

MRI CHARACTERIZATION OF CLEFT LIP AND PALATE RESULTING FROM HEDGEHOG SIGNALING ANTAGONISM IN MICE

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Introduction: Cleft lip and/or palate is the most common birth defect, occurring in approximately 1 in 700 births [1]. Of those affected, 20% present with cleft lip (CL), 30% with cleft palate (CP), and 50% with cleft lip and palate (CLP). This spectrum of defects cause significant morbidity and may require extensive medical intervention. The underlying causes of CLP are not well understood and it is hypothesized that multifactorial etiologies involving the interaction of genetic predisposition with environmental factors and/or teratogenic insults are responsible for the majority of cases. CLP is also associated with a spectrum of abnormalities termed holoprosencephaly (HPE), characterized by median forebrain deficiency, typically co-occurring with median facial deficiencies, reduced interocular distance, high arching or clefting of the palate and median or lateral clefts of the lip and [2,3]. While HPE occurs infrequently in live births (1.3/10,000), its prevalence as well as its prenatal lethality or co-occurrence with lethal birth defects, is illustrated by an incidence in conceptuses of approximately 1 in 250 [4,5]. While it is established that interference with the Hedgehog (Hh) signaling pathway can yield HPE phenotypes, we have investigated the extent by which Hh signaling may induce HPE and CLP abnormalities. Consequently, in this study, we have employed cyclopamine and a potent cyclopamine-analog to examine a phenotypic spectrum of abnormalities resulting from transient *in utero* Hh signaling inhibition in mice. High resolution MRI was utilized to facilitate the assessment of craniofacial and CNS abnormalities.

Methods: *Animals:* Cyclopamine was administered by subcutaneous infusion via micro-osmotic pump at a rate of 160 mg/kg/d between GD8.25 and GD9.5 as previously described [6]. AZ75, a synthetic cyclopamine-analog (AstraZeneca, Waltham, MA) was administered at GD8.5 as a single oral gavage dose of 40 or 80 mg/kg. *Specimen preparation:* Fixed (10% formalin) fetal specimens were dehydrated in a 4% saline solution for 24 h, and subsequently allowed to rehydrate in 5 mM Multihance® (Bracco Diagnostics Inc., Princeton, NJ) solution for at least 10 days prior to imaging. *MRI:* Fetuses were placed in Fluorinert (3M, St. Paul, MN) and positioned centrally in a Varian 3 cm ID quadrature coil. Images were acquired at 4.7T using a Varian Inova imaging and spectroscopy system. A 3D gradient echo sequence (Tr=20ms, Te=6.5 ms, Fl=65, FOV= 24x12x12mm, Ma=512x256x256, NT=32) was used to acquire images with an isotropic resolution of approximately 47 μm (Figure 1). ImageJ was used to construct 2D images and produce linear measurements. 3D volume and surface renderings were obtained using Osirix4. Brain segmentation (Figure 2) was performed using Amira® (version 4.1.1)

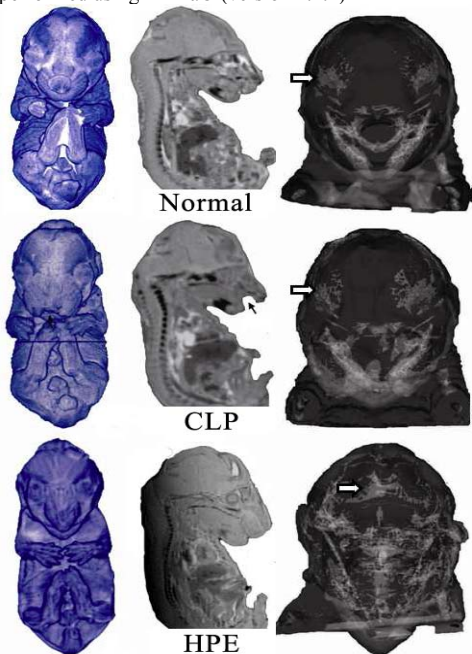


Figure 1: MRI-based fetal phenotypic analysis: Typical morphological data showing 3D volume (left column), 2D section (middle column) and surface rendering (right column) reveal external and internal CLP- and HPE-associated dysmorphology. Cleft lip is indicated by the black arrow while white arrows indicate cranial frontal bones which are abnormally fused (metopic craniosynostosis) in the HPE fetus.

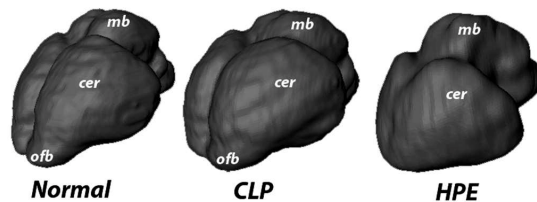


Figure 2: Segmented fetal brain morphology: A subtle but consistent hypoplasia of the olfactory bulbs was found in the CLP group. The HPE group exhibited medial union and rostro-caudal foreshortening of the cerebral hemispheres together with a gross reduction in brain volume. (ofb – olfactory bulb; cer – cerebral hemisphere; mb – midbrain)

Results and Discussion: High resolution MRI, confirmed by standard histological methodologies (data not shown), were used to characterize the CNS phenotype of GD16.5 mouse fetuses exposed to Hh antagonists. Holoprosencephalic fetuses exhibited incompletely separated cerebral hemispheres and complete pituitary and olfactory bulb agenesis. Those with CLP exhibited olfactory bulb hypoplasia and anterior pituitary aplasia. This work provides new insights into cyclopamine-induced CNS and facial defects, highlighting lateral cleft lip and palate as a manifestation of Hh pathway interference. These results demonstrate phenotypic fidelity of the mouse model to known clinical phenotypes and highlight subtle CNS abnormalities as are expected to occur in a subset of clinical CLP populations. In conclusion, craniofacial development in man and mouse [7] appear quite similar supporting our findings here, where the mouse appears to be an ideal model system in which to investigate the interaction of chemical and genetic factors in the etiopathology of complex birth defects such as CLP and HPE.

References: [1] Vieira, AR, J Dent Res 87:119 (2008) [2] Cohen, M. M., Jr. 2006. Holoprosencephaly: clinical, anatomic, and molecular dimensions. Birth Defects Res A Clin Mol Teratol 76: 658-73. [3] Dubourg C, et al., Orphanet J Rare Dis. 2:8 (2007), [4] Matsunaga, E. & K. Shiota, K, Teratology 16: 261 (1977) [5] Leoncini, E, et al., Birth Defects Res A Clin Mol Teratol 82:585 (2008), [6] Lipinski, RJ, et al., Toxicol Sci 104:189 (2008), [7] Diwert, VM & Wang KY, Crit Rev Oral Biol Med 4:111 (1992)

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