ARTICLE

Neuropathology of Holoprosencephaly

PASCALE MARCORELLES* AND ANNIE LAQUERRIERE

Holoprosencephaly (HPE) is a brain malformation which results from a primary defect in induction and patterning of the rostral neural tube during early embryogenesis and usually considered as an impaired cleavage of the prosencephalon. The review of neuropathologic findings highlights a complex malformation involving not only the prosencephalon but also the whole brain, the eyes, and the cerebral vascularization. The classical form of HPE is divided in three sub-types according to DeMyer classification, although the spectrum is far wider, ranging from the most severe, aprosencephaly/atelencephaly, to milder forms such as syntelencephaly and to the less severe ends of the spectrum. Macroscopy and microscopy abnormality patterns are described extensively, allowing a comparison of the anatomic features between each form. Disturbances observed in the main cerebral structures including the basal ganglia, the commissures, the hippocampus, the brainstem, the cerebellum, and spinal cord are reviewed. Macroscopic and microscopic features of the ophthalmic anomalies are described, as well as brain vascular and associated central nervous system malformations.

KEY WORDS: holoprosencephaly spectrum; aprosencephaly/atelencephaly; syntelencephaly; mild forms; central nervous system pathology; eye pathology

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INTRODUCTION

The first neuropathological description of holoprosencephaly (HPE) was reported by Kundrat [1882], who recognized aplasia of the olfactory bulbs and tracts as the common denominator of this group of malformations and named it arhinencephaly [Kundrat, 1882]. The early literature concerning arhinencephaly and cyclopia in humans and animals was reviewed by Kohn [1952]. More extensive morphological studies were described by Yakolev [1959] for whom "arhinencephaly" was a misnomer. Although this author was the first to recognize a common pattern in the altered cytoarchitectonic fields of HPE brains, little advancement occurred until Mizuguchi and Morimatsu [1989a,b] and Mizuguchi et al. [1994] performed exhaustive histological analyses of HPE cortical alterations. A comprehensive case series was published by DeMyer et al. [1963, 1964], who termed the malformation "holoprosencephaly," then by Robain and Gorce [1972], by Jellinger and Gross [1973] who introduced the concept of a midline series of defects. This concept was further extended by Cohen [1989] who defined HPE as a single developmental field defect. Additional descriptions were made by Friede [1989] and Norman and Muenke [1995]. Based on the gross neuroanatomy degree of hemispheric non-separation, DeMyer and Zeman [1963] proposed a classification into three "classic" types according to a decreasing degree of severity and to the absence or the presence of the interhemispheric fissure and the extent of separation of both hemispheres: alobar, semilobar, and lobar. Other methods of grading HPE have been subsequently developed [Cohen, 1989; Golden, 1999]. A more complex classification was proposed by Probst [1979], in which HPEs are classified according to the presence or absence of a dorsal sac, to the presence or absence of a cleavage of basal structures with formation of an interhemispheric fissure. However, these classifications do not include all variants of the HPE spectrum, in particular aprosencephaly/atelencephaly (AP/AT), the most severe defect of prosencephalon development. About 15 years ago, a mild variant called syntelencephaly or middle interhemispheric variant of HPE (MIH) has also been identified [Barkovich and Quint, 1993] indicating that HPE apparently represents a continuum of forebrain malformations with no clear-cut distinction among the different subcategories [Leech and Shuman, 1986; Golden, 1999; Barkovich, 2005].

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CEREBRAL LESIONS IN "CLASSICAL" FORMS OF HOLOPROSENCEPHALY

Gross Examination

The most severe expression of classical HPE occurs in alobar HPE. In this form, the brain is often smaller than normal and consists of a holosphere with no interhemispheric fissure, sagittal sinus,

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[DeMyer, 1977; Jellinger et al., 1981; Cohen, 1989; Barkovich, 2005]. On the superior and posterior views of the brain, the telencephalon is uncleaved, usually horseshoe shaped and in its posterior part, the single ventricle is roofed with a thin pia arachnoid membrane (Fig. 1a). This membrane may be distended by hydrocephalus which in turn is responsible for apparently normal-sized head. The cyst occupies the space between the holosphere and the caudally displaced tentorium and cerebellum. The roof of the third ventricle forms the apex of this cyst whose walls contain choroids plexuses (Fig. 1b). In some instances, the cyst appears to have no connection with the cerebrospinal fluid (CSF) spaces. The Sylvian fissure is not formed, with no distinction between the cerebral lobes. The frontal and inferior faces of the holosphere are either smooth or covered with gyri which can have a normal size but run disorderly, traversing from one side to the other and running from anterior to posterior [Norman and Muenke, 1995]. Olfactory bulbs and tracts are missing. Optic nerves can be normal, fused, or absent [Barkovich, 2005]. On brain sections, a single crescent-shaped large ventricle without midline structures is bounded by a fine smooth layer of white matter. The common ventricle communicates with the aqueduct by way of the distended cystic upper part of the third ventricle. The corpus callosum (CC), the anterior commissure, and the septum pellucidum are not visible. The most median gyri frequently contain the hippocampus and entorhinal cortex. The striatum is absent in the most severely malformed brains. In the other cases, the deep gray nuclei are fused and located under the cyst-like membrane. The mamillary bodies may be fused [Jellinger et al., 1981]. The pineal gland is most often absent. The brainstem and cerebellum are usually normal, but a single cerebral peduncle may be found, and in many cases, the aqueduct is not macroscopically recognizable [Friede, 1989]. Pes pedonculi and pyramids are often hypoplastic.

In the semilobar form, affected fetuses or children present with less reduction in brain weight than in the alobar one. Macrocephaly is observed in case of hydrocephalus associated with a dorsal cyst. The main characteristic consists in an incomplete interhemispheric fissure and falx cerebri. When the interhemispheric fissure is rudimentary, its greatest depth is nearly always occipital, forming a short occipital cleft into the holosphere resulting in partly separated V-shaped lobes frontally fused (Fig. 1c,d) [Friede, 1989]. The gyral pattern remains abnormal [DeMyer and Zeman, 1964]. The frontal lobes may be underdeveloped with rudimentary temporal horns and incompletely

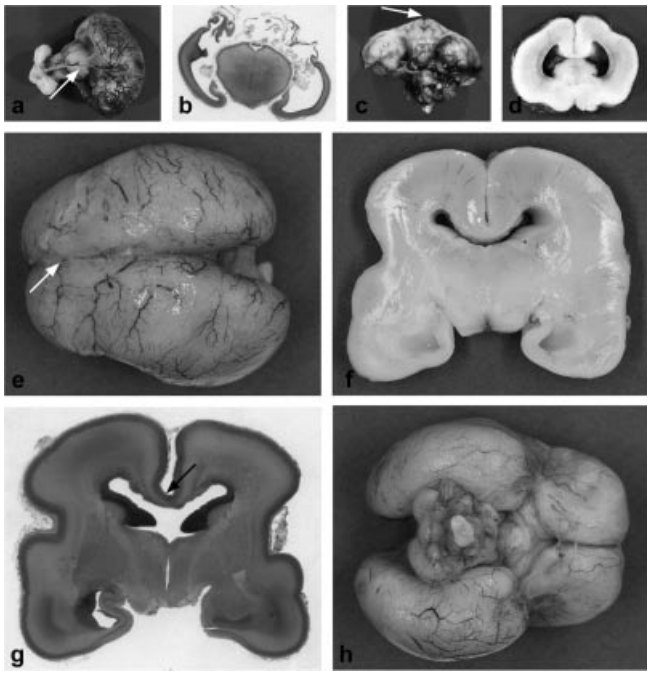

Figure 1. Macroscopic patterns of "classical" HPE brains. **a**: Superior view of alobar HPE. Ball shaped telencephalon with dorsal cyst (arrow) covering the diencephalon. **b**: Cross section at the level of the mesencephalic–diencephalic junction. Choroid plexuses filling the dilated posterior horns of the lateral ventricles. **c**: Inferior view of semilobar HPE. Presence of an interhemispheric fissure at the most rostral level of the frontal lobes (arrow). **d**: Cross section passing through the diencephalon (same brain). Fused thalami, absence of the third ventricle, with well-formed dorsal interhemispheric but with continuity of the frontal cortex on the midline. **e**: Superior view of lobar HPE, showing a fusion of the meninges only. **f**,**g**: Cross sections passing through the diencephalon. Well-formed CC, with continuous overlying cortex (arrow). **h**: Inferior view of lobar HPE. Normal olfactory bulbs and tracts.

formed hippocampi. The Sylvian fissure is abnormally anteriorly positioned. On cross sections, the septum pellucidum is absent but the CC can be observed even incomplete in the region of the separated hemispheres. Dorsal cysts are observed in particular when the thalami are fused. The deep nuclei display various degrees of separation, but the hypothalamus and thalami remain often unseparated resulting in a small, sometimes atretic third ventricle (Fig. 1d).

In the lobar form, the interhemispheric fissure is present along the entire midline. The cerebral lobes are fully developed, in particular the frontal ones. On coronal sections, the cortex crosses the midline at the level of the median fissure, forming a bridge between both hemispheres. The frontal horns of the lateral ventricles are present although dysplastic. The temporal horns and the third ventricle are better defined. The deep gray nuclei are nearly completely formed, but the caudate nuclei may remain fused anteriorly. The thalami are separated or may remain joined by a mass broader than the normal interthalamic commissure [Norman and Muenke, 1995]. The CC may be present, normal, incomplete, or hypoplastic, but the septum pellucidum is never seen. The hippocampi are normal or mildly affected (Fig. 1e–h).

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All these anomalies in hemispheric cleavage associate with aplasia of the olfactory bulbs and tracts except for the lobar form, where olfactory bulbs can be identified.

Histological Studies

Histological analyses have revealed that the cortex bordering the orifice of the ventricle corresponds to the Ammon horn, the presubiculum and the entorhinal cortex (Fig. 2a) [Yakolev, 1959]. Although olfactory bulbs and tracts are missing, the rhinencephalon is always present. Within the same brain, the cytoarchitectony of the cortex may differ

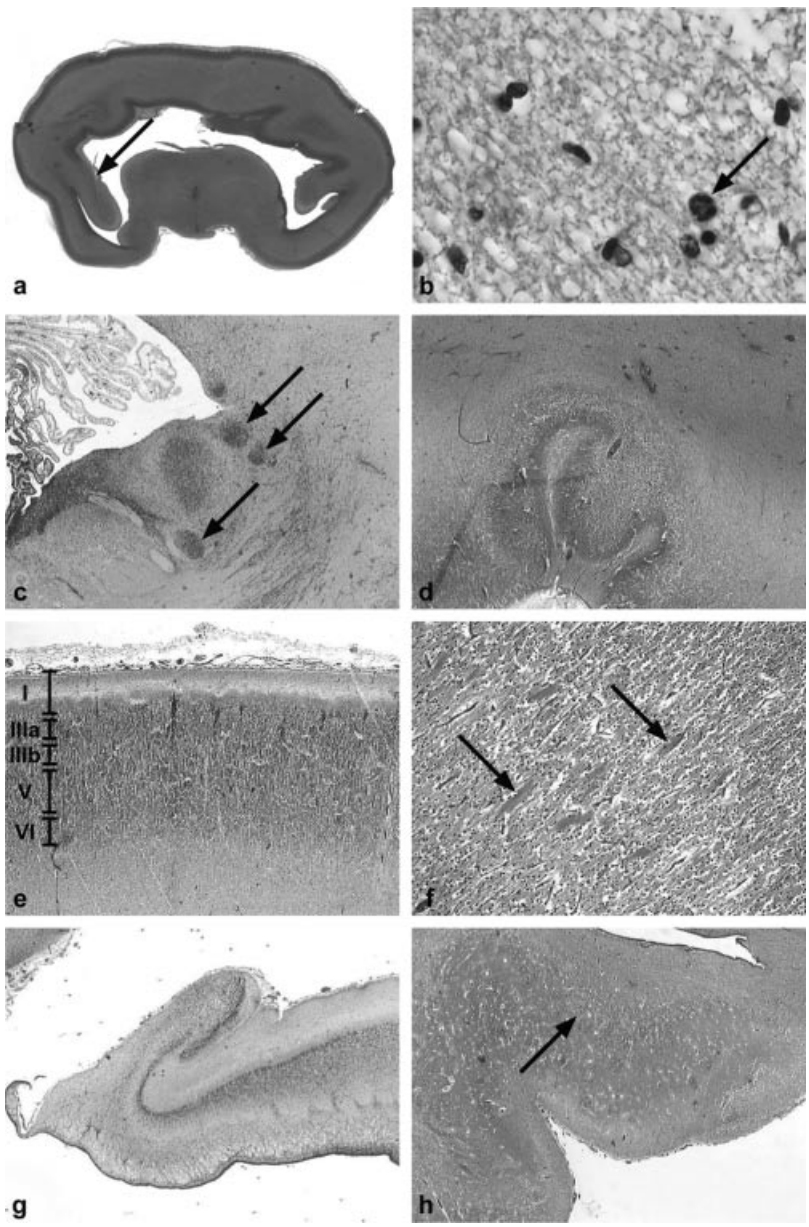

Figure 2. Microscopic lesions in classical HPE. **a**: Lateral ventricles lined by malpositioned hippocampus and entorhinal cortex (arrow). **b**: Paucicellular layer 1, displaying several apoptotic neurons (arrow). **c**: Neuron depletion in the ganglionic eminences, with small dispersed nodules of post-mitotic neuroblasts (arrows). **d**: Micropolygyria on the surface of the ventral frontal forebrain. **e**: "Five layered" cortex, containing no external and internal granular cell layers. **f**: Presence of multiple neuropil islands in layer V, with decreased density of immature pyramidal neurons. **g**: Hypoplastic hippocampus in a case of semilobar HPE. **h**: Dysplastic hippocampus, with dysplastic and hypoplastic dentate gyrus (arrow), and hypoplasia of the pyramidal cell layer.

from place to place and all major cytoarchitectural fields of the cortex may be identified [Yakolev, 1959]. According to other authors, the cortex is severely disorganized in some areas [Kobori et al., 1987]. The cortical layers have been described as "bizarre" with gray matter heterotopias and cortical cell loss disturbing the architectonic pattern (Fig. 2b) [Larroche, 1977; Norman and Muenke, 1995]. Norman and Muenke [1995] as well as Golden [1999] stated that all neurons seemed to have reached the cortical plate, conversely to Mizuguchi and Morimatsu [1989a] who extensively described cortical anomalies related with a defect of both radial and tangential migration. In HPE, the cortex is significantly thicker, arguing for impaired radial neuronal migration, and the constant lack of external and internal granular cell layers (II and IV) as well as the depletion of progenitors in the ganglionic eminences, for a defect of tangential GABAergic migrating interneurons, which represent most of the cells in these two layers and originate from the ganglionic eminences [Fertuzinhos et al., 2009]. Additional findings resulting from a failure of proper neuronal migration are diffuse mats of periventricular germinal layer cells (Fig. 2c), white matter heterotopic nodules and abnormal gyral patterns mainly consisting in polymicrogyria (Fig. 2d) or dysplasia, especially observed on the ventral surface of the forebrain [Jellinger et al., 1981]. The other cortical plate abnormalities consist in a five-layered laminar architecture, although each of the layers varies among cases (Fig. 2e). The molecular layer contains very few Cajal Retzius cells and a small number of tangential fibers, underlined by radially arranged clusters of neurons (layer IIIa), covering a less cellular band. Underneath, clusters of small to medium sized neurons are observed, corresponding to layer IIIb. The pyramidal cell layer (layer V) is sparce in cellularity and contains spherical or oval fibrillary structures corresponding to islands of neuropil which are likely to reflect alterations of cytoskeletal proteins secondary to inappropriate connections between cortical neurons (Fig. 2f). Claustrum and insula may be dysmorphic with a failure of operculization [Jellinger et al., 1981]. The cytoarchitecture of the hippocampus, although malpositioned or hypoplastic on gross examination, is most often normal. Nevertheless, it may be histologically hypoplastic and/or dysplastic (Fig. 2g,h).

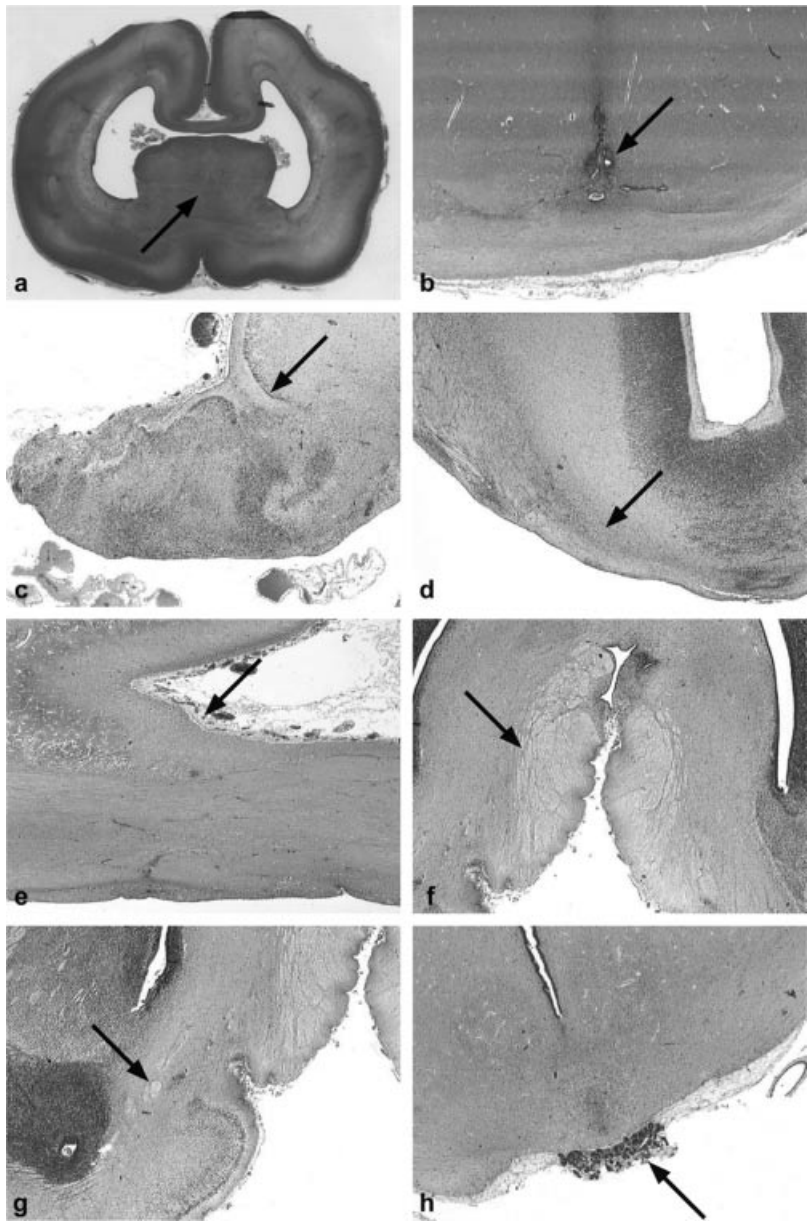

Figure 3. Microscopic anomalies in the ventral forebrain and of the commissures. **a**: Fusion of the thalami which consist in an indistinct cellular mass, with no discernible third ventricle (arrow). **b**: Atretic third ventricle composed of multiple small ependymal channels (arrow). **c**: Interruption of the frontal cortex (arrow) in a case of alobar HPE, with complete absence of CC which is replaced by dysplastic dorsally placed hippocampus, and with no Probst bundles. **d**: Disappearance of the CC near the midline (arrow), where the cortex passes through the middle interhemispheric fissure. **e**: Presence of CC, made of several irregularly oriented fascicles, covered by a rudimentary cortical ribbon containing the molecular layer only (arrow). **f**: Dysplastic and misoriented anterior commissure. **g**: Rudimentary fornical commissure (arrow). **h**: Hypoplastic and flattened pituitary gland.

White matter abnormalities have seldom been described. Nevertheless, using imaging techniques, the white

matter myelination has been found to be delayed in the majority of children with classical forms of HPE [Kinsman, 2004].

In the ventral forebrain, the caudate and lentiform nuclei are hypoplastic and dysmorphic, the septum is indiscernible, and the thalami form an indistinct fused mass containing an atretic third ventricle which is replaced by a single or by multiple ependymal tubules, responsible for hydrocephalus (Fig. 3a,b) [Larroche, 1977; Jellinger et al., 1981; Hayashi et al., 2004]. The internal capsule may be missing, hypoplastic, or fragmented.

Although constantly absent in alobar HPE (Fig. 3c), the CC displays different types of abnormalities in the semilobar or lobar forms, ranging from more or less severe hypoplasia to dysplastic patterns consisting of disorganized axonal bundles covered by a more or less thick continuous cortical ribbon crossing the midline (Fig. 3d,e). Otherwise, agenesis of the CC may be only partial, the callosal splenium being present without callosal body or genu. HPE is the only brain anomaly in which the posterior CC forms in the absence of anterior callosal formation [Barkovich, 2005]. Furthermore, Probst bundles are never observed in HPE conversely to other isolated or syndromic CC ageneses. Jellinger et al. [1981] found

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constant agenesis, hypoplasia, or dysplasia of the anterior and fornical commissures in their series (Fig. 3f,g). The pituitary gland may be found within the sella turcica or in aberrant positions and may be flattened and hypoplastic (Fig. 3h) [Ikeda et al., 1987; Cohen, 2006]. Even though HPE is thought to be restricted to the forebrain, brainstem and cerebellar anomalies have been identified by several authors. In the brainstem, the most frequently observed anomaly concerns the pyramidal tracts [Jellinger et al., 1981]. Corticospinal tracts may be missing or hypoplastic but abnormalities of decussation at caudal medulla level have never been found (Fig. 4a,b). At the mesencephalic level, aqueductal stenosis has been described [Jellinger et al., 1981]. Larroche [1977] emphasized that cerebellar cell migration abnormalities are almost always associated, such as misplaced nodules of various types of cells including granular, spindle, or ganglionic, which are aberrantly located in the white matter or within the dentate nuclei and/or the roof nuclei. Dentate nuclei have been described as unconnected clusters of cells or as smooth bands of neurons with absence of convolutions [Ikeda et al., 1987].

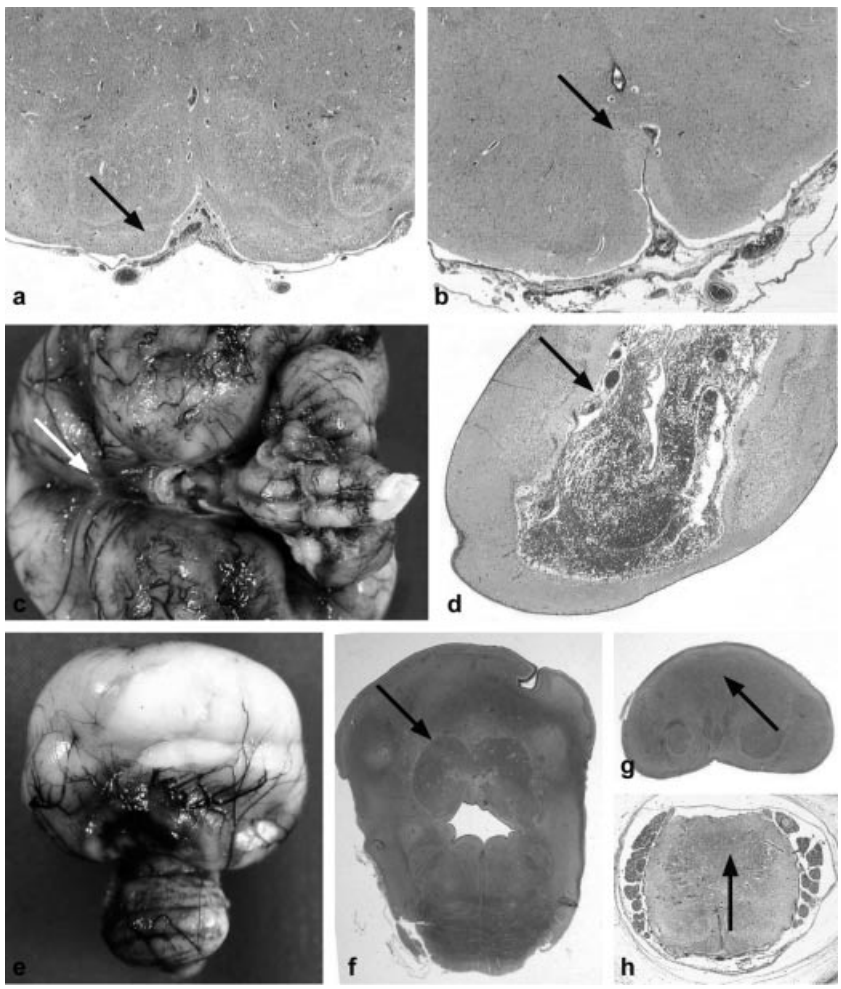

Figure 4. Associated histological abnormalities in the brainstem and the meninges. **a**: Hypoplastic corticospinal tracts in the medulla oblongata (arrow). **b**: Decussation of the hypoplastic corticospinal tracts at the level of the bulbospinal junction (arrow). **c**: Absence of the anterior part of the circle of Willis, with multiple vessels arising from the carotid arteries (arrow). **d**: Pseudoangiomatous pattern of the meninges containing multiple dilated and congestive vessels in the interhemispheric fissure of a semilobar HPE brain. **e**: Holoencephaly: coexistence of alobar HPE and rhombencephalosynapsis. Courtesy of Dr. Marie Gonzales, Armand Trousseau Hospital, Paris, France. **f**: Same case: cross section passing through the pons. Fused cerebellar hemispheres and dentate nuclei (arrow), with absent vermis. **g**: And with fusion of the superior colliculi (arrow). **h**: Holomyelia in a case of Smith–Lemli–Opitz syndrome. Fusion of the anterior horns of the spinal cord (arrow).

Absence of inferior vermis has also been reported [Cohen, 2006].

Vascular anomalies are most severe in the alobar form. Instead of a well-formed anterior half of the circle of Willis, the vascular supply to the cerebrum consists of multiple small vessels arising directly from the internal carotids and basilar arteries (Fig. 4c) [Van Overbeeke et al., 1994]. Other vascular malformations comprise agenesis or dysplasia of the cerebral sinuses, arteries, and veins [Jellinger et al., 1981; Norman and Muenke, 1995]. In the alobar form as well as in milder forms, the middle cerebral arteries are bilateral but the anterior cerebral artery is sometimes single [Friede, 1989].

Meningeal vascular proliferation or pseudoangiomatous lesions have been observed (Fig. 4d) [Jellinger et al., 1981]. Meningeal crusts forming thick grayish fibrous membrane covering the ventral forebrain and brainstem, and arising from the paramedian area of the rostral diencephalons, are histologically composed of leptomeningeal glial neuronal heterotopias [Jellinger et al., 1981; Mizuguchi and Morimatsu, 1989b; Mizuguchi et al., 1994; Norman and Muenke, 1995]. These heterotopias have also been observed in the infundibulum, the lateral surface of the mesencephalon, and the dorsolateral surface of the pons. A retroprosencephalic extracerebral cyst has been reported in one single case of lobar HPE [Rössing and Friede, 1992]. The cyst wall was composed of ependymal-glial tissue, arguing for a maldevelopmental origin from heterotopic cerebral tissue, in contrast to true arachnoid cysts, which were also been reported in association with HPE [Jellinger et al., 1981].

ASSOCIATED CNS LESIONS

Various CNS malformations have been reported by Jellinger et al. who found more than 90% of associated CNS lesions in HPE. Nearly all CNS structures may be affected [Jellinger et al., 1981].

Neural tube defects are the most frequently reported malformations, comprising exencephalies, meningoencephaloceles, myelomeningoceles, and myeloceles [Jellinger et al., 1981; Lemire et al., 1981].

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An association between HPE and Dandy–Walker malformation has also been reported, although both malformations are not developmentally and anatomically related [McCormack et al., 2002].

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Otherwise, HPE has been described in association with rhombencephalosynapsis, which could be termed "holoencephaly," a neologism used to describe the association between HPE and infratentorial midline anomalies characterized by cerebellar hypoplasia with aplasia of the vermis and fusion of the cerebellar hemispheres and underlying dentate nuclei across the midline (Fig. 4e,f) [Gross, 1959; Isaac and Best, 1987]. Associated infratentorial lesions consist in Purkinje cell heterotopias, atresia-forking or stenosis of the aqueduct of Sylvius, and fusion of the colliculi (Fig. 4g). To our knowledge, only six cases of holoencephaly have been published so far, in which HPE spectrum varies from aprosencephaly to lobar HPE. It is worth noting that all these holoencephaly cases were associated with several intra and extra-neural anomalies. In the two most recent cases, karyotype analysis was normal and the screening for HPE genes failed to detect either mutations or subcryptic genomic rearrangements [Pasquier et al., 2009].

Spinal cord lesions have seldom been described. Hydromyelia is very uncommon as well as holomyelia which consists in partial or complete fusion of both anterior horns of the spinal cord and has not yet been related with the HPE spectrum [Jellinger et al., 1981; Friede, 1989]. Our single case concerned a female fetus who had a mild form of HPE due to a 7-dehydrocholesterol reductase deficit (Fig. 4h).

EXPANSION OF THE NEUROPATHOLOGICAL PHENOTYPES

There are several lines of evidence that HPE corresponds to a wide continuous spectrum, with no clear-cut boundaries, of abnormal separation of the prosencephalon, rather than to the three neuroanatomical forms. Since the first classification proposed by DeMyer, other entities have emerged, ranging from the most severe end of the spectrum, AP/AT, to intermediate and milder forms.

Aprosencephaly, which represents the continuum between anencephaly and HPE, is defined by a lack of prosencephalic derivates [Harris et al., 1994]. This extremely rare malformation was first described in 1977 [Garcia and Duncan, 1977; Iavanainen et al., 1977]. In aprosencephaly, the prosencephalon is absent, resulting in severe microcephaly (Fig. 5a), while in atelencephalic prosencephaly, which represents a subset of aprosencephaly, a rudimentary medial vesicle resembling the diencephalon with rudimentary lateral telencephalic vesicle expansion exists [Harris et al., 1994]. In terms of timing, AP/AT is considered as an early failure of the closed neural tube in forming a normal prosencephalon, HPE resulting more likely from the

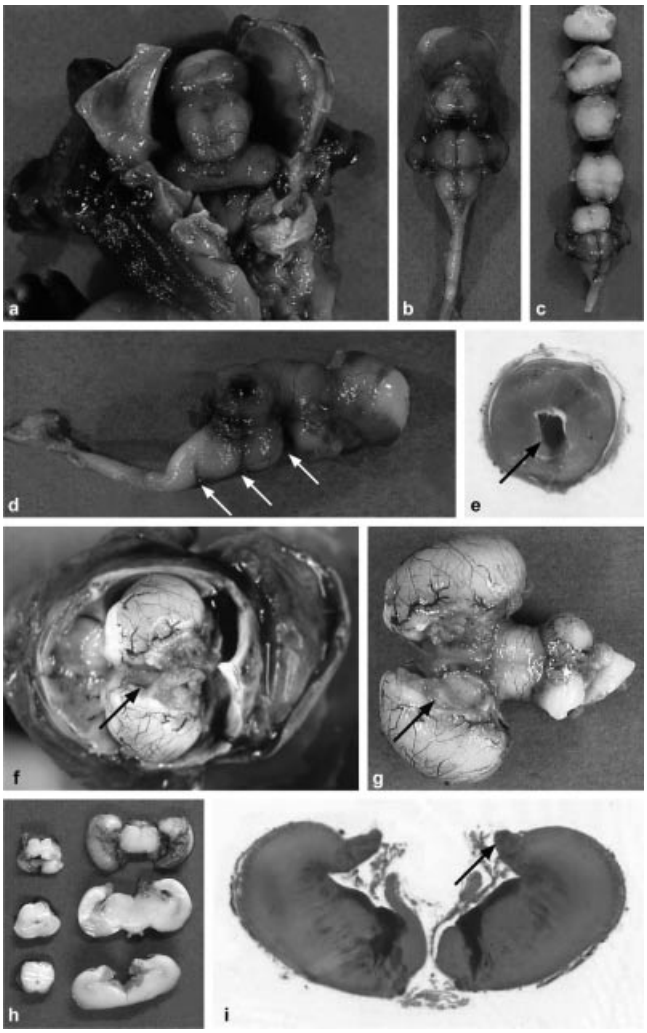

Figure 5. Morphological patterns of aprosencephaly/atelencephaly. **a**: In situ view of an aprosencephalic brain, consisting in a single prosencephalic vesicle. **b**: Same case: well-developed infratentorial structures. **c**: Cross sections: the prosencephalon forms a rudimentary and indistinct mass, with no recognizable ventricles. **d**: Presence of the medullary, pontic, and diencephalic curvatures (arrows). **e**: Absence of telencephalic structures, with a single central germinative zone (arrow). **f**: Superior in situ view of an atelencephalic/aprosencephalic brain, with a dorsal cyst covering the diencephalon (arrow). **g**: Superior in situ view after extraction of the brain, showing two rudimentary telencephalic vesicles, partly covered by the dorsal cyst (arrow). **h**: Cross sections of the same brain: fusion of the deep gray nuclei, single ventricle, but with presence of the optic chiasm. **i**: Absence of the CC, the dorsal hippocampus (arrow), and of Probst bundles.

inability of the telencephalon to give rise to hemispheric vesicles. Craniofacial malformations involving the frontonasal eminence are similar to those observed in HPE, and recently an inherited mutation in the *SIX3* gene also implicated in HPE has been evidenced in aprosencephaly, supporting the hypothesis of common pathogenetic mechanisms [Pasquier et al., 2005]. The hindbrain and midbrain are morphologically normal (Fig. 5b), conversely to the rudimentary forebrain where olfactory bulbs and tracts are missing (Fig. 5c,d). Cortical plate, basal ganglia, and ventricles are virtually absent but in contrast to anencephaly, the calvarium is intact. Diencephalic structures including eyes, optic nerves, mamillary bodies, hypothalamus, and hypophysis may also be affected [Harding and Copp, 2002]. In the *SIX3* mutated reported cases, aprosencephaly was associated with cyclopia, a single optic nerve being found in one case and a synophthalmia with two hypoplastic optic nerves in the other case. Histological examination of the rudimentary forebrain discloses a diencephalic pattern (Fig. 5e) [Pasquier et al., 2005]. In other cases, the globular or sometimes multinodular forebrain remnant is histologically composed of totally disorganized structures and of a prominent leptomeningeal calcified gliomesodermal proliferation [Harding and Copp, 2002]. In atelencephaly, the prosencephalon, although rudimentary, presents morphological features similar to classical alobar HPE (Fig. 5f–i).

Middle interhemispheric variant, also called syntelencephaly, is a subtype of HPE in which the posterior frontal and parietal areas are fused, whereas the frontal and occipital lobes are separated, and portions of the genu and splenium are intact [Barkovich and Quint, 1993]. Although the brain is generally small, the basal forebrain has a normal appearance with normal olfactory sulci. The interhemispheric fissure and falx cerebri are normal or hypoplastic. On superior and lateral views, the Sylvian fissures are continuous or nearly continuous across the midline (Fig. 6a,b). On cross sections of the posterior frontal and parietal lobes, the interhemispheric fissure disappears as the two hemispheres become continuous across the midline (Fig. 6c,d). The lentiform nuclei and the hypothalamus are separated, but the most commonly affected deep gray nucleus is the thalamus, which is not separated in one-third

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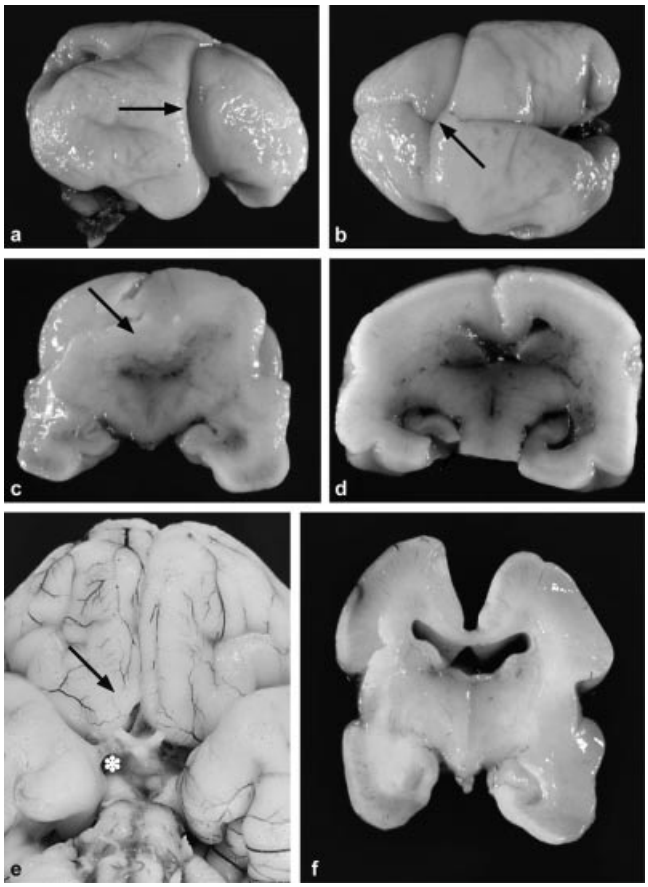

Figure 6. Other HPE phenotypes. **a**: Middle interhemispheric variant: vertically placed Sylvian fissure (arrow). Courtesy of Prof. Catherine Godfraind, Catholic University Louvain, Brussels, Belgium. **b**: Same case: early continuous Sylvian fissure on the midline (arrow). **c**: Same case: cross section at the level of the Sylvian fissure, showing fusion of both cerebral hemispheres (arrow). **d**: Same case: cross section at the level of the uncus hippocampi: well separated midline structures. **e**: Isolated arhinencephaly with no other obvious abnormality. **f**: Primary septal agenesis, with normal CC.

of the cases. This malformation has been interpreted as resulting from a defect of dorsal–ventral induction of the telencephalon and more specifically from a lack of induction of the dorsal midline structures [Lewis et al., 2002].

Although the classic definition is not ambiguous, diagnosis difficulties arise at the less severe ends of the spectrum, which include arhinencephaly, CC agenesis, septal agenesis, and septo-optic dysplasia [Hahn and Barnes, 2010]. Olfactory aplasia alone may occur as a minimal lesion without any sign of fusion of the hemispheres (Fig. 6e) [Harding and Copp, 2002]. Nevertheless, most patients have associated CNS anomalies [Friede, 1989]. Aplasia of the olfactory bulbs can be found in patients with callosal agenesis alone [Jellinger and Gross, 1973] but is most of the time found to be associated with multiple congenital CNS and extra-CNS anomalies [Barkovich, 2005]. When isolated, arhinencephaly may represent the mildest form of HPE spectrum.

Defective formation of the CC results from different mechanisms [DeMyer, 1977; Cohen and Sulik, 1992] and may form a part of a more extensive malformative complex, in particular HPE. In partial isolated forms, the posterior portion is usually missing, while when related with HPE spectrum, the posterior part forms in the absence of anterior callosal formation [Volpe et al., 2009]. Moreover, Probst bundles, which run longitudinally and represent misdirected callosal fibers, are classically not identified in HPE cases [Harding and Copp, 2002].

Primary septal agenesis is a rare malformation that can be isolated or part of developmental CNS abnormalities (Fig. 6f). If isolated, the absence of the septum pellucidum has usually only subtle neurologic consequences [Belhocine et al., 2005]. Nevertheless, this anomaly should serve as a clue to the presence of associated anomalies such as Chiari II malformation, CC agenesis, and particularly HPE [Siebert et al., 1981; Barkovich and Norman, 1989]. It is also associated with septo-optic dysplasia (SOD), where absence or severe malformations of the sella and the pituitary gland occur. The olfactory tracts may be present. In SOD, whose diagnosis is based on the recognition of the following triad: aplasia of optic disk, hypopituitarism and absence of the septum pellucidum, the optic chiasm and/or the optic nerves are atrophic similar to some forms of HPE, with sparse or absent myelination. The geniculate bodies are also atrophic. In the most severe forms, uni- or bilateral optic aplasia along with anophthalmia can be observed [De Morsier, 1956].

EYE PATHOLOGY

In the most severe forms of HPE, the orbits and globes are fused resulting in cyclopia (Fig. 7a) [Kakita et al., 1997]. Optic nerves and chiasm are hypoplastic and poorly myelinated. The eyes may be completely absent or can be represented by a midline cyst at the anterior edge of the holosphere which contains disorganized retinal tissue corresponding to an abortive eye [Norman and Muenke, 1995; Kakita et al., 2001]. In other cases with no optic nerve, a small cyclopic eye contains a fibrous scleral remnant lined by pigmented epithelium or hyaloid tissue [Lurie et al., 1980; Kim et al., 1990; Harris et al., 1994]. Cyclopia is a failure of the primary eye field division and has been evaluated as occurring in 2% of HPE cases [Yamada et al., 2004]. In classical HPE, ocular anomalies may also consist in synophthalmia, apposed eyeballs, or microphthalmia

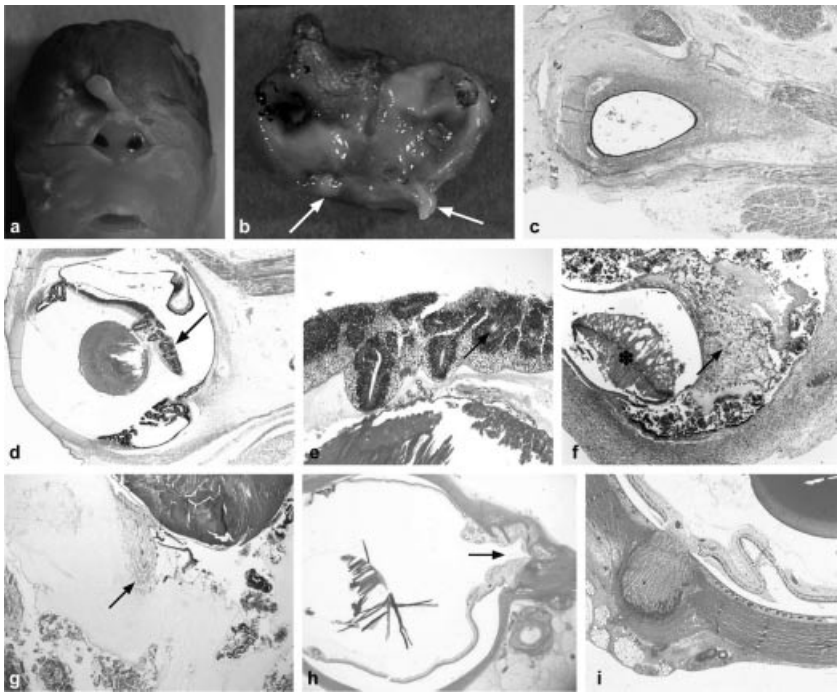

Figure 7. Ophthalmic pathology in HPE. **a**: Cyclopia, with fusion of the orbits and eyelids, but with two distinct optic canals. **b**: Synophthalmia: medial fusion of the globes, but with two distinct optic nerves (arrow). **c**: Microphthalmia: absence of differentiated structures. The globe is composed of pigmented epithelium, surrounded by a loose connective tissue. **d**: Microphthalmos eye with areas of retinal dysplasia (arrow). **e**: Retinal dysplasia, consisting in the presence of retinal rosettes, whose outer membrane is displaced at the center of the lumen, forming an eosinophilic inner border (arrow). **f**: Complex malformation of eye, with retinal dysplasia, cataracts (asterisk), and persistence of the primitive vitreous (arrow). **g**: Persistence of the hyaloid artery at the posterior level of the lens (arrow). **h**: Optic nerve coloboma, with colobomatous cyst (arrow). **i**: Normal eye (for comparison).

with various microscopic abnormal features. Synophthalmia is more frequent and characterized by two eyeballs partially fused on the midline (Fig. 7b). The eyelids are also more or less fused. In a single orbital cavity and behind a single eyelid, two small eyeballs may be present. The eyes are better developed interiorly and laterally than posteriorly and medially [Torczynski et al., 1977]. The fused eyes may contain two juxtaposed cornea, pupils, and lenses. A single or two optic nerves can emerge posteriorly from the middle of the fused eyeballs and traverse a single or two separate optic canals [Kokich et al., 1982]. Immunohistochemical studies of single optic nerves make it possible to see that axons occupy a marginal position arising from the retina of each eyeball, while the central area is more cellular and composed of glial cells [Kakita et al., 1997]. When eyeballs are separate, they are often asymmetrical and microphthalmos [Offret et al., 1986].

On microscopic examination, eye malformation identification is often complex. Each component can be hypoplastic in the anterior chamber as well as in the posterior one. Most of the normal structures are present but are disorganized or fail to differentiate properly (Fig. 7c).

In some cases, the retina is well differentiated, but most of the time, the ordered arrangement of the retina is disrupted (Fig. 7d). Areas of normal layered retina are intermingled with foci of retinal dysplasia, characterized by the presence of tubular structures reminiscent of Flexner–Wintersteiner rosettes or fleurettes of retinoblastoma. The rosettes are composed of differentiated retinal neurons, which have failed to establish the normal orientated layers, with an inside–out pattern, the outer limiting membrane being displaced in the central lumen of the rosettes (Fig. 7e). Other dysplasic features such as glial tissue or cartilage can be found [Chan et al., 2007]. Other anomalies consist in the persistence of the primitive vitreous (Fig. 7f) and of the hyaloid artery, corresponding to the persistent fetal vasculature (Fig. 7f) [Offret et al., 1986; McPherson et al., 2004]. Other anomalies such as absence of cornea, corneal epithelium, stroma, and Descemet's membrane have also been reported [Offret et al., 1986].

Colobomata are mainly observed within the retina and optic nerve, other locations being more uncommon (Fig. 7h,i). Eye coloboma, which is defined by a failure of the optic fissure to close completely, can be highly variable in size, ranging from a minimal defect to a cystic colobamous microphthalmos [Muenke and Beachy, 2001]. The small eye and the coloboma are always linked by a gap. The internal surface of the colobomatous cavity is covered with an abnormal inversed retina [Chan et al., 2007].

Ophthalmic pathology in aprosencephaly reveals the presence of normally formed eyes, suggesting that aprosencephaly occurs after the development of optic vesicles; nevertheless, some cases have been described with various abnormalities of ocular development, such as retinal dysplasia, optic nerve hypoplasia, or colobomatous defect [Harris et al., 1994; Kakita et al., 2001].

CONCLUSION

Neuropathology studies have demonstrated that complex and multiple malformations may be observed in the HPE sequence. These anomalies can be restricted to the brain, or involve multiple other variable locations, indicating that complete morphologic examination, whenever possible, is required to better delineate the different underlying diseases and pathophysiological mechanisms. It is also essential for genetic counseling improvement, owing to extreme phenotype variability,

genetic heterogeneity, and risk of recurrence even in apparently sporadic cases. Recent advances in pathogenesis and genetics have led to the idea that HPE encompasses a wide continuous spectrum of lesions, which should contribute to a more rational morphologic classification of this brain malformation.

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