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Developmental Biology: Frontiers for Clinical Genetics

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The ups and downs of holoprosencephaly: dorsal versus ventral patterning forces

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Holoprosencephaly (HPE), characterized by incomplete separation of forebrain and facial components into left and right sides, is a common developmental defect in humans. It is caused by both genetic and environmental factors and its severity covers a wide spectrum of phenotypes. The genetic interactions underlying inherited forms of HPE are complex and poorly understood. Animal models, in particular mouse mutants, are providing a growing understanding of how the forebrain develops and how the cerebral hemispheres become split into left and right sides. These insights, along with the characterization to date of some of the genes involved in human HPE, suggest that two distinct mechanisms underlie the major classes of HPE, 'classic' and midline interhemispheric (MIH). Disruption either directly or indirectly of the ventralizing effect of sonic hedgehog signaling appears central to all or most forms of classic HPE, while disruption of the dorsalizing effect of bone morphogenetic protein signaling may be key to cases of MIH HPE.

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Holoprosencephaly (HPE) is the most frequent developmental forebrain defect described in humans, with an estimated 1 in 5000 to 10,0000 live births and 1 in 200–250 cases of spontaneous abortions (1–5). A common feature defining HPE is the incomplete separation of the anterior part of the forebrain, or telencephalon, into left and right hemispheres, which normally occurs between the 18 and 28 days of gestation. This defect is due to a failure to form midline structures. HPE is also usually associated with craniofacial abnormalities, but the focus of this review is on the forebrain defects.

The etiology of HPE is very heterogeneous due to the involvement of both environmental and genetic factors, as well as the interactions between them (6–8). The wide spectrum of HPE phenotypes can be observed within a single family in which individuals who carry an identified HPE mutation are very severely affected or clinically normal (9–11). The extreme heterogeneity within HPE families and among sporadic cases makes it difficult at present to establish clear genotype–phenotype correlations and to counsel potential parents who may carry an HPE mutation about the risks involved in having an affected child. Our knowledge about the genetic pathways involved in normal forebrain development comes mainly from studies on animal models. The increasing number of mouse mutants with telencephalic midline defects that mimic human HPE continues to lend key insights into the ontology of human HPE and raises new questions that will need to be solved before we can more fully understand this devastating human birth defect.

HPE: two classes and four types

In humans, an increasing knowledge of HPE comes from magnetic resonance imaging and

high-quality X-ray computerized tomography scans. These analyses define two major classes of HPE that encompass four types. The two classes are 'classic' HPE, in which the most severely affected region of the hemispheres is the basal/ventral forebrain, and midline interhemispheric HPE (MIH HPE), or syntelencephaly, in which the cortical/dorsal part of the hemispheres fails to separate and in which the basal forebrain can be normal (8, 12–14).

Classic HPE is composed of three different types. At the most severe end of the phenotypic spectrum of malformations is alobar HPE, in which the ventral forebrain is unseparated, the whole forebrain is monoventricular and small, and the face can be cyclopic. In the second type, semilobar HPE, the anterior regions of the hemispheres fail to cleave but the posterior regions are often normal. The third ventricle is small and partially formed, and the cortex, basal ganglia, and thalamus are significantly fused. Finally, in lobar HPE, the anterior forebrain is incompletely separated but to a lesser degree than in semilobar cases. Individuals have a fully formed third ventricle, although dysmorphic, and anterior structures such as the corpus callosum, and the olfactory bulbs may be missing or hypoplastic.

The second class of HPE known as MIH HPE is rarer and milder than classic HPE, in some cases only affecting the dorsal forebrain. The dorsal part of the hemispheres fail to divide in the posterior frontal and parietal regions, and in many cases, the caudate nuclei and thalami are also incompletely separated. Nevertheless, there is an interhemispheric separation of the basal forebrain, the anterior frontal lobes, and the occipital regions. It has been suggested that genetic pathways necessary for normal development of the dorsal forebrain are impaired in MIH HPE, whereas in classic HPE, genetic pathways important for the development of the ventral forebrain may be more often defective (15).

Genetic heterogeneity of HPE

The genetics of human HPE are complex, and only a few mutated genes that underlie familial cases of HPE have been identified. Up to 45% of patients with HPE display clear cytogenetic abnormalities such as trisomy 13, trisomy 18, and triploidy (16). From karyotype analyses, at least 12 genomic regions spread over 11 different chromosomes (loci HPE1 to HPE12) have been described as containing HPE candidate genes (8, 17). Pedigree studies support autosomal dominant, recessive, and X-linked inheritance (17, 18). Moreover, HPE can sometimes be associated with other congenital syndromes such as Smith–Lemli–Opitz and Pallister–Hall syndromes (19, 20). The heterogeneity in familial HPE from severely affected to clinically normal individuals carrying the same mutation may be due to the influence of environmental or teratogenic factors [e.g. alcohol, diabetes, cholesterol, retinoic acid (21–23)] or modifier genes (24). Consistent with the existence of modifier genes and multiple interacting loci, heterozygous mutations in two HPE genes are required to produce a severe phenotype in three human cases to date [sonic hedgehog (SHH) and TGIF (25), SHH and ZIC2 (25), and PTC1 and GLI2 (26)], but this number is likely to rise as more candidate HPE genes are characterized and their interactions understood.

Keys to this characterization are studies using mouse models. Note that in mice, in contrast to humans, heterozygous mutations in single genes associated with HPE do not usually display a phenotype but only the homozygous mutants produce the phenotype (Table 1). This suggests that mice are less prone to haploinsufficiency than humans. Nevertheless, genes associated with human cases of HPE, when homozygously mutated in mice, more often than not also result in HPE. Furthermore, mouse mutants with HPE phenotypes, for which the gene has not yet been associated with HPE in humans, provide new candidate HPE and modifier genes. Another advantage of the mouse is that mutations in two or more genes can readily be combined to study and understand the genetic interactions that cause HPE (Table 1).

Several genes when mutated on their own in mouse [e.g. *Cdo* and *Tgif* (27–29)] exhibit HPE with variable expressivity or penetrance, mimicking what is observed in familial cases of HPE in humans. For instance, in *Cdo* mutants, the phenotypes range from a normal forebrain to semilobar HPE. The spectrum of variability in these mutant mice is strain dependent, suggesting that modifier genes account for the heterogeneity (28–30). It should be possible to identify these modifier genes through quantitative trait loci analyses. The genetic interactions underlying the heterogeneity of HPE phenotypes in humans are just beginning to be understood, and mouse models will continue to be instrumental in elucidating the genetic pathways that regulate the formation of the telencephalic midline and that lead to HPE when disrupted.

Table 1. HPE genes in humans and mice

Genes	Phenotypes in humans	Phenotypes in mice	References
Classic HPE			
SHH	HPE3: large spectrum from cyclopia and alobar HPE to normal individuals	*Shh*−/−: cyclopia, lack of ventral telencephalon, HPE	(25, 53, 56, 57, 63, 76, 77, 80, 100)
GLI2	HPE9: pituitary anomalies, craniofacial abnormalities, alobar HPE, microcephaly, hydrocephalus	*Gli2*−/−: normal telencephalon, hypothalamus defects	(79, 101, 102)
PTC	HPE7: large spectrum from semilobar HPE to lobar HPE with craniofacial defects to normal individuals	*Ptc*−/−: no HPE, failure to close the neural tube	(75, 81)
GLI2 and *PTC*	HPE-like and craniofacial defects	ND	(26)
SHH and *TGIF*	Semilobar HPE, craniofacial defects	ND	(25)
SHH and *ZIC2*	Semilobar HPE, microcephaly, craniofacial defects	ND	(25)
SMO	ND	*Smo*−/−: cyclopia, lack of ventral telencephalon, HPE	(82)
		Smo cKO: lack of ventral telencephalon with normal DM	(59)
DISPA	ND	*Disp*−/−: cyclopia, lack of ventral telencephalon, HPE	(70)
CDO	ND	*Cdo*−/−: from severe to microform of HPE (strain dependence), lack of ventral telencephalon	(28, 29, 71)
GAS1	ND	*Gas1*−/−: microform of HPE	(73)
CDO and *GAS1*	ND	*Cdo*−/−;*Gas1*−/−: craniofacial defects, lack of ventral telencephalon	(72)
GAS1 and *SHH*	ND	*Gas1*−/−;*Shh*−/+: craniofacial defects	(73)
FGF8	ND	*Fgf8* hypomorph: HPE, lack of ventral telencephalon, lack of the DM; *Fgf8* cKO: HPE, lack of ventral telencephalon	(43)
FGFR1 and *FGFR2*	ND	*Fgfr1*−/−;*Fgfr2*−/− cKO: HPE, lack of ventral telencephalon	(65)
NODAL	ND	*Nodal* cKO: small head, no midline separation of the forebrain	(85, 86)
NODAL and *SMAD2*	ND	*Nodal*+/−;*Smad2*+/−: cyclopia, head truncation	(91)
NODAL and *ActRIIA*	ND	*ActrIIA*−/−;*Nodal*+/−: cyclopia, head truncation	(90)
NODAL and *GDF1*	ND	*Gdf1*−/−;*Nodal*+/−: HPE, head truncation	(89)
TGIF	HPE4: lobar and semilobar HPE, agenesis of the corpus callosum, microcephaly, craniofacial defects	*Tgif*−/−: normal telencephalon *Tgif*Δexon3: HPE, exencephaly, microcephaly	(27, 88, 103)
FOXH1 (FAST1)	HPE	*Foxh1*−/−: lethal prior to forebrain formation	(8, 12, 24, 104, 105)
TDGF1 (CRIPTO)	HPE: minor craniofacial abnormalities, small head size, single ventricle	*Cripto*−/−: lethal prior to forebrain formation	(87, 106, 107)
OTX2 and *FOXA2*	ND	*Otx2*+/−;*Foxa2*+/−: HPE, cyclopia, anterior forebrain truncation	(108)
MEGALIN/LRP2	ND	*Lrp2* cKO: HPE, craniofacial defects, lack of ventral telencephalon	(84)

Table 1. Continued

Genes	Phenotypes in humans	Phenotypes in mice	References
NOGGIN and *CHORDIN*		*Chrd−/−;Nog+/−*: HPE, cyclopia, craniofacial defects, truncation of the rostral forebrain, lack of the ventral telencephalon	(83)
DKK-1	ND	*Dkk1−/−*: from cyclopia to lack of anterior head structure; *Dkk1+/−;Nog+/−:* from cyclopia to lack of anterior head structure, lack of ventral forebrain	(109, 110)
SIX3	HPE2: semilobar, alobar, cyclopia, microcephaly, craniofacial defects	*Six3* hypomorph: lack of eyes and forebrain	(92–95)
ZIC2	See below		
MIH HPE			
BMPs	ND	*Bmpr1a−/−;Bmpr1b−/−* cKO: MIH HPE, normal ventral telencephalon, lack of CPe and CH	(40)
ZIC2	HPE5: alobar, semilobar, MIH HPE, microcephaly, hydrocephaly, agenesis of corpus callosum, mild face dysmorphism	*Zic2* hypomorph: HPE, abnormal ventral telencephalon, lack of the DM	(15, 44, 94, 96–98)
FGF8	See above		
RFX4_v3	ND	*Rfx4_v3−/−*: HPE, normal ventral telencephalon, severe reduction of the CPe and CH, hypoplasia of the dorsal telencephalon	(42)
LHX5	ND	*Lhx5−/−*: lack of CPe and CH, expansion of cortical primordium	(111)

CH, cortical hem; CPe, choroid plexus; cKO, conditional knockout; DM, dorsal midline; HPE, holoprosencephaly; MIH, midline interhemispheric; ND, not determined.

Development of the telencephalic midline

The mammalian forebrain is derived from the embryonic prosencephalon located at the most anterior part on the neural tube. Shortly after neural tube closure at around embryonic day 9.5 in the mouse (E9.5) and 3.5 weeks of gestation in humans, the prosencephalon starts to differentiate into telencephalon, future cerebral hemispheres, and diencephalon, future thalamus. By E10.5 in mouse and 35 days in human, the telencephalon undergoes dramatic morphological changes, becoming split medially into two bilateral vesicles (Fig. 1). In contrast to their more lateral neighbors, midline cells undergo higher levels of cell death and reduced proliferation, leading to a pinching off of the expanding cerebrum into right and left hemispheres. Dorsal midline cells differentiate medially into the choroid plexus, which secretes the cerebrospinal fluid, and into the adjacent cortical hem, which induces formation of the hippocampus and is also a source Cajal–Retzius neurons (31–34). Ventrally and rostrally, midline cells contribute to the septum and ganglionic eminences, which give rise to parts of the basal ganglia. Several secreted signaling molecules are expressed by telencephalic midline cells. Dorsally, the midline expresses bone morphogenetic proteins (BMPs) and wingless-Int proteins (WNTs), while the rostral and ventral midline express fibroblast growth factors (FGFs) and SHH, respectively (Fig. 1). These factors are hypothesized to interact in forming and patterning the telencephalic hemispheres (35–37).

The dorsal midline

BMP signaling is necessary for dorsal midline development. At least five BMP genes are expressed in the dorsal midline [*Bmp2*, *Bmp4*, *Bmp5*, *Bmp6*, and *Bmp7*; (38)]. BMP4-soaked beads implanted on cultured explants of lateral telencephalon induce dorsal midline features such as cell death, low levels of proliferation, expression of the midline marker *Msx1* and repression of the non-midline marker *Foxg1*

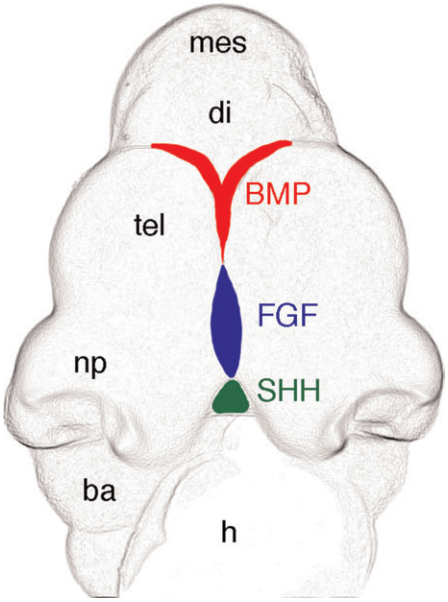

Fig. 1. Schematic representation of an E10.5 mouse head (frontal view, dorsal up). Telencephalic midline areas are highlighted in color, with the dorsal midline expressing bone morphogenetic proteins (red), the rostral midline expressing fibroblast growth factors (blue), and the ventral midline expressing sonic hedgehog (green). ba: branchial arch, di: diencephalon, h: heart, mes: mesencephalon, np: nasal process, tel: telencephalon.

(38). Telencephalon-specific knockouts of type I BMP receptor genes demonstrate that BMP signaling is required for the formation of a dorsal midline (39, 40). Notably, double *Bmpr1a* and *Bmpr1b* mutants fail to separate the telencephalon into left and right hemispheres, mimicking MIH HPE (40). A role for disrupted BMP signaling in human HPE, however, has yet to be identified.

In addition to BMP genes, WNT genes, *Wnt2b*, *Wnt3a*, *Wnt5a*, and *Wnt8b* are expressed in the dorsal midline (31, 32). Although *Wnt3a* is essential for hippocampal development (32, 41), WNTs have not been implicated in midline formation. Interestingly, however, a knockout of *Rfx4-v3*, a transcript variant encoding a winged-helix transcription factor, results in loss of *Wnt3a* expression and HPE (42). Nevertheless, a direct role for WNT genes in dorsal midline formation and in HPE has not been demonstrated.

Other than BMP receptor genes, mutations in only two other genes to date cause the loss of the dorsal midline, *Fgf8* and *Zic2*, and in both these cases, the mutations are hypomorphic (43, 44). *Zic2* encodes a zinc finger transcription factor expressed in both the dorsal and ventral midline (40, 45–47). *Zic2* expression is not dependent on BMP signaling (40) but can be induced by ectopic FGF8 application (47), suggesting that it acts downstream of FGFs and in parallel or upstream of BMPs (Fig. 3). Although the ventral telencephalon, which appears grossly normal in the *Zic2* mutant, has not been examined in detail (44), *ZIC2* mutations in humans can lead to both classic and MIH HPE (see below) (15).

The rostral midline

The rostral midline expresses at least five *Fgf* genes: *Fgf3*, *Fgf8*, *Fgf15* (*Fgf19* in humans), *Fgf17*, and *Fgf18* (48–51). FGF8-soaked beads placed in dorsolateral areas of the chick telencephalon can induce an ectopic sulcus with rostral midline features (52), suggesting that FGFs may play a role in the formation of the rostral midline. However, no mutations in the FGF pathway have yet been linked to the loss of the rostral midline in mice or humans.

The ventral telencephalon, including the midline

Shh is essential for ventral forebrain development, and mutations of this gene in mouse or human lead to classic HPE phenotypes [(53–57); see below]. In the forebrain of the *Shh* mouse mutant, only dorsal precursors remain and all ventral precursors that normally give rise to the basal ganglia are missing (53, 58–60). Along with its role as an inducer of ventral neural cells, *Shh* is also required to maintain the proliferation and survival of ventral precursor cells (54, 61–63). In the *Shh* mutant, the loss of ventral structures is accompanied by an apparent lack of separation of the dorsal telencephalic hemispheres (53, 63). This is likely not due to a failure of the dorsal midline to initially form and is probably due instead to a lack of overall growth and expansion of the hemispheres (40, 60).

The loss of ventral cells observed in the *Shh* mutant is rescued if its downstream antagonist *Gli3* is also mutated, indicating that factors other than SHH can induce ventral development (58, 64). Indeed, FGFs are also required for this process. In *Fgfr1* and *Fgfr2* double mutants or *Fgf8* single mutants, ventral cells fail to be generated (43, 65). Moreover, SHH acts genetically upstream of FGFs in forming the ventral telencephalon. SHH not only regulates the expression of several *Fgf* genes, *Fgf3*, *Fgf8*, *Fgf15*, *Fgf17*, and *Fgf18* (60, 63, 64), but also depends on FGF signaling to form all ventral regions because even when SHH is expressed and active, no ventral structures develop if FGF signaling is disrupted (65). Conversely, FGF signaling can ectopically induce ventral gene expression even

when SHH signaling is disrupted (66). The regulation of FGF expression and signaling by SHH is indirect through *Gli3*. In *Gli3* mouse mutants, *Fgf* gene expression is expanded and the telencephalon is ventralized (31, 64, 66, 67). Moreover, unlike for the *Shh* mutant, loss of *Gli3* does not rescue loss of FGF signaling, placing FGFs downstream of *Gli3* (65).

The current understanding of the genetic regulation of cerebral midline development is clearly incomplete, but it provides a useful framework in which to place future components of these and other genetic pathways found to be required to form the midline.

SHH: the central player in classic HPE?

Several mouse models mimicking classic forms of HPE have been studied. All these models, as well as the human mutations identified to date that lead to classic HPE, suggest that the common denominator may be disruption of SHH signaling, whether directly or indirectly, which leads primarily to a lack of ventral cell types.

The SHH pathway

The SHH pathway has been extensively dissected in several vertebrate and invertebrate species [Fig. 2; reviewed by Fuccillo et al. and Chen et al. (68, 69)]. In the absence of extracellular SHH, the transmembrane protein Smoothened (SMO) is inhibited by the SHH receptor, Patched (PTC). Binding of SHH to PTC relieves the inhibition on SMO and promotes the function of the GLI2 transcriptional activator while inhibiting the repressor form of GLI3. Several transmembrane proteins also promote SHH signaling. In SHH-producing cells, DispatchedA (DISPA) increases the amount of the cleaved, active form of SHH (SHH-N) (70), whereas in the SHH-responding cell, GAS1, CDO, and BOC are all thought to promote SHH signaling through the PTC receptor (29, 71–74).

Mutations in the SHH pathway lead to classic HPE in mouse and human

In humans, mutations in three genes that encode components of the SHH signaling pathway, *SHH* (HPE3), *PTC* (HPE7), and *GLI2* (HPE9), lead to classic HPE (56, 57, 75–80). Only one of these genes, *Shh*, when deleted in mice also leads to HPE. Loss of mouse *Gli2* does not appear to affect forebrain development. The human *PTC* mutations are hypothesized to be gain-of-function mutations rather than loss-of-function mutations because PTC antagonizes SHH signaling (75). Hence, it is not surprising that deleting the *Ptc* gene in mice does not result in HPE (81). Generating mouse models using the human *PTC* missense mutations would be informative in testing the nature and effect of these mutations on SHH signaling.

Other mouse genes that encode components of SHH signaling also lead to HPE when deleted, namely, *Smo*, *Disp*, *Cdo*, and *Gas1* (29, 70, 71, 73, 82). The phenotype of these mice suggests that these genes, if not genes that can cause HPE when mutated on their own in humans, may at least act as modifier loci in the presence of another mutated gene. In fact, in mice, some of these genes, *Gas1* and *Cdo* as well as *Gas1* and *Shh*, have been shown to interact genetically to worsen at least the craniofacial and neural tube defects (72, 73). *Cdo* mutants themselves display widely varying phenotypes depending on the strain background, indicating the existence of as yet unidentified modifier loci (28, 29). In humans, to date, there are three examples of genetic interactions between two loci leading to HPE: SHH and TGIF (25), SHH and ZIC2 (25), and GLI2 and PTC (26).

Disruption of other pathways that modulate SHH signaling results in HPE

Genes in mice that are not directly implicated in SHH signaling, but indirectly modulate it, also result in HPE when mutated. For example, mice deficient for genes that inhibit BMP activity, *Noggin* and *Chordin* and *Megalin*/*Lrp2*, display reduced SHH signaling and a loss of ventral cell types that can resemble classic HPE (83, 84). Other examples are components of the FGF signaling pathway. As shown with *Fgf8* and *Fgfr1*;*Fgfr2* mutants, FGF signaling, like SHH, is required to generate ventral cell types in the telencephalon (43, 65) and SHH depends on FGFs for this process to occur (65). These mouse studies provide additional candidates for genes associated with classic HPE in humans.

Components of the Nodal signaling pathway (Fig. 2), when mutated, have also been associated with HPE in humans and mice. Loss of Nodal signaling leads to loss of *Shh* expression (85, 86), suggesting that disruption of the Nodal pathway indirectly results in HPE through loss of SHH signaling. In humans, mutations in *TDGF1* (87), which encodes an extracellular cofactor that facilitates Nodal binding to its

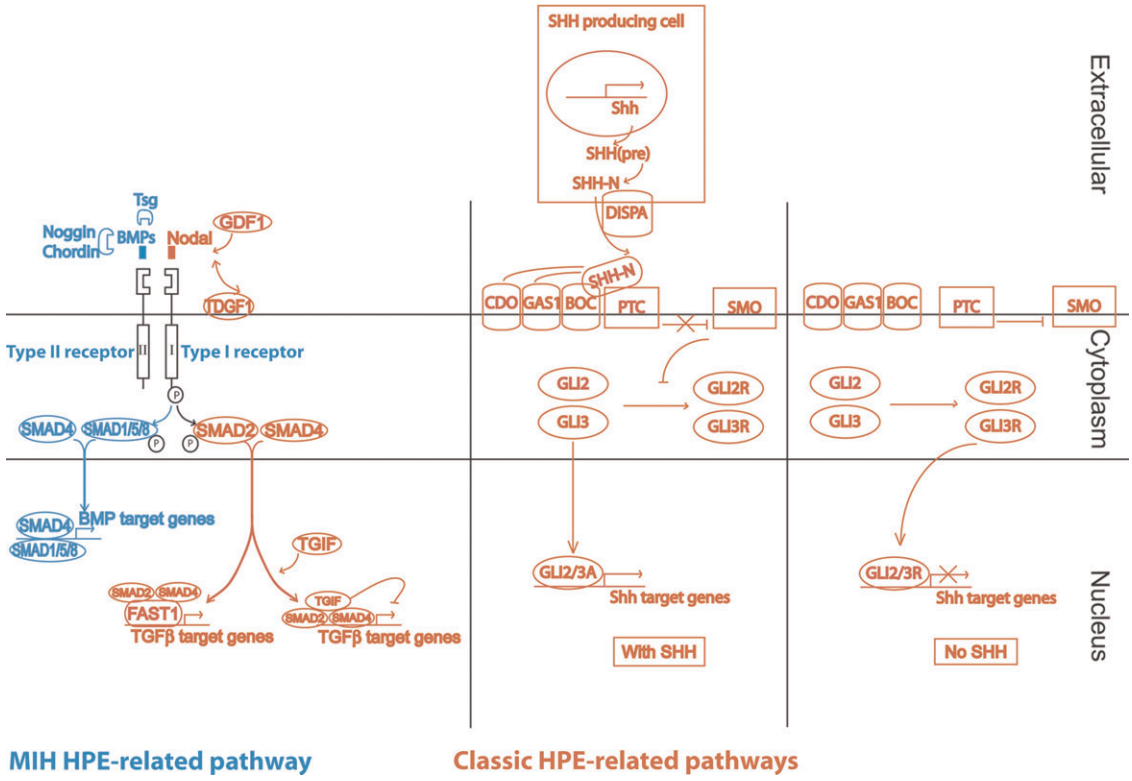

Fig. 2. Schematic representation of the pathways involved in MIH (blue) and classic (orange) holoprosencephaly in humans and mice [Adapted from Ming and Muenke (24) and Krauss (30)]. BMP, bone morphogenetic protein.

receptor, *FOXH1* (*FAST1*) (8, 12, 24), which encodes a transcription factor that promotes expression of Nodal-responsive genes, and *TGIF* [HPE4, (88)], which encodes a transcription cofactor that inhibits the Nodal pathway, have all been associated with HPE. Similarly in mouse, mutations in genes associated with Nodal signaling, *Nodal* itself as well as *Nodal* with its extracellular cofactor gene *Gdf1*, its receptor gene *ActrIIA*, or its downstream effector gene *Smad2*, can result in phenotypes with features of classic HPE (86, 89–91) (Table 1).

Six3 encodes a homeodomain transcription factor of the Six/*sine oculis* family. In humans, *SIX3* maps to the HPE2 locus (92–94). In mice, a partial loss of function of *Six3* displays a total lack of eyes and forebrain (95), precluding the identification of a link between *Six3*, SHH signaling, and ventral telencephalon development. However, it is likely that even milder mutations of *Six3* would also result in loss of SHH signaling and classic HPE-like phenotypes in mouse.

MIH HPE, a different molecular basis

In humans, *ZIC2* (HPE5), although most often associated with classic HPE features, is the only gene in which mutations are also associated with MIH HPE (15, 94, 96–98). In mouse, a hypomorphic allele of *Zic2* largely recapitulates the human phenotypes (44). Other than *Zic2*, however, only genes linked with BMP signaling have shown MIH-like phenotypes in mice (Figs 2 and 3). In particular, a double mutant of *Bmpr1a* and *Bmpr1b* leads to MIH HPE with a loss of dorsal midline cell types, while *Shh* expression and ventral development are normal (40).

In fact, the molecular causes for classic and MIH HPE can be considered opposite because BMP signaling and SHH signaling throughout the neural axis have opposing, if not, antagonistic effects on dorsal–ventral patterning. Consistent with this, mutations that lead to increased BMP signaling, such as those in *Megalin*/*Lrp2* as well as *Noggin* and *Chordin*, disrupt SHH expression and lead to classic HPE phenotypes (83, 84). Conversely, increasing the amount of active SHH leads to decreased *Bmp* gene expression and features of MIH HPE (99).

Perspective

A growing understanding of the genetic pathways regulating forebrain development, in

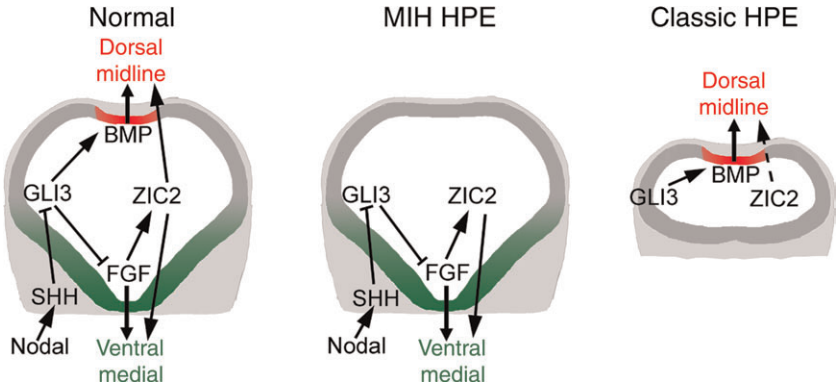

Fig. 3. Highly streamlined model of the genetic interactions for normal midline development (left), for midline interhemispheric holoprosencephaly (MIH HPE) (middle), and for classic HPE (right). In MIH HPE, the ventral telencephalon can develop normally through active SHH signaling and associated pathways, whereas the dorsal midline is absent, perhaps in some cases due to a lack of BMP signaling. In classic HPE, the ventral telencephalon is missing due to direct or indirect disruption of SHH function, while the dorsal midline at least initially develops normally [Adapted from Fernandes et al. (40)].

particular midline and ventral regions, along with the identification of genes that lead to HPE in both humans and mice, suggests that the genetic complexity underlying HPE phenotypes in the forebrain can perhaps be simplified to few signaling pathways: those that modulate SHH ventrally and those that modulate BMP signaling dorsally (Fig. 3). Moreover, studies using animal models are pointing toward candidate genes in humans that may act as modifiers and account for the wide spectrum of HPE phenotypes. Identification of these genes is essential in improving prenatal diagnoses and prognoses for HPE.

References

1. Cohen MM Jr. Perspectives on holoprosencephaly: part I. Epidemiology, genetics, and syndromology. Teratology 1989: 40: 211–235.
2. Muenke M, Beachy PA. Holoprosencephaly. In: Scriver CR et al., eds. The metabolic and molecular bases of inherited disease, 8th edn. New York, NY: McGraw-Hill, 2001: 6203–6230.
3. Roach E, Demyer W, Conneally PM, Palmer C, Merritt AD. Holoprosencephaly: birth data, genetic and demographic analyses of 30 families. Birth Defects Orig Artic Ser 1975: 11: 294–313.
4. Matsunaga E, Shiota K. Holoprosencephaly in human embryos: epidemiologic studies of 150 cases. Teratology 1977: 16: 261–272.
5. Cohen MM Jr. Perspectives on holoprosencephaly: part III. Spectra, distinctions, continuities, and discontinuities. Am J Med Genet 1989: 34: 271–288.
6. Barr M Jr, Hanson JW, Currey K et al. Holoprosencephaly in infants of diabetic mothers. J Pediatr 1983: 102: 565–568.
7. Croen LA, Shaw GM, Lammer EJ. Risk factors for cytogenetically normal holoprosencephaly in California: a population-based case-control study. Am J Med Genet 2000: 90: 320–325.
8. Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. Orphanet J Rare Dis 2007: 2: 8.
9. Lazaro L, Dubourg C, Pasquier L et al. Phenotypic and molecular variability of the holoprosencephalic spectrum. Am J Med Genet A 2004: 129: 21–24.
10. Ming JE, Muenke M. Holoprosencephaly: from homer to hedgehog. Clin Genet 1998: 53: 155–163.
11. Muenke M, Gurrieri F, Bay C et al. Linkage of a human brain malformation, familial holoprosencephaly, to chromosome 7 and evidence for genetic heterogeneity. Proc Natl Acad Sci U S A 1994: 91: 8102–8106.
12. Cohen MM Jr. Holoprosencephaly: clinical, anatomic, and molecular dimensions. Birth Defects Res A Clin Mol Teratol 2006: 76: 658–673.
13. Barkovich AJ, Quint DJ. Middle interhemispheric fusion: an unusual variant of holoprosencephaly. AJNR Am J Neuroradiol 1993: 14: 431–440.
14. Demyer W, Zeman W, Palmer CD. Familial alobar holoprosencephaly (Arhinencephaly) with median cleft lip and palate. Report of patient with 46 chromosomes. Neurology 1963: 13: 913–918.
15. Simon EM, Hevner RF, Pinter JD et al. The middle interhemispheric variant of holoprosencephaly. AJNR Am J Neuroradiol 2002: 23: 151–156.
16. Olsen CL, Hughes JP, Youngblood LG, Sharpe-Stimac M. Epidemiology of holoprosencephaly and phenotypic characteristics of affected children: New York State, 1984–1989. Am J Med Genet 1997: 73: 217–226.
17. Roessler E, Muenke M. Holoprosencephaly: a paradigm for the complex genetics of brain development. J Inherit Metab Dis 1998: 21: 481–497.
18. Hockey A, Crowhurst J, Cullity G. Microcephaly, holoprosencephaly, hypokinesia – second report of a new syndrome. Prenat Diagn 1988: 8: 683–686.
19. Kelley RL, Roessler E, Hennekam RC et al. Holoprosencephaly in RSH/Smith-Lemli-Opitz syndrome: does abnormal cholesterol metabolism affect the function of sonic hedgehog? Am J Med Genet 1996: 66: 478–484.
20. Hall JG, Pallister PD, Clarren SK et al. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly – a new syndrome? Part I: clinical, causal, and pathogenetic considerations. Am J Med Genet 1980: 7: 47–74.

21. Edison R, Muenke M. The interplay of genetic and environmental factors in craniofacial morphogenesis: holoprosencephaly and the role of cholesterol. Congenit Anom (Kyoto) 2003: 43: 1–21.
22. Muenke M, Cohen MM Jr. Genetic approaches to understanding brain development: holoprosencephaly as a model. Ment Retard Dev Disabil Res Rev 2000: 6: 15–21.
23. Cohen MM Jr, Shiota K. Teratogenesis of holoprosencephaly. Am J Med Genet 2002: 109: 1–15.
24. Ming JE, Muenke M. Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. Am J Hum Genet 2002: 71: 1017–1032.
25. Nanni L, Ming JE, Bocian M et al. The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly. Hum Mol Genet 1999: 8: 2479–2488.
26. Rahimov F, Ribeiro LA, de Miranda E, Richieri-Costa A, Murray JC. GLI2 mutations in four Brazilian patients: how wide is the phenotypic spectrum? Am J Med Genet A 2006: 140: 2571–2576.
27. Kuang C, Xiao Y, Yang L et al. Intragenic deletion of Tgif causes defects in brain development. Hum Mol Genet 2006: 15: 3508–3519.
28. Cole F, Krauss RS. Microform holoprosencephaly in mice that lack the Ig superfamily member Cdon. Curr Biol 2003: 13: 411–415.
29. Zhang W, Kang JS, Cole F, Yi MJ, Krauss RS. Cdo functions at multiple points in the sonic hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly. Dev Cell 2006: 10: 657–665.
30. Krauss RS. Holoprosencephaly: new models, new insights. Expert Rev Mol Med 2007: 9: 1–17.
31. Grove EA, Tole S, Limon J, Yip L, Ragsdale CW. The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. Development 1998: 125: 2315–2325.
32. Lee SM, Tole S, Grove E, McMahon AP. A local Wnt-3a signal is required for development of the mammalian hippocampus. Development 2000: 127: 457–467.
33. Takiguchi-Hayashi K, Sekiguchi M, Ashigaki S et al. Generation of reelin-positive marginal zone cells from the caudomedial wall of telencephalic vesicles. J Neurosci 2004: 24: 2286–2295.
34. Zhao C, Guan W, Pleasure SJ. A transgenic marker mouse line labels Cajal-Retzius cells from the cortical hem and thalamocortical axons. Brain Res 2006: 1077: 48–53.
35. Hebert JM. Unraveling the molecular pathways that regulate early telencephalon development. Curr Top Dev Biol 2005: 69: 17–37.
36. Wilson SW, Houart C. Early steps in the development of the forebrain. Dev Cell 2004: 6: 167–181.
37. Monuki ES, Walsh CA. Mechanisms of cerebral cortical patterning in mice and humans. Nat Neurosci 2001: 4 (Suppl.): 1199–1206.
38. Furuta Y, Piston DW, Hogan BL. Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. Development 1997: 124: 2203–2212.
39. Hebert JM, Mishina Y, McConnell SK. BMP signaling is required locally to pattern the dorsal telencephalic midline. Neuron 2002: 35: 1029–1041.
40. Fernandes M, Gutin G, Alcorn H, McConnell SK, Hebert JM. Mutations in the BMP pathway in mice support the existence of two molecular classes of holoprosencephaly. Development 2007: 134: 3789–3794.
41. Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. Development 2000: 127: 469–482.
42. Blackshear PJ, Graves JP, Stumpo DJ, Cobos I, Rubenstein JL, Zeldin DC. Graded phenotypic response to partial and complete deficiency of a brain-specific transcript variant of the winged helix transcription factor RFX4. Development 2003: 130: 4539–4552.
43. Storm EE, Garel S, Borello U et al. Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. Development 2006: 133: 1831–1844.
44. Nagai T, Aruga J, Minowa O et al. Zic2 regulates the kinetics of neurulation. Proc Natl Acad Sci U S A 2000: 97: 1618–1623.
45. Nagai T, Aruga J, Takada S et al. The expression of the mouse Zic1, Zic2, and Zic3 gene suggests an essential role for Zic genes in body pattern formation. Dev Biol 1997: 182: 299–313.
46. Cheng X, Hsu CM, Currle DS, Hu JS, Barkovich AJ, Monuki ES. Central roles of the roof plate in telencephalic development and holoprosencephaly. J Neurosci 2006: 26: 7640–7649.
47. Hayhurst M, Gore BB, Tessier-Lavigne M, McConnell SK. Ongoing sonic hedgehog signaling is required for dorsal midline formation in the developing forebrain. Dev Neurobiol 2008: 68: 83–100.
48. Crossley PH, Martin GR. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 1995: 121: 439–451.
49. Maruoka Y, Ohbayashi N, Hoshikawa M, Itoh N, Hogan BL, Furuta Y. Comparison of the expression of three highly related genes, Fgf8, Fgf17 and Fgf18, in the mouse embryo. Mech Dev 1998: 74: 175–177.
50. McWhirter JR, Goulding M, Weiner JA, Chun J, Murre C. A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. Development 1997: 124: 3221–3232.
51. Shinya M, Koshida S, Sawada A, Kuroiwa A, Takeda H. Fgf signalling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. Development 2001: 128: 4153–4164.
52. Crossley PH, Martinez S, Ohkubo Y, Rubenstein JL. Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. Neuroscience 2001: 108: 183–206.
53. Chiang C, Litingtung Y, Lee E et al. Cyclopia and defective axial patterning in mice lacking sonic hedgehog gene function. Nature 1996: 383: 407–413.
54. Ericson J, Muhr J, Placzek M, Lints T, Jessell TM, Edlund T. Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube. Cell 1995: 81: 747–756.
55. Belloni E, Muenke M, Roessler E et al. Identification of sonic hedgehog as a candidate gene responsible for holoprosencephaly. Nat Genet 1996: 14: 353–356.
56. Roessler E, Belloni E, Gaudenz K et al. Mutations in the human sonic hedgehog gene cause holoprosencephaly. Nat Genet 1996: 14: 357–360.
57. Roessler E, Ward DE, Gaudenz K et al. Cytogenetic rearrangements involving the loss of the sonic hedgehog gene at 7q36 cause holoprosencephaly. Hum Genet 1997: 100: 172–181.

58. Rallu M, Machold R, Gaiano N, Corbin JG, McMahon AP, Fishell G. Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and hedgehog signaling. Development 2002: 129: 4963–4974.
59. Fuccillo M, Rallu M, McMahon AP, Fishell G. Temporal requirement for hedgehog signaling in ventral telencephalic patterning. Development 2004: 131: 5031–5040.
60. Rash BG, Grove EA. Patterning the dorsal telencephalon: a role for sonic hedgehog? J Neurosci 2007: 27: 11595–11603.
61. Litingtung Y, Chiang C. Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. Nat Neurosci 2000: 3: 979–985.
62. Rowitch DH, S-Jacques B, Lee SM, Flax JD, Snyder EY, McMahon AP. Sonic hedgehog regulates proliferation and inhibits differentiation of CNS precursor cells. J Neurosci 1999: 19: 8954–8965.
63. Ohkubo Y, Chiang C, Rubenstein JL. Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles. Neuroscience 2002: 111: 1–17.
64. Aoto K, Nishimura T, Eto K, Motoyama J. Mouse GLI3 regulates Fgf8 expression and apoptosis in the developing neural tube, face, and limb bud. Dev Biol 2002: 251: 320–332.
65. Gutin G, Fernandes M, Palazzolo L et al. FGF signalling generates ventral telencephalic cells independently of SHH. Development 2006: 133: 2937–2946.
66. Kuschel S, Ruther U, Theil T. A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon. Dev Biol 2003: 260: 484–495.
67. Theil T, Alvarez-Bolado G, Walter A, Ruther U. Gli3 is required for Emx gene expression during dorsal telencephalon development. Development 1999: 126: 3561–3571.
68. Fuccillo M, Joyner AL, Fishell G. Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. Nat Rev Neurosci 2006: 7: 772–783.
69. Chen MH, Wilson CW, Chuang PT. SnapShot: hedgehog signaling pathway. Cell 2007: 130: 386.
70. Ma Y, Erkner A, Gong R et al. Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. Cell 2002: 111: 63–75.
71. Tenzen T, Allen BL, Cole F, Kang JS, Krauss RS, McMahon AP. The cell surface membrane proteins Cdo and Boc are components and targets of the hedgehog signaling pathway and feedback network in mice. Dev Cell 2006: 10: 647–656.
72. Allen BL, Tenzen T, McMahon AP. The hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development. Genes Dev 2007: 21: 1244–1257.
73. Seppala M, Depew MJ, Martinelli DC, Fan CM, Sharpe PT, Cobourne MT. Gas1 is a modifier for holoprosencephaly and genetically interacts with sonic hedgehog. J Clin Invest 2007: 117: 1575–1584.
74. Martinelli DC, Fan CM. The role of Gas1 in embryonic development and its implications for human disease. Cell Cycle 2007: 6: 2650–2655.
75. Ming JE, Kaupas ME, Roessler E et al. Mutations in PATCHED-1, the receptor for SONIC HEDGEHOG, are associated with holoprosencephaly. Hum Genet 2002: 110: 297–301.
76. Maity T, Fuse N, Beachy PA. Molecular mechanisms of sonic hedgehog mutant effects in holoprosencephaly. Proc Natl Acad Sci U S A 2005: 102: 17026–17031.
77. Schell-Apacik C, Rivero M, Knepper JL, Roessler E, Muenke M, Ming JE. SONIC HEDGEHOG mutations causing human holoprosencephaly impair neural patterning activity. Hum Genet 2003: 113: 170–177.
78. Traiffort E, Dubourg C, Faure H et al. Functional characterization of sonic hedgehog mutations associated with holoprosencephaly. J Biol Chem 2004: 279: 42889–42897.
79. Roessler E, Du YZ, Mullor JL et al. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. Proc Natl Acad Sci U S A 2003: 100: 13424–13429.
80. Roessler E, Belloni E, Gaudenz K et al. Mutations in the C-terminal domain of sonic hedgehog cause holoprosencephaly. Hum Mol Genet 1997: 6: 1847–1853.
81. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 1997: 277: 1109–1113.
82. Zhang XM, Ramalho-Santos M, McMahon AP. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R symmetry by the mouse node. Cell 2001: 106: 781–792.
83. Anderson RM, Lawrence AR, Stottmann RW, Bachiller D, Klingensmith J. Chordin and noggin promote organizing centers of forebrain development in the mouse. Development 2002: 129: 4975–4987.
84. Spoelgen R, Hammes A, Anzenberger U et al. LRP2/megalin is required for patterning of the ventral telencephalon. Development 2005: 132: 405–414.
85. Rohr KB, Barth KA, Varga ZM, Wilson SW. The nodal pathway acts upstream of hedgehog signaling to specify ventral telencephalic identity. Neuron 2001: 29: 341–351.
86. Lowe LA, Yamada S, Kuehn MR. Genetic dissection of nodal function in patterning the mouse embryo. Development 2001: 128: 1831–1843.
87. de la Cruz JM, Bamford RN, Burdine RD et al. A loss-of-function mutation in the CFC domain of TDGF1 is associated with human forebrain defects. Hum Genet 2002: 110: 422–428.
88. Gripp KW, Wotton D, Edwards MC et al. Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. Nat Genet 2000: 25: 205–208.
89. Andersson O, Reissmann E, Jornvall H, Ibanez CF. Synergistic interaction between Gdf1 and Nodal during anterior axis development. Dev Biol 2006: 293: 370–381.
90. Song J, Oh SP, Schrewe H et al. The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. Dev Biol 1999: 213: 157–169.
91. Nomura M, Li E. Smad2 role in mesoderm formation, left-right patterning and craniofacial development. Nature 1998: 393: 786–790.
92. Wallis DE, Roessler E, Hehr U et al. Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. Nat Genet 1999: 22: 196–198.
93. Ribeiro LA, El-Jaick KB, Muenke M, Richieri-Costa A. SIX3 mutations with holoprosencephaly. Am J Med Genet A 2006: 140: 2577–2583.
94. Dubourg C, Lazaro L, Pasquier L et al. Molecular screening of SHH, ZIC2, SIX3, and TGIF genes in patients with features of holoprosencephaly spectrum: mutation review and genotype-phenotype correlations. Hum Mutat 2004: 24: 43–51.

95. Carl M, Loosli F, Wittbrodt J. Six3 inactivation reveals its essential role for the formation and patterning of the vertebrate eye. Development 2002: 129: 4057–4063.
96. Brown SA, Warburton D, Brown LY et al. Holoprosencephaly due to mutations in ZIC2, a homologue of Drosophila odd-paired. Nat Genet 1998: 20: 180–183.
97. Brown LY, Odent S, David V et al. Holoprosencephaly due to mutations in ZIC2: alanine tract expansion mutations may be caused by parental somatic recombination. Hum Mol Genet 2001: 10: 791–796.
98. Marcorelles P, Loget P, Fallet-Bianco C, Roume J, Encha-Razavi F, Delezoide AL. Unusual variant of holoprosencephaly in monosomy 13q. Pediatr Dev Pathol 2002: 5: 170–178.
99. Huang X, Litingtung Y, Chiang C. Ectopic sonic hedgehog signaling impairs telencephalic dorsal midline development: implication for human holoprosencephaly. Hum Mol Genet 2007: 16: 1454–1468.
100. Odent S, Atti-Bitach T, Blayau M et al. Expression of the sonic hedgehog (SHH) gene during early human development and phenotypic expression of new mutations causing holoprosencephaly. Hum Mol Genet 1999: 8: 1683–1689.
101. Park HL, Bai C, Platt KA et al. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. Development 2000: 127: 1593–1605.
102. Ding Q, Motoyama J, Gasca S et al. Diminished sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. Development 1998: 125: 2533–2543.
103. Shen J, Walsh CA. Targeted disruption of Tgif, the mouse ortholog of a human holoprosencephaly gene, does not result in holoprosencephaly in mice. Mol Cell Biol 2005: 25: 3639–3647.
104. Yamamoto M, Meno C, Sakai Y et al. The transcription factor FoxH1 (FAST) mediates Nodal signaling during anterior-posterior patterning and node formation in the mouse. Genes Dev 2001: 15: 1242–1256.
105. Hoodless PA, Pye M, Chazaud C et al. FoxH1 (Fast) functions to specify the anterior primitive streak in the mouse. Genes Dev 2001: 15: 1257–1271.
106. Ding J, Yang L, Yan YT et al. Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. Nature 1998: 395: 702–707.
107. Liguori GL, Echevarria D, Improta R et al. Anterior neural plate regionalization in cripto null mutant mouse embryos in the absence of node and primitive streak. Dev Biol 2003: 264: 537–549.
108. Jin O, Harpal K, Ang SL, Rossant J. Otx2 and HNF3beta genetically interact in anterior patterning. Int J Dev Biol 2001: 45: 357–365.
109. Mukhopadhyay M, Shtrom S, Rodriguez-Esteban C et al. Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. Dev Cell 2001: 1: 423–434.
110. del Barco Barrantes I, Davidson G, Grone HJ, Westphal H, Niehrs C. Dkk1 and noggin cooperate in mammalian head induction. Genes Dev 2003: 17: 2239–2244.
111. Zhao Y, Sheng HZ, Amini R et al. Control of hippocampal morphogenesis and neuronal differentiation by the LIM homeobox gene Lhx5. Science 1999: 284: 1155–1158.