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Alobar holoprosencephaly, cyclopia and enigmatic associations in newborn rabbits

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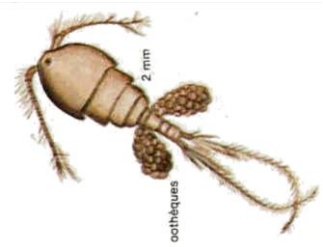
Holoprosencephaly is a serious and complex congenital malformation of the brain caused by a defect of the medial cleavage of the forebrain into two cerebral hemispheres. It is characterized by typical craniofacial abnormalities.

The word cyclopia comes from the ancient Greek Κύκλωψ (Kyklops), meaning "round eye". It refers to strange beings in the Greek mythology, Giants with only one eye in the middle of their faces, whose aspect is otherwise normal. Their parents from Ouranos (Heaven) and Gaia (Earth) gave birth to three children with particularly dangerous strength, power and weapons. There were the Uranian Cyclops, blacksmiths, builders and pastors. Some see it as an analogy with the central eye of volcanoes rather than living things. Other civilizations also have beliefs or legends in which beings appear with only one central eye. This is the case with the Irish and the Ossetians. The reality of a man or animal born with cyclopia is, unfortunately, tragic,

Figure 1: Extremely rare discovery: Flemish Giant newborn presenting a combination of major cerebral and facial morphological abnormalities.

as most cases are not viable. But they can be the source of these terrifying tales of giant monsters.

There is also a small freshwater crustacean – the Cyclops, which has only a simple median eye. Hence its name.



Cyclopia in vertebrates

Embryonal development of the nervous system and the brain is complex. It is formed from the notochord, a cartilaginous structure that will become the neural plate and the vertebral bodies. The neural induction mechanism will result in the formation of a neural tube, which will gradually close along the entire length from the cervical region to the caudal region. Closure defects cause different pathologies in the newborn:

- A failure to close the anterior opening of the neural tube (neuropore) is characterized by the absence of a brain in the embryo (anencephaly), i.e. the appearance of a brain outside the skull (hernia of the brain or encephalocele) (Figure 2).
- A failure to close the posterior opening of the neural tube causes a malformation of the spine called *spina bifida*.

Coordination of gene expression

The processes involved in the formation of the brain and the development of motor neurons are under the control of the Sonic Hedgehog glycoprotein (SHH). The Hedgehog protein signaling pathway (formation of organs during embryonic development) plays, indeed, a vital role in the regulation of digits formation (number of fingers and toes) and the brain organization. A concentration gradient appears when the molecule spreads throughout the embryo creates. This contributes to the appearance of the dorso-ventral axis. The effect on cells depends on its concentration.

An abnormal concentration of the Sonic Hedgehog protein will affect the brain development. It can have a genetic origin like, e.g., a mutation of genes involved in the regulation of SHH. Fibroblast growth factor (FGF) genes also appear to play a role

Figure 2: Newborn rabbits presenting a failure to close the neural tube during the embryonic development, causing an encephalocele (left) or a *spina bifida* (right, https://www.devtox.org/nomenclature/ml_imag_im.php?img=1.1117.5226-3-01).

	Embryologic divisions			Anatomic Divisions
Encephalon	**Prosencephalon**	Telencephalon		Brain
		Diencephalon		
	Mesencephalon	Mesencephalon		Brain stem
	Rhombencephalon	Metencephalon	Annular protuberance (pons)	
			Cerebellum	Cerebellum
		Myelencephalon	Medullary brain	Brain stem
Spinal cord				Spinal cord

Table 1: Division of the brain during the development of the vertebrate embryo. https://fr.wikipedia.org/wiki/Rhombencéphale

in controlling the concentration of SHH in the developing brain. Finally, a plant alkaloid steroid is able to block the SHH signaling pathway.

Brain formation

The development of the brain is gradual and takes place above the spinal cord (Table 1, Figure 3). Different structures are formed by budding.

Prosencephalon: this structure will divide into two secondary vesicles, the diencephalon and the telencephalon. The latter will divide into approximately symmetric left and right cerebral hemispheres.

Mesencephalon: (midbrain) derives from the middle cerebral vesicle of the primary encephalon. It provides the motor control of muscles and the sensory reflexes of vision and hearing.

Rhombencephalon: represents the posterior part of the brain. It is subdivided into two portions (metencephalon and

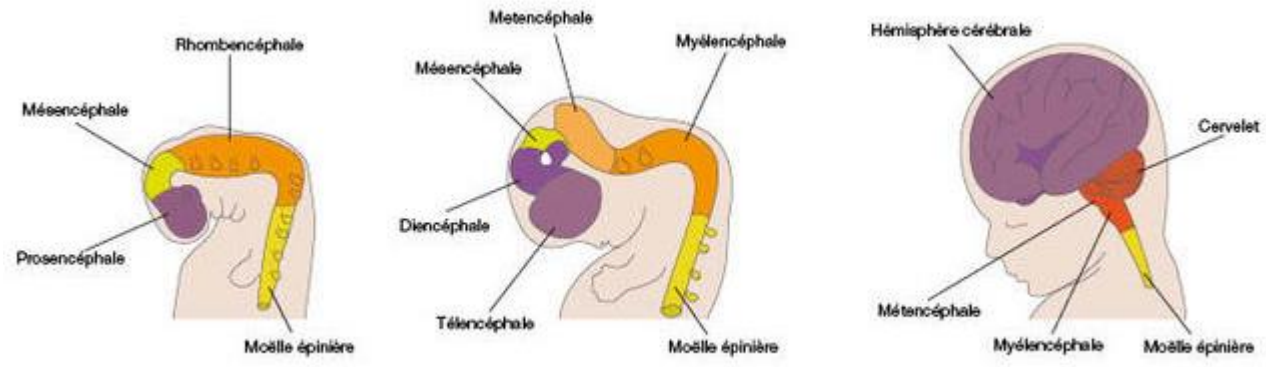


Figure 3: Development and division of the neural tube and formation of different parts of the brain.

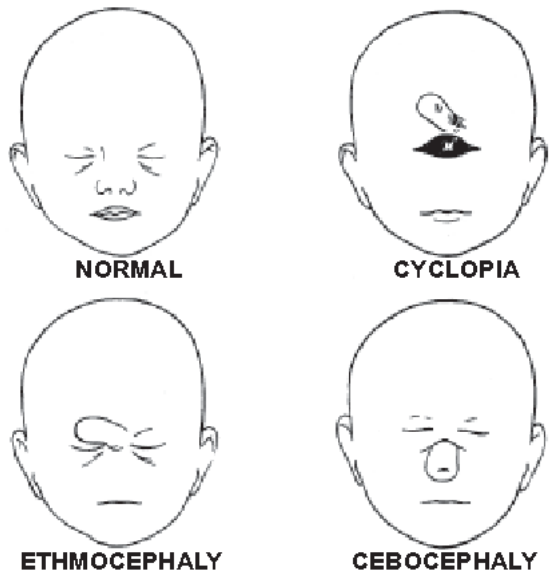


Figure 4: Craniofacial features suggestive of alobar holoprosencephaly in humans.

myelencephalon) which will give rise to the cerebellum and the medullary brain (*medulla oblongata*).

Median longitudinal fissure

During embryogenesis, the longitudinal division of the primitive brain into two cerebral hemispheres and two ventricles is closely coordinated by different genes. These regulate the synthesis of signaling proteins that will tell cells of the primitive brain to form the right and left hemispheres. The inactivation of several genes or the mutation of a gene will have serious structural consequences on the brain. These are associated with specific and more or less serious congenital malformations of the skull and face. Depending on the severity of the anomaly, the alteration of the brain development leads to a partial, longitudinal cleavage or a complete absence of cleavage of the forebrain into two distinct cerebral hemispheres

This set of birth defects is called holoprosencephaly. Individuals born with such a defect are usually not viable.

Holoprosencephaly exhibits a great variability of phenotypes. The earlier the alteration of the forebrain cleavage appears in the development of the embryo, the more the pathology will be severe.

Alobar holoprosencephaly

Alobar holoprosencephaly is the most severe condition of birth defects of the brain and face (Figure 4). Individuals affected by this malformation are generally not viable.

Causes

The causes have various origins.

Alobar holoprosencephaly is often associated with chromosomal aberration. It can also have a multigenic origin in humans. Forty percent of cases are caused by mutations in genes involved in the production and spread of a specific molecule in the neural tube. The latter induces the correct differentiation of the ventral structures of the brain. Sixteen mutated target genes have been identified to date in man. They act together to regulate the activity of this molecule. Any defect at this embryonic stage will result in the appearance of a cyclopia-like holoprosencephalic phenotype. The disease is, thus, characterized by great genetic complexity

It has also been suggested that cyclopia may be caused by the recessive cy gene in rabbits. Other genetic causes include high inbreeding, e.g. between members belonging to the same nest (2nd degree).

A plant can, moreover, cause holoprosencephaly when ingested by pregnant females whose embryos are at an early stage of development. it is the Californian corn lily, also called California

false hellebore (*Veratrum californicum*). This plant contains steroidal alkaloids: cyclopamine and jervine (Figure 5). These molecules block the Hedgehog signaling pathway and, thus, induce a developmental delay in embryos. Teratogenic molecules like thalidomide are also known to induce holosencephaly in rabbits

Finally, environmental causes cannot be ruled out, such as a pre-existing disease linked to the cholesterol metabolism, hyperglycemia or a viral infection in pregnant rabbits

Pathogenesis

The absence of cleavage and diverticulation of the primitive brain into two cerebral hemispheres results in alobar holoprosencephaly. The brain is, thus, organized as a single ventricular cavity shaped like a horseshoe. The longitudinal fissure between the cerebral hemispheres is absent. As a consequence, nervous fibers interconnecting the two cerebral hemispheres (*corpus callosum*) are also absent. The basal ganglia and rudimentary thalami (diencephalon) are fused in the midline. The brainstem and cerebellum are usually present, but they are malformed. There is a single anterior cerebral artery.

The brain is lined with cerebral parenchyma at the front and demarcated by a thin wall at its posterior. The ventricular system, which represents a collection of chambers located inside the brain, merges and forms a single chamber (monoventricle). At the back of the brain, this monoventricle may become a fluid pocket (dorsal sac) that puts pressure on the peripheral brain parenchyma. The size of the monoventricle varies. Indeed, the amount of cerebrospinal fluid (CSF) in the cerebral ventricular system is regulated by the Sylvius aqueduct. In individuals with alobar holoprosencephaly, the opening of this aqueduct is absent (atresia), leading to an accumulation of fluids and, consequently, a dilation of the single cerebral ventricle (hydrocephalus).

The Lamb and the Flower

The sheep, born in Idaho, were a victim of the wild corn lily (inset). This flower contains a potent chemical called cyclopamine that was eaten by the lambs' mother, causing a birth defect. The chemical caused lambs to be born with a single eye and a malformed brain, but now it is serving as the blueprint for new cancer drugs.

Figure 5: Californian corn lily (*Veratrum californicum*) causes holoprosencephaly when ingested by gestating females whose embryos are at an early stage of development.

This malformation also causes facial abnormalities of the midline (Figures 6, 7, 8). The most evocative are:

- The presence of a single eye socket located on the midline. In the case of cyclopia, it contains a single eye. In synophthalmia, there is a doubling of the internal structures of the eye to varying degrees, or the presence of two joined eyes. In the case of cebocephaly, the two hypoteloric eyes are distinct and very close together. The optic nerves are

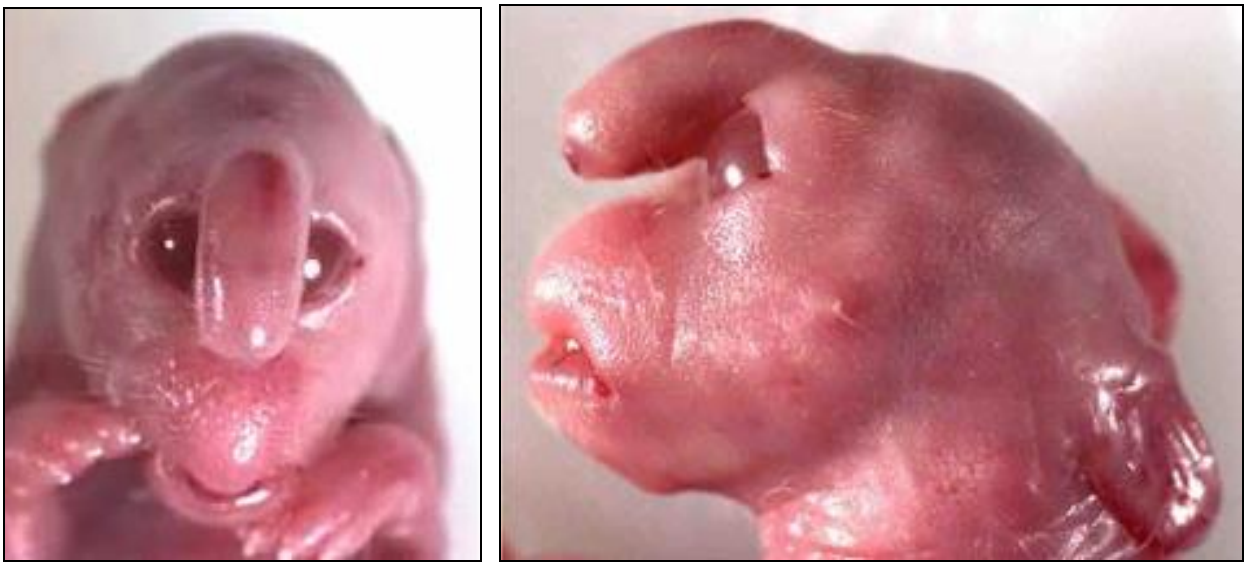


Figure 6: Newborn rabbit with ethmocephaly-appearing alobar holoprosencephaly, with a proboscis above a single orbit, a duplex eye formation and an atrophied nasal apparatus. https://www.devtox.org/nomenclature/ml_manus.php?mno=110325066&spc=3).

usually fused into a single nerve, but may also be absent;

- Absence of the olfactory system and the nose (arrhinia). The olfactory tracts are merged into a single tract, but may also be absent;
- Presence of a proboscis, also called tubular nose. It results from the absence of development of the lining of the frontal bud. This appendix is located on the supraorbital midline of the head (ethmocephaly), above the eye. Indeed, due to the non-separation of the ocular buds, the nose cannot migrate down the face during embryonic development.

Enigmatic association

Alobar holoprosencephaly with cyclopia is

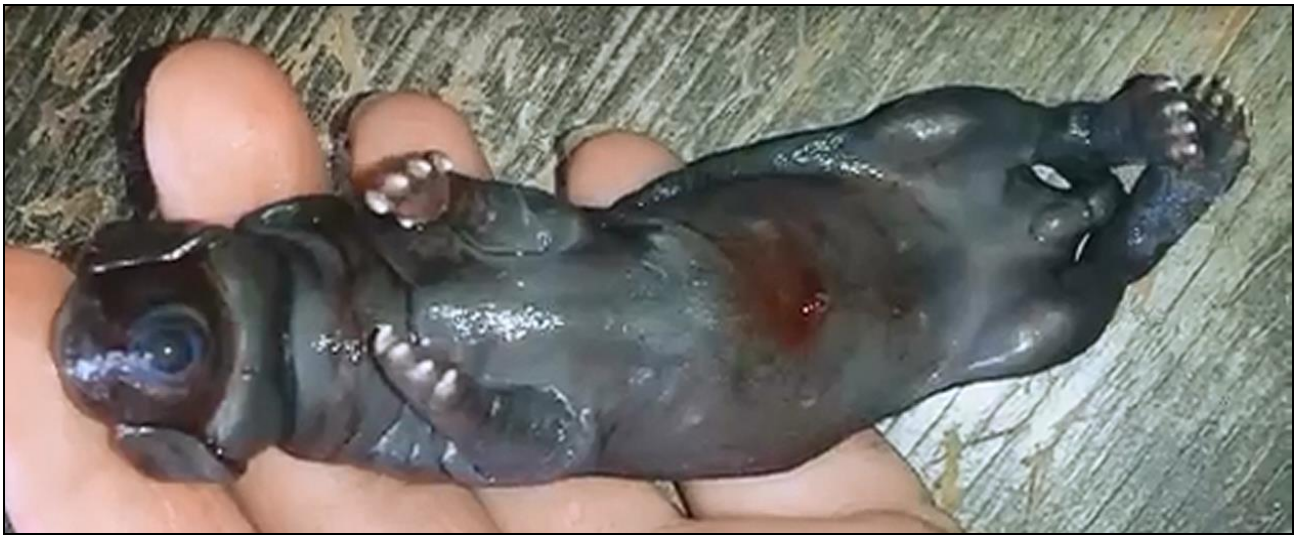


Figure 7: Cyclopia in a rabbit newborn, presenting the *Astomia agnathia* type of malformation, with a single eye on the face, no nose, atresia of the jaws and rotation of the ears. Video: JoAnna Bova https://www.youtube.com/watch?v=vQwMhyuUUwA

Figure 8: Severe craniofacial malformations in a newborn rabbit, with caudal positioning of the ears, no nose (arrhinia), no mouth (astomy) and no jaws (agnatia).

exceptionally associated with other malformations ranging from absence of the jaw to a failure of ears migratation from a prone position to a cranial position during embryogenesis (otocephaly). The ears remain at the level of the cheeks, on the side of the head. Studies in humans have shown that this very serious form of

holoprosencephaly affects mostly females. The animal world is not spared. Such individuals have been observed in guinea pigs, sheep, mice and rabbits (Figures 7, 8). They frequently suffer from other birth defects. The most common one is an abnormal spatial arrangement of the internal paired or single organs in the thorax and/or abdominal cavity (heterotaxis), relative to the right/left symmetry axis in a normal individual. Causes and pathogenesis of this association remain enigmatic. It may be linked to the lack of inducing activity of the prechordial plaque (which will later develop into the mouth opening) on tissues of the prosencephalic portion of the neural tube and the cells of the neural crest. In the skull, part of these cells normally differentiates into cartilage, bone and ganglia. Others contribute to the formation of the jaw and the middle ear, among others. Not in these individuals. This does, however, not explain all the clinical manifestations observed in those affected individuals

Conclusion

The development of cranial and facial structures and those of the brain is the result of complex interactions between gene coordination and different signaling mechanisms. An anomaly or defect can occur at any time during this crucial stage of embryonic development, explaining the broad clinical spectrum with a great variability of phenotypes in affected newborn rabbits.

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