Brain Dysplasia Associated with Ciliary Dysfunction in Infants with Congenital Heart Disease

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Objective To test for associations between abnormal respiratory ciliary motion (CM) and brain abnormalities in infants with congenital heart disease (CHD)

Study design We recruited 35 infants with CHD preoperatively and performed nasal tissue biopsy to assess respiratory CM by videomicroscopy. Cranial ultrasound scan and brain magnetic resonance imaging were obtained pre- and/or postoperatively and systematically reviewed for brain abnormalities. Segmentation was used to quantitate cerebrospinal fluid and regional brain volumes. Perinatal and perioperative clinical variables were collected.

Results A total of 10 (28.5%) patients with CHD had abnormal CM. Abnormal CM was not associated with brain injury but was correlated with increased extraaxial cerebrospinal fluid volume ($P < .001$), delayed brain maturation ($P < .05$), and a spectrum of subtle dysplasia including the hippocampus ($P < .0078$) and olfactory bulb ($P < .034$). Abnormal CM was associated with higher composite dysplasia score ($P < .001$), and both were correlated with elevated preoperative serum lactate ($P < .001$).

Conclusions Abnormal respiratory CM in infants with CHD is associated with a spectrum of brain dysplasia. These findings suggest that ciliary defects may play a role in brain dysplasia in patients with CHD and have the potential to prognosticate neurodevelopmental risks. *(J Pediatr 2016;178:141-8).*

The most common sequelae following congenital heart disease (CHD) surgical palliation during infancy are neurodevelopmental disabilities, with survivors reported to develop executive, attention, and social-emotional problems, with deficits observed in school performance and overall competence.[1] Recent studies have focused on the potential role of specific surgical techniques and/or related white matter injury as mediators of poor neurocognitive outcome in patients with CHD.[2] The overall presumption in these studies has been that surgical injury or secondary effects from hypoxic-ischemic injury from the structural heart defects may be the drivers of poor neurodevelopmental outcomes in patients with CHD.

We investigated here a novel hypothesis that patients with CHD may have brain abnormalities of a developmental etiology involving motile cilia defects independent of surgical or hypoxic brain injury. In the brain, motile cilia in the ependymal exhibit coordinated ciliary beat that provides directional flow of the cerebrospinal fluid (CSF) and play an important role in neurogenesis.[3-7] The important role of cilia in CHD pathogenesis has been demonstrated recently in a large-scale mouse forward genetic screen that showed a significant enrichment for cilia-related genes among 61 genes identified to cause CHD.[8] In parallel to these murine findings, clinical studies have shown a high prevalence of abnormal respiratory ciliary motion (CM) in patients with heterotaxy, as well as patients with CHD of a broad spectrum without heterotaxy.[9,10] The finding of abnormal CM in the respiratory epithelia provides a proxy for CM in the brain ependymal cilia, as mice studies have shown abnormal CM in the respiratory epithelia is highly correlated with abnormal CM in the brain ependyma.[11-14] It is unknown, however, if there is an association between ciliary dysfunction and abnormal brain development in CHD. In this study, we recruited infants with CHD preoperatively, and obtained nasal scrapes to assess respiratory cilia motion, and conducted cranial ultrasound (CUS) and conventional and volumetric brain magnetic resonance imaging (MRI) to assess for brain abnormalities.

BBS	Bardet-Biedl syndrome
CHD	Congenital heart disease
CM	Ciliary motion
CP	Choroid plexus
CSF	Cerebrospinal fluid
CUS	Cranial ultrasound
MRI	Magnetic resonance imaging
RACHS	Risk-Adjusted Classification for Congenital Heart Surgery
TE	Echo time
TR	Repetition time

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Methods

We prospectively recruited neonates and infants >36 weeks' gestational age with complex CHD requiring palliative surgery and informed consent was obtained.

Exclusion criteria included presence of a major congenital brain malformation (not subtle brain dysplasia), known chromosomal anomalies/syndromes, documented prenatal/perinatal brain injury, or central nervous system infections. All study protocols were approved by the University of Pittsburgh's Institutional Review Board. Clinical variables that were collected included perinatal variables (birth weight, birth length, and head circumference); preoperative variables (arterial blood gas pH and partial pressure of O_2 in arterial blood and serum lactate); heart lesion category (single vs double and cyanotic vs acyanotic); Risk-Adjusted Classification for Congenital Heart Surgery (RACHS) score; intraoperative variables (cardiopulmonary bypass time and deep hypothermic circulatory arrest time); and postoperative variables (seizures, ventilator dependency, gastrostomy, extracorporeal membrane oxygenation days, total intensive care unit days, total hospital days, delayed sternal closure, and number of lifetime surgical procedures).

Nasal Tissue Sampling, Reciliation, and CM Analysis

Nasal scrape was obtained with curettage of the inferior nasal turbinate using a Rhino-Probe (Arlington Scientific, Springville, Utah). No sedation or anesthesia was required. CM in the nasal epithelia was examined using high-speed videomicroscopy, and CM phenotype was classified as normal or abnormal based on consensus review by a panel of 3 investigators blinded to patient phenotype as previously described.[9] As infection potentially may affect CM, patients with known active infections, viral or bacterial, were excluded and scraped at a later time point when clinically stable. To exclude secondary ciliary dyskinesia, after videomicroscopy, all patient nasal tissues were cultured over a period of 4-6 weeks using methods previously described.[9] This entails a cycle of deciliation, proliferative expansion, and reciliation of the epithelium, followed by similar analysis of CM by videomicroscopy of the reciliated patient nasal epithelia (**Table I**; available at www.jpeds.com).

Serial Cranial Ultrasound

A General Electric Logic 9 ultrasound machine (General Electric, Boston, Massachusetts) was used with 9 L Mhz and 6-15 Mhz linear transducers. Linear measurement of CSF and ventricular volume have been previously described and validated.[15-19]

Neonatal Brain MRI Protocol

Pre- and postoperative brain MRI studies were conducted using no sedation. Newborns and infants who were not clinically sedated were quieted by feeding and swaddling, provided ear protection, and were immobilized through using an infant vacuum immobilizer (Newmatic Medical, Caledonia, Michigan). A 3T Skyra Siemens (Siemens, Erlangen, Germany) with multichannel head coil was used and the following pulse sequences were obtained: (1) volumetric T1 Magnetization-Prepared Rapid Gradient-Echo at echo time (TE)/repetition time (TR): 418/3100 ms, $1.0 \times 1.0 \times 1.0$ mm^3, and matrix size 320×320; (2) volumetric T2 Sampling Perfection with Application optimized Contrasts using different flip angle Evolution sequence at TE/TR: 2.56/2400 ms, $1.0 \times 1.0 \times 1.0$ mm^3, and matrix size 256×196; (3) axial susceptibility weighted at TE/TR: 20/27 ms, slice thickness 2.0 mm with 0 skip, and in-plane matrix resolution 200×256; and (4) axial diffusion-weighted image at TE/TR: 96.6/8000 ms, slice thickness 5.0 mm with 6.0 mm skip, and in-plane matrix resolution 192×192.

Neuroimaging Analyses

A wide spectrum of potential brain abnormalities, including increased intracranial CSF, brain dysplasia, brain maturation deficit, and brain injury patterns were assessed by 2 experienced pediatric neuroradiologists (>12 years experience) blinded to clinical and respiratory CM findings. Intracranial CSF was assessed in 2 separate regions, including region surrounding the surface of the cerebral cortex (extraaxial CSF) and CSF within the intraventricular system (ventriculomegaly). Basic pediatric neuroradiologic definitions and criteria were used from Barkovich et al[20] for overall assessment of brain abnormalities. Brain dysplasia was assessed using criteria and definitions previously reported and focused on brain regions known to be associated with abnormal ciliary function (both motile and primary).[21] For olfactory abnormalities, we assessed for aplasia/hypoplasia of the olfactory bulb within the olfactory groove and aplasia/hypoplasia of the olfactory sulcus on high resolution coronal T2 images.[22] Hippocampal abnormalities (hypoplasia/malrotation/inversion) were identified as previously described on coronal T1 and T2 images.[23-27] Brainstem dysplasia, including either hyperplasia or hypoplasia and asymmetry/disproportion of the any part of the brainstem (medulla, pons, midbrain) using sagittal and axial T1/T2 imaging, was based on prior studies by Barkovich et al.[28] Corpus callosum dysplasia included asymmetry/disproportion of different portions of the corpus callosum (genu, body, splenium, rostrum), or overall abnormal "arching" or morphology best identified on sagittal T1/T2 imaging as previously described by Hetts et al.[29] A composite brain dysplasia index was created with 1 point given for each positive finding in any of 13 measurements including hypoplasia in cerebellar hemispheres and vermis; dysplasia in cerebellar hemispheres and vermis; supratentorial extraaxial fluid; dysmorphometry of left and right olfactory bulbs and sulci; abnormalities in hippocampus and choroid plexus (CP); malformation of corpus callosum; and brainstem dysplasia. Brain maturation and injury were assessed using the method described by Licht et al[30] to score 2 measurements: myelination and cortical in-folding. Regional brain morphometric techniques included volumetric segmentation of total intracranial CSF, cortical gray matter, cortical white matter, deep gray nuclei, brainstem, and cerebellum using a neonatal and infant brain segmentation age-specific atlas customized by our group.[31-33]We further segmented total intracranial CSF into 3 compartments: supratentorial extraaxial CSF, infratentorial extraaxial CSF, and intraventricular CSF.