both sides elevated to a horizontal position dorsal to the tongue and abutted each other (Fig. 4*C*). By E16.5, the medial-edge epithelia approximating the palatal shelves from the two sides completed fusion and then degenerated, resulting in an intact palate with continuous mesenchyme (Fig. 4*E*). In *Lhx8*-mutant embryos, the palatal shelves initially formed (Fig. 4*B*, eight embryos examined) and elevated (Fig. 4*D*, seven embryos examined), but they often failed to contact each other and fuse properly (Fig. 4*D*), resulting in a cleft of the palate (Fig. 4*F*). Despite the defect in palatal closure, development of other oral structures, including the mandible, the tongue, the molar teeth (Fig. 4 *B*, *D*, and *F*), the incisor teeth, and the Meckel's cartilage (Fig. 4*H*), all appeared normal in mutant embryos as compared with wild-type embryos (Fig. 4 *A*, *C*, *E*, and *G*).

## Discussion

Expression of *Lhx8* mRNA during mouse embryogenesis has been characterized previously by *in situ* hybridization. It has been reported that *Lhx8* transcripts are expressed in the neural crest-derived ectomesenchyme of the first branchial arch from E9.5 to E12; the expression persists in the developing teeth until the postnatal period. In addition, *Lhx8* is also expressed in the medial ganglionic eminence of the developing ventral telencephalon (39, 40). In this study, a similar pattern of *Lhx8* expression was observed. However, our experiments further revealed that

*Lhx8* mRNA was also continuously expressed in the developing secondary palate, suggesting that *Lhx8* may play a role in palatal development.

Our analysis of mice with a targeted deletion of the *Lhx8* gene showed that *Lhx8* was indeed involved in palatal development. However, other than the developing palate, histological staining and *in situ* hybridization analysis of expression of molecular markers such as *Dlx1*, *Dlx2*, and *Msx1* revealed no clear defects in the rest of the *Lhx8*-expressing structures (data not shown). It is possible that the function of *Lhx8* is partially redundant and can be substituted for by other genes. One likely candidate is *Lhx6*, another LIM homeobox gene that shares a similar expression pattern and a high sequence homology with *Lhx8* (40).

Formation of the mammalian secondary palate is a dynamic process (1). It begins with migration of the cranial neural crest cells from the posterior midbrain and rhombomeres 1 and 2 of the hindbrain to form the first branchial arch (45, 46). The primordial palatal shelves emerge as a bilateral outgrowth from the maxillary processes of the first arch. The palatal shelves first grow vertically downward along the sides of the tongue. They later elevate to a horizontal position, make contact, and finally fuse at the midline to form a complete palate. The phenotype of cleft palate can result from impairments at any of the steps in palatal development. In *Lhx8* homozygous mutant mice, formation and elevation of the palatal shelves appeared to proceed normally. However, the palatal shelves often failed to make contact and to fuse. This indicates that *Lhx8* plays a specific role at this particular stage in palatal development.

Cleft palate has been observed in a number of mice carrying mutations in various genes. Although the phenotype may appear similar, the mechanisms that cause this phenotype could be
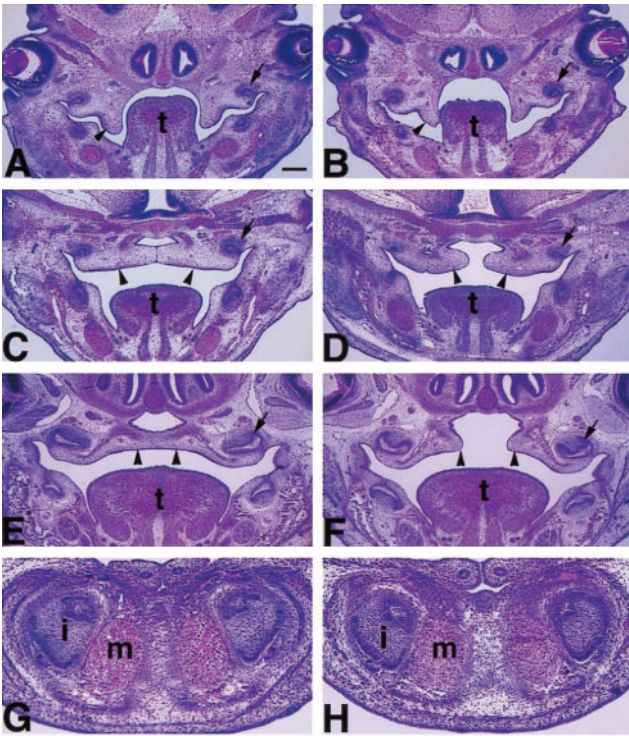
**Fig. 4.** Palate shelves elevated but failed to make contact and fuse in *Lhx8* homozygous mutant mouse without detectable defects in the mandible. H&E staining of coronal sections through the palatal region of the homozygous mutant embryos (*B*, *D*, and *F*) at different developmental stages as compared with that of wild-type controls (*A*, *C*, and *E*). (*A* and *B*) E13.5; (*C* and *D*) E14.5; (*E* and *F*) E16.5. Arrowheads point at the palatal shelves that were not in contact (*A*, *B*, *D*, and *F*), were in contact (*C*), or were completely fused (*E*). Arrows point at one of the upper molar tooth buds in all panels. (*G* and *H*) H&E-stained coronal sections through the distal region of the mandible of a wild-type (*G*) and an *Lhx8* homozygous mutant embryo with cleft palate at E16.5, showing normal development of the mandible, the incisor teeth (i), and the Meckel's cartilage (m) in the mutant. Scale bar in *A* represents 200 $\mu$m for panels *A–F* and 80 $\mu$m for panels *G–H*. t, tongue.

different. Impairments in palatal development can derive from intrinsic defects of the palatal shelves themselves. For example, in mice with mutations of genes encoding transforming growth factor-$\beta$3 (9, 10), epidermal growth factor receptor (15), or transcription factor TTF2 (8), the palatal shelves are able to elevate and abut each other, but they fail to fuse because the medial-edge epithelia overlying the mesenchyme between the opposing palatal shelves do not adhere and disappear promptly. In addition, palate formation is also dependent on normal development of other oral or craniofacial structures. The cleft palate observed in many mice with mutations of homeobox genes, like *Hoxa2* (2, 3), *Msx1* (4), *Mhox* (5), *Dlx1* (6), *Dlx2* (6), and *Pax9* (7), is accompanied by gross craniofacial and skeletal

malformations. The palatal defect in those mice can be attributed, at least partially, to those malformations, which often cause mechanical hindrance in elevation, contact, or fusion of the palatal shelves. In *Lhx8*-mutant mice, despite the defect in the secondary palate, the gross morphology of the skull and other oral structures such as the mandible, tongue, molar and incisor teeth, and Meckel's cartilage all appeared normal. This indicates that the cleft palate observed in those mutant mice is most likely caused by an intrinsic primary defect in development of the palatal shelves.

The expression of *Lhx8* mRNA in the mesenchymal cells of the developing palatal shelves suggests that its role is played out in those cells. Contact and fusion of the palatal shelves can be impaired by a reduction of their size, which in turn can result from a reduction in cell proliferation (1). Previously, the LIM homeobox gene *Lhx2* has been shown to be involved in controlling the proliferation of neural precursor cells in the developing forebrain (32). However, we analyzed the palatal mesenchymal cell proliferation with bromodeoxyuridine pulse labeling and detected no clear differences between *Lhx8* homozygous mutant and wild-type embryos (data not shown). Fusion of the palatal shelves also requires interactions between the medial-edge epithelia and the underlying mesenchyme (1, 47). Previous knockout studies have demonstrated that molecules expressed in the palatal epithelia, like transforming growth factor-$\beta$3 (9, 10), epidermal growth factor receptor (15), and TTF2 (8), are required for the fusion of the palatal shelves. The cleft palate phenotype observed in the *Lhx8*-mutant mouse is similar to those observed in transforming growth factor-$\beta$3 (9, 10), epidermal growth factor receptor (15), and TTF2 (8) knockout mice, which suggests that *Lhx8* may play a role in the mesenchyme in mediating the epithelia-mesenchyme interactions that are required for the fusion of the palatal shelves. It has been shown, in fact, that the expression of *Lhx8* in the ectomesenchyme of the developing tooth bud is induced by signals from the overlying oral epithelia (40). Further studies of the *Lhx8* and other mutant mice that have been generated will help to elucidate the molecular and cellular mechanisms underlying the formation of the mammalian secondary palate.

The incomplete penetrance of the cleft palate phenotype observed in the *Lhx8*-mutant mouse suggests that the *Lhx8* gene may interact with other cleft palate susceptibility genes or teratogens. Cleft palate phenotype with incomplete penetrance has also been observed in other mutant mice (15, 48). Consistent with these studies in mice, isolated nonsyndromic cleft palate in humans has been found to have a highly complex multifactorial etiology that involves both genetic and environmental factors (49). Previous studies have identified the association of cleft palate with *TGF-A* and *MSX1* loci (16, 17). The cleft palate phenotype we observe here identifies *Lhx8* as another potential candidate gene for human cleft palate.

1. Ferguson, M. W. (1988) *Development (Cambridge, U.K.)* **103** Suppl., 41–60.
2. Gendron-Maguire, M., Mallo, M., Zhang, M. & Gridley, T. (1993) *Cell* **75,** 1317–1331.
3. Rijli, F. M., Mark, M., Lakkaraju, S., Dierich, A., Dolle, P. & Chambon, P. (1993) *Cell* **75,** 1333–1349.
4. Satokata, I. & Maas, R. (1994) *Nat. Genet.* **6,** 348–356.
5. Martin, J. F., Bradley, A. & Olson, E. N. (1995) *Genes Dev.* **9,** 1237–1249.
6. Qiu, M., Bulfone, A., Ghattas, I., Meneses, J. J., Christensen, L., Sharpe, P. T., Presley, R., Pedersen, R. A. & Rubenstein, J. L. (1997) *Dev. Biol.* **185,** 165–184.
7. Peters, H., Neubuser, A., Kratochwil, K. & Balling, R. (1998) *Genes Dev.* **12,** 2735–2747.
8. De Felice, M., Ovitt, C., Biffali, E., Rodriguez-Mallon, A., Arra, C., Anastassiadis, K., Macchia, P. E., Mattei, M. G., Mariano, A., Scholer, H., Macchia,

V. & Di Lauro, R. (1998) *Nat. Genet.* **19,** 395–398.
9. Kaartinen, V., Voncken, J. W., Shuler, C., Warburton, D., Bu, D., Heisterkamp, N. & Groffen, J. (1995) *Nat. Genet.* **11,** 415–421.
10. Proetzel, G., Pawlowski, S. A., Wiles, M. V., Yin, M., Boivin, G. P., Howles, P. N., Ding, J., Ferguson, M. W. & Doetschman, T. (1995) *Nat. Genet.* **11,** 409–414.
11. Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R. G., Kanbara, N., Kuzume, H., Sanbo, M., Yagi, T. & Obata, K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6496–6499.
12. Condie, B. G., Bain, G., Gottlieb, D. I. & Capecchi, M. R. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 11451–11455.
13. Culiat, C. T., Stubbs, L. J., Woychik, R. P., Russell, L. B., Johnson, D. K. & Rinchik, E. M. (1995) *Nat. Genet.* **11,** 344–346.

14. Homanics, G. E., DeLorey, T. M., Firestone, L. L., Quinlan, J. J., Handforth, A., Harrison, N. L., Krasowski, M. D., Rick, C. E., Korpi, E. R., Makela, R., et al. (1997) Proc. Natl. Acad. Sci. USA 94, 4143–4148.
15. Miettinen, P. J., Chin, J. R., Shum, L., Slavkin, H. C., Shuler, C. F., Derynck, R. & Werb, Z. (1999) Nat. Genet. 22, 69–73.
16. Shiang, R., Lidral, A. C., Ardinger, H. H., Buetow, K. H., Romitti, P. A., Munger, R. G. & Murray, J. C. (1993) Am. J. Hum. Genet. 53, 836–843.
17. Lidral, A. C., Romitti, P. A., Basart, A. M., Doetschman, T., Leysens, N. J., Daack-Hirsch, S., Semina, E. V., Johnson, L. R., Machida, J., Burds, A., Parnell, T. J., Rubenstein, J. L. & Murray, J. C. (1998) Am. J. Hum. Genet. 63, 557–568.
18. Jurata, L. W., Kenny, D. A. & Gill, G. N. (1996) Proc. Natl. Acad. Sci. USA 93, 11693–11698.
19. Agulnick, A. D., Taira, M., Breen, J. J., Tanaka, T., Dawid, I. B. & Westphal, H. (1996) Nature (London) 384, 270–272.
20. Bach, I., Carriere, C., Ostendorff, H. P., Andersen, B. & Rosenfeld, M. G. (1997) Genes Dev. 11, 1370–1380.
21. Morcillo, P., Rosen, C., Baylies, M. K. & Dorsett, D. (1997) Genes Dev. 11, 2729–2740.
22. Breen, J. J., Agulnick, A. D., Westphal, H. & Dawid, I. B. (1998) J. Biol. Chem. 273, 4712–4717.
23. Taira, M., Otani, H., Saint-Jeannet, J. P. & Dawid, I. B. (1994) Nature (London) 372, 677–679.
24. Shawlot, W. & Behringer, R. R. (1995) Nature (London) 374, 425–430.
25. Way, J. C. & Chalfie, M. (1988) Cell 54, 5–16.
26. Freyd, G., Kim, S. K. & Horvitz, H. R. (1990) Nature (London) 344, 876–879.
27. Cohen, B., McGuffin, M. E., Pfeifle, C., Segal, D. & Cohen, S. M. (1992) Genes Dev. 6, 715–729.
28. Bourgouin, C., Lundgren, S. E. & Thomas, J. B. (1992) Neuron 9, 549–561.
29. Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T. & Jessell, T. M. (1996) Cell 84, 309–320.
30. Sheng, H. Z., Zhadanov, A. B., Mosinger, B., Jr., Fujii, T., Bertuzzi, S., Grinberg, A., Lee, E. J., Huang, S. P., Mahon, K. A. & Westphal, H. (1996) Science 272, 1004–1007.
31. Sheng, H. Z., Moriyama, K., Yamashita, T., Li, H., Potter, S. S., Mahon, K. A.

32. Porter, F. D., Drago, J., Xu, Y., Cheema, S. S., Wassif, C., Huang, S. P., Lee, E., Grinberg, A., Massalas, J. S., Bodine, D., Alt, F. & Westphal, H. (1997) Development (Cambridge, U.K.) 124, 2935–2944.
33. Thor, S. & Thomas, J. B. (1997) Neuron 18, 397–409.
34. Hobert, O., Mori, I., Yamashita, Y., Honda, H., Ohshima, Y., Liu, Y. & Ruvkun, G. (1997) Neuron 19, 345–357.
35. Hobert, O., D'Alberti, T., Liu, Y. & Ruvkun, G. (1998) J. Neurosci. 18, 2084–2096.
36. Duggan, A., Ma, C. & Chalfie, M. (1998) Development (Cambridge, U.K.) 125, 4107–4119.
37. Sharma, K., Sheng, H. Z., Lettieri, K., Li, H., Karavanov, A., Potter, S., Westphal, H. & Pfaff, S. L. (1998) Cell 95, 817–828.
38. Zhao, Y., Sheng, H. Z., Amini, R., Grinberg, A., Lee, E., Huang, S., Taira, M. & Westphal, H. (1999) Science 284, 1155–1158.
39. Matsumoto, K., Tanaka, T., Furuyama, T., Kashihara, Y., Mori, T., Ishii, N., Kitanaka, J., Takemura, M., Tohyama, M. & Wanaka, A. (1996) Neurosci. Lett. 204, 113–116.
40. Grigoriou, M., Tucker, A. S., Sharpe, P. T. & Pachnis, V. (1998) Development (Cambridge, U.K.) 125, 2063–2074.
41. Kitanaka, J., Takemura, M., Matsumoto, K., Mori, T. & Wanaka, A. (1998) Genomics 49, 307–309.
42. Mitchell, L. E., Healey, S. C. & Chenevix-Trench, G. (1995) Am. J. Hum. Genet. 57, 1130–1136.
43. Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W. & Roder, J. C. (1993) Proc. Natl. Acad. Sci. USA 90, 8424–8428.
44. Robinson, G. W., Wray, S. & Mahon, K. A. (1991) New Biol. 3, 1183–1194.
45. Osumi-Yamashita, N., Ninomiya, Y., Doi, H. & Eto, K. (1994) Dev. Biol. 164, 409–419.
46. Kontges, G. & Lumsden, A. (1996) Development (Cambridge, U.K.) 122, 3229–3242.
47. Sharpe, P. M. & Ferguson, M. W. (1988) J. Cell Sci. Suppl. 10, 195–230.
48. Wilson, J. B., Ferguson, M. W., Jenkins, N. A., Lock, L. F., Copeland, N. G. & Levine, A. J. (1993) Cell Growth Differ. 4, 67–76.
49. Houdayer, C. & Bahuau, M. (1998) Ann. Genet. 41, 89–117.

& Westphal, H. (1997) Science 278, 1809–1812.