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

Ashok Panigrahy^{1,7}, Vincent Lee¹, Rafael Ceschin^{1,7}, Giulio Zuccoli¹, Nancy Beluk¹, Omar Khalifa², Jodie K Votava-Smith⁶, Mark DeBrunner³, Ricardo Munoz⁴, Yuliya Domnina⁴, Victor Morell⁵, Peter Wearden⁵, Joan Sanchez De Toledo⁴, William Devine², Maliha Zahid², and Cecilia W. Lo²

¹Department of Pediatric Radiology, Childrens Hospital of Pittsburgh of UPMC and University of Pittsburgh School of Medicine

²Dept. of Developmental Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

³Division of Pediatric Cardiology, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA

⁴Cardiac Intensive Care Division, University of Pittsburgh School of Medicine, Pittsburgh, PA

⁵Division of Pediatric Cardiothoracic Surgery, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA

⁶Department of Pediatric, Division of Cardiology, Childrens Hospital of Los Angeles., Los Angeles, CA

⁷Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA

Abstract

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 and brain magnetic resonance imaging were obtained pre- and/or post-operatively 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 extra-axial CSF volume ($p < 0.001$), delayed brain maturation ($p < 0.05$), and a spectrum of subtle dysplasia including the hippocampus

Corresponding author: Ashok Panigrahy, MD, Department of Pediatric Radiology, Children's Hospital of Pittsburgh of UPMC, 45th Street and Penn Avenue, Pittsburgh PA 15201. panigrahy@upmc.edu, Phone: 412-692-5510, Fax: 412-692-6929.

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($p < 0.0078$) and olfactory bulb ($p < 0.034$). Abnormal CM was associated with higher composite dysplasia score ($p < 0.001$) and both were correlated with elevated pre-operative serum lactate ($p < 0.001$).

Conclusion—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.

Keywords

extra-axial CSF; olfactory; choroid plexus; hippocampus; motile cilia

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 (WMI) 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 ependyma 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 heterotaxy patients 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, 12],[13, 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.

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); pre-operative variables (arterial blood gas pH and PaO₂ and serum lactate); heart lesion category (single vs double and cyanotic vs acyanotic; RACHS (Risk-Adjusted Classification for Congenital Heart Surgery) score; intraoperative variables (cardiopulmonary bypass time and deep hypothermic circulatory arrest [DHCA] time) and postoperative variables (seizures, ventilator dependency, gastrostomy, extracorporeal membrane oxygenation (ECMO) days, total intensive care unit (ICU) days, total hospital days, delayed sternal closure and number of lifetime surgical procedures).

Nasal Tissue Sampling, Reciliation and Ciliary Motion Analysis

Nasal scrape was obtained with curettage of the inferior nasal turbinate using a Rhino-Probe (Arlington Scientific, Springville, UT). 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 three 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 (GE) Logic 9 ultrasound machine was used with 9L 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

(2) Pre- and post-operative 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 with multi-channel head coil was used and the following pulse sequences were obtained: (1) Volumetric T1 MPRAGE at TE/TR: 418/3100 ms, 1.0×1.0×1.0 mm³, and matrix size 320×320; Volumetric T2 SPACE at TE/TR: 2.56/2400 ms, 1.0×1.0×1.0 mm³, and matrix size 256×196; (3) axial susceptibility weighted (SWI) at TE/TR: 20/27 ms, slice thickness 2.0mm with 0 skip, and in-plane matrix resolution 200×256; and (4) axial diffusion weighted DWI at TE/TR: 96.6/8000 ms, slice thickness 5.0mm with 6.0mm 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 two, experienced pediatric neuroradiologists (>12 years experience) blinded to clinical and respiratory CM findings. Intracranial CSF was assessed in two separate regions, including region surrounding the surface of the cerebral cortex (extra-axial CSF) and CSF within the intraventricular system (ventriculomegaly). Basic pediatric neuroradiological definitions and criteria were used from Barkovich et al for overall assessment of brain abnormalities.[20] 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/hypoplasia and asymmetry/disproportion of the any part of the brainstem (medulla, pons, midbrain) using sagittal and axial T1/T2 imaging based on prior studies by Barkovich et al.[28] Corpus callosum dysplasia included asymmetry/disproportion of different portions of the corpus callosal (genu, body, splenium, rostrum), or overall abnormal “arching” or morphology best identified on Sagittal T1/T2 imaging as previously described by Barkovich et al. [29] A Composite Brain Dysplasia (CBD) index was created with one point given for each positive finding in any of thirteen measurements including: hypoplasia in cerebellar hemispheres and vermis; dysplasia in cerebellar hemispheres and vermis; supratentorial extra-axial fluid; dysmorphometry of left and right olfactory bulbs and sulci; abnormalities in hippocampus and choroid plexus; 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 three compartments: supratentorial extra-axial CSF, infratentorial extra-axial CSF and intraventricular CSF.

Statistical Analyses

A univariate regression analysis was conducted to assess if there were any correlations between neuroimaging data and abnormal CM. For volumetric measurements, post-conceptional age was used as a covariate. In order to test for the effect of surgical timing on neuroimaging variables, a mixed-effect model was used. Inter-reader reliability for conventional MRI findings was assessed by calculating the Cohen’s kappa coefficients between readers for each category. Post hoc analysis using False Discovery Rate (FDR) was conducted to minimize Family Wise Error rates, with FDR corrected p-values of <0.05 considered statistically significant. All analyses were performed using SAS 9.3 (SAS Institute Inc. 2010. SAS/STAT ® 9.3).

RESULTS

Thirty five neonates with a wide spectrum of CHD were consecutively recruited prior to surgical palliation (Table I). CUS was obtained in all patients. Usable conventional MRI data were obtained for 28 patients and volumetric MRI for 19 patients, with each patient having a least one pre-operative or post-operative scan. CM was determined to be normal for 25 of the 35 patients, and 10 had abnormal CM (Video; available at www.jpeds.com). Abnormal CM was correlated with the cyanotic CHD lesion type ($p < 0.045$) and elevated serum pH ($p < 0.001$). Abnormal CM was not correlated with single ventricle CHD lesion type, RACHS score, or any of the perinatal, pre-operative, intra-operative or post-operative clinical variables collected (Table II; available at www.jpeds.com). There was no significant difference for post-conceptual age (PCA) at the time of CUS or brain MRI imaging for patients with abnormal vs. normal CM (p value = 0.1184).

Increased Extra-axial CSF with Abnormal Ciliary Motion

Our patient cohort showed an increase in extra-axial CSF with a spectrum of severity ranging from relatively mild to severe accumulation of extra-axial CSF (Figures 1 and 2). The CSF increase was observed both pre- and post-operatively and was significantly associated with abnormal CM (Figure 1, B and Table III). However, intraventricular CSF as assessed by the incidence of ventriculomegaly showed no difference between patients with abnormal vs. normal CM, or pre- vs. post-operatively (Table III and Figure 1, D). As CUS data were available for our entire patient cohort, we also searched for increased extra-axial CSF using the CUS data. This analysis confirmed the MRI findings, showing a higher incidence of increased extra-axial CSF in patients with CHD with abnormal CM. An increase in supratentorial extra-axial CSF volume using 3D volumetric techniques was found to be significantly correlated with abnormal CM ($p < 0.024$), but this was not observed for intraventricular CSF volume. The latter is consistent with the failure to observe association of ventriculomegaly with abnormal CM with the qualitative analysis.

The finding of extra-axial CSF prompted an examination for possible abnormalities associated with the choroid plexus (CP), given its essential role in regulating CSF production. Using imaging data obtained by both CUS (Figure 1, G and H) and MRI conducted at the level of the atrium/trigone of the lateral ventricle[17], we observed CP abnormality comprising either diffuse thickening and/or nodularity (Figure 1, H). The choroid plexus was also significantly increased in thickness using CUS quantitative measurements in patients with abnormal CM (Table III), supporting the qualitative observations. Overall, we found CUS provided more accurate assessment of CP abnormality as compared with conventional MRI. This was indicated by the higher inter-rater reliability (kappa score) for CUS as compared with MRI (Table IV; available at www.jpeds.com).

High Prevalence of Brain Dysplasia and Correlation with Abnormal Ciliary Motion

A spectrum of subtle brain dysplasia was observed (Figures 1 and 2 and Table V). Most prominent were the finding of olfactory bulb hypoplasia, hippocampal abnormalities encompassing both dysplasia and hypoplasia, dysplasia of the corpus callosum, and brain stem (Figures 1 and 2). As with the extra-axial CSF findings, these brain abnormalities

showed a spectrum of severity, spanning from “mild”, “moderate”, to “severe” (Figure 2). The brain abnormalities (hypoplasia and/or dysplasia) with the highest incidence in this cohort included hippocampal, corpus callosal, cerebellar (vermis), olfactory, and choroid plexus (Table V). The brain dysplasia findings were significantly correlated with abnormal CM ($p<0.05$ -corrected for pre/postoperative status; Table III). There was no difference in the incidence of the brain dysplasia findings between the pre-operative and post-operative scans using the mixed-effect model. We also used our volume segmentation pipeline to quantitate brain parenchyma, including supratentorial (grey and white matter) and subcortical (deep grey, cerebellum and brainstem) structures. This analysis showed no significant changes in brain parenchymal volume correlated with abnormal CM, except for an increase in brainstem volume ($p=0.042$), a finding that is concordant with the brainstem dysplasia (hyperplasia) noted in patients with abnormal CM. We did not observe any correlation between abnormal CM with multiple other structural brain abnormalities, including defects involving the orbital and facial regions.

Scoring each patient using a composite brain dysplasia score comprising all of the possible brain dysplasia findings (Table V) also showed a significant increase in the brain dysplasia score in patients with CHD with abnormal CM ($p<0.0001$). Increased brain dysplasia score was correlated with elevated pre-operative serum lactate level ($p<0.001$) (similar to abnormal CM) and increased number of lifetime surgical procedures. Brain dysplasia score was not correlated with CHD lesion type, RACHS score, or any other of the perinatal, pre-operative, intra-operative or post-operative clinical variables collected.

We also assessed for brain maturation and brain injury using conventional brain MRI data. This analysis showed significantly reduced myelination score ($p<0.0341$) and cortical infolding score ($p<0.0341$) with abnormal CM, indicating greater brain immaturity in patients with abnormal CM (Table III). In contrast, individual analysis of brain injury types, or using a composite score comprising all four lesions combined did not show any difference in the prevalence of injury among patients with abnormal vs. normal CM (Table VI; available at www.jpeds.com).

DISCUSSION

We demonstrate that patients with CHD have a high prevalence of subtle brain dysplasia involving the olfactory bulb, hippocampus, corpus callosum, brain stem and choroid plexus. This was observed in conjunction with delayed brain maturation. We further showed that these brain dysplasia and the cortical maturation defects were correlated with abnormal CM. Additionally, we observed increased supratentorial extra-axial CSF volume correlated with abnormal CM, but this was not associated with ventriculomegaly. As these brain abnormalities were not surgery or age related, this would suggest they may be intrinsic to the patients with CHD and may involve the perturbation of cilia function.

Because the original description of major brain malformation in the autopsy of patients with CHD,[34] little or no effort has been devoted to examining for more subtle brain dysplasia in patients with CHD over the last two decades. The possibility of intrinsic brain defects in patients with CHD has been suggested recently by prenatal brain imaging studies showing

brain abnormalities in subjects with CHD, even as fetuses. [35] [36] [37] Most of the structural imaging of neonates with CHD performed up to this point have focused more on volumetric alterations of relatively larger intracranial structures, instead of examining for more subtle dysplastic structures of relatively smaller structures including olfactory bulbs, hippocampus, and cerebellum. We note that some of the abnormalities that we detected in these structures appeared to represent a spectrum including small volumes (hypoplasia) vs alteration in shape or contours (dysplasia). For example, our detected hippocampal abnormalities ranged from small size (but normal shape) to actual alterations in shape/orientation (also known as hippocampal malrotation). [23–27] Recent neuropathology literature demonstrates that a range of subtle hippocampal asymmetries or microdysgenesis exist that involve alterations of the degree of convolutions and histological alterations of the dentate gyrus that lead to gross morphological changes that overlap with our identified neuroimaging spectrum of abnormalities.[38–41]

Together with the present study showing abnormal CM correlating with brain dysplasia, these findings raise the possibility that defects involving cilia may play a role in brain abnormalities in patients with CHD. Human diseases associated with cilia defects, referred to collectively as ciliopathies, are commonly associated with brain abnormalities and diverse neurodevelopmental anomalies, including mental retardation and autism. [42, 43] Olfactory abnormalities are commonly seen in the ciliopathy known as Bardet-Biedl syndrome (BBS) syndrome and in Kallman syndrome (anosomia). [44, 45] Hippocampal abnormalities also are observed in BBS patients and mouse models of BBS. [46] Abnormalities involving the corpus callosum, such as in acrocallosal syndrome, are associated with mutations affecting cilia transduced cell signaling. [47]

Although these ciliopathies are predominantly thought to affect only primary ciliary function, functional overlap between components of the primary and motile cilia is likely given the observation that many proteins expressed in motile cilia also are found in primary cilia [48]. One demonstration of this is our recent study showing motile respiratory cilia defects in a patient with cranioectodermal dysplasia, a ciliopathy thought to affect only primary cilia function.[49] Conversely, mouse studies have also shown mutations affecting motile cilia can perturb primary cilia function. [50] Furthermore, as primary and motile cilia are both known to play important roles in neurogenesis and axon path finding, the brain abnormalities in patients with CHD may involve both motile and primary cilia defects.

Both primary and motile ependymal cilia in the brain regulate neuronal cell migration and maintenance of neural progenitors. [3–6] Primary cilia have been shown to regulate radial glial deployment and axonal outgrowth. [42, 43] We note *Pde2a*, a dual specificity phosphodiesterase [51] known to mediate long-term potentiation and synaptic plasticity [52] was recovered among cilia related genes causing CHD in our mouse mutagenesis screen. Also among CHD genes recovered from the mouse screen were many involved in Shh signaling - a cilia transduced cell signaling pathway with essential roles in both heart and brain development and also in adult neurogenesis in the olfactory bulb, hippocampus, and cerebellum, all structures affected in patients with CHD with abnormal CM. [3–7],[53, 54] These findings suggest cilia defects can not only underlie the brain dysplasia and cortical maturation defect observed in the infants with CHD, but also may have long term impact on

postnatal neurodevelopmental outcomes. In total, these findings point to a possible shared genetic etiology for the brain abnormalities and structural heart defects in patients with CHD involving perturbation of cilia and cilia transduced cell signaling.

The high incidence of increased extra-axial CSF in patients with CHD with abnormal CM were associated with the supratentorial extraaxial CSF, but not with ventriculomegaly. CSF increase in neonates with CHD has been suggested to arise from loss of brain parenchymal volume and/or “external hydrocephalus” due to CSF absorption deficiency or flow abnormality. Although reduced regional brain volumes have been reported in patients with CHD [55], our volumetric analysis showed that the increase in extraaxial CSF was not secondary to reduction in brain parenchymal volume.

The increased extraaxial fluid observed in the patients with CHD represents CSF accumulation in the subarachnoid spaces, particularly in the region of the frontal convexity. Whether this reflects aberrant CSF flow and/or absorption is not known, but we note similar findings have been previously described as benign extra-axial fluid of infancy, benign external hydrocephalus, communicating hydrocephalus, and subarachnoid fluid collections [56]. The finding of choroid plexus hypertrophy in the patients with CHD with abnormal CM would suggest alterations in CSF production might contribute to increased extra-axial CSF. Mouse studies have shown respiratory cilia defects are highly correlated with brain ependymal cilia defects, suggesting patients with abnormal respiratory CM may also have ependymal cilia defects that could disrupt CSF flow and contribute to the extra-axial CSF.

Extra-axial CSF have been reported in third trimester fetuses with complex CHD [35] [36, 37], and preoperative increase in extra-axial CSF also have been associated with neurobehavioral deficits [55]. Our finding that extra-axial CSF and brain dysplasia are both associated with abnormal CM may reflect the importance of CSF flow in providing growth factors and guidance cues for neurogenesis and neuronal cell migration. Thus, CSF flow is thought to generate concentration gradients of guidance molecules required for directional migration of neuronal cells, and stagnant extraaxial CSF [3–7] due to CM defects would be predicted to cause aberrant brain development. This could account for the correlation of abnormal CM with brain dysplasia and extra-axial CSF in patients with CHD. This model can be tested in the future with studies to assess CSF flow in patients with CHD to determine whether those with abnormal CM may have abnormal CSF flow.

Our study was limited by the small size of the cohort with CHD, and its heterogeneity with respect to cardiac lesions, age at MRI scanning, and surgical procedures. We also lacked analyzable serial MRI dataset in a subset of patients due to data corruption from motion artifacts. However, our findings were statistically corrected for pre- and post-operative status and age at brain imaging and we used a mixed effect model post-hoc to examine for possible pre-operative and post-operative difference. Our segmentation analysis of the MRI data did not employ more sensitive surface-based deformation techniques needed to distinguish dysplasia from hypoplasia, especially for the subcortical structures, which is part of ongoing analyses in our group. Given that we did find that both ciliary motion abnormality and brain dysplasia was associated with increased pre-operative serum lactate (indicator of hemodynamic instability and overall more critically ill patients), it is possible that our

spectrum of brain dysplasia abnormalities does include both intrinsic and acquired brain abnormalities (i.e. volume loss vs hypoplasia). Another limitation is the poor initial kappa score for certain brain structures like the choroid plexus using MRI, which was improved after better delineation of the pattern of abnormality (nodular vs. thickening) and supplementation by CUS imaging. CUS is a valuable cost effective brain imaging tool, given it can be performed at the bedside and provide brain imaging data on patients too unstable for MRI scanning. Moreover, hemorrhage within the choroid plexus may confound our findings. Finally, our analysis did not include healthy neonates, but this does not affect the overall conclusions of our study examining the effects of abnormal CM on brain abnormalities in infants with CHD.

The mechanism for poor neurodevelopmental outcomes associated with CHD is unknown, but has largely been attributed to poor brain perfusion from the cardiac lesion and/or surgical intervention. In this study, we show a high prevalence of subtle brain abnormalities and increased extra-axial CSF in infants with CHD. These were significantly associated with respiratory ciliary dysfunction, but not correlated to surgery or type of cardiac lesions, nor with brain injury. Given the increasing appreciation of the role of cilia in brain development and adult neurogenesis, our findings would suggest the identification of neonates and infants with CHD with CM abnormalities may provide opportunities for prognostication of long-term neurodevelopmental outcomes. Further studies are needed with longitudinal follow up with neurocognitive and functional assessments to determine whether abnormal respiratory CM can prognosticate neurodevelopmental risks among patients with CHD. Other future studies not only include more detailed quantitative analyses of the brain dysplasia structures, but also the relationship between brain dysplasia and brain connectivity and/or metabolism. In additional, whole exome sequencing results of this cohort is in progress, which will allow for further confirmation and delineation of underlying genetic mechanisms.

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Figure 1.

Constellation of brain abnormalities in term infant with CHD 7430 with abnormal ciliary motion. MRI obtained postoperatively showed thin and dysplastic corpus callosum (CC) in Sagittal T1 (A, B) and coronal T2 views (E/F). Also observed were hippocampal abnormalities (C, D) and severely hypoplastic olfactory bulb (OB)(E,F). Postoperative cranial US also showed both nodular and hypertrophied choroid plexus (G, H).

Figure 2.

Spectrum of brain abnormalities of varying severity in infants with CHD with abnormal ciliary motion. MRI imaging showed brain abnormalities ranging from mild, moderate to severe in four infant cases (52 – 80 weeks PCA) with abnormal CM as compared with a healthy infant control (60 weeks PCA). Note the degree of increased extra-axial fluid, hippocampal dysplasia, and brainstem/cerebellar dysplasia. The mild case is pre-operative, and the three other moderate-severe cases are postoperative.

Table 1

(on-line). CHD Diagnosis and Ciliary Function in Patients with CHD

Patient ID*	Cardiac Lesions [†]	Cyanotic	Ciliary Motion ^{‡‡}
7004	DILV, D-TGA, IAA-Type A	+	Abn
7058	TOF	+	Abn
7106	HLHS, COA, DORV, VSD	+	Nml
7134	DORV/subaortic VSD, PS	+	Abn
7136	HLHS, DORV, HTX	+	Nml
7151	D-TGA	+	Nml
7208	D-TGA	+	Abn
7288	Truncus arteriosus	+	Abn
7289	HLHS	+	Abn
7302	HLHS	+	Nml
7306	D-TGA	+	Abn
7328	Unbal AVSD, PA, TAPVR, DORV, HTX	+	Abn
7334	ASD, VSD, PDA, Interrupted IVC	–	Nml
7336	D-TGA	+	Nml
7351	HLHS	+	Nml
7374	L-TGA, tricuspid atresia	+	Nml
7376	Truncus arteriosus	+	Nml
7381	D-TGA	+	Nml
7383	TOF, PA, VSD, MAPCA	+	Nml
7386	HLHS	+	Nml
7389	HLHS	+	Nml
7397	TOF, PA	+	Nml
7400	HLHS	+	Nml
7401	DORV, D-TGA	+	Nml
7405	TAPVR	+	Nml
7409	D-TGA, tricuspid atresia, hypo Ao	+	Abn
7411	IAA-Type A, fenestrated ASD, PDA	–	Nml
7414	CoA, Inlet VSD	–	Nml
7415	TOF, PA	+	Nml
7417	VSD, PFO	–	Nml
7419	DORV, PA, AVSD, TAPVR, HTX	+	Nml
7421	TOF	+	Nml
7422	D-TGA	+	Nml
7425	CoA, Hypoplastic AoA (Shone's complex)	–	Nml
7430	Unbalanced AVSD, PA, TAPVR L-TGA, HTX	–	Abn

* Grey fill are patients with reciliated nasal tissue analysis - only patient 7151 had ciliary motion diagnosis changed (from abnormal to normal)

[†] AoA: aortic arch; AVSD: atrioventricular septal defect; CoA: coarctation, DILV: double inlet left ventricle, DORV: double outlet right ventricle, D or L-TGA: dextro or levo-transposition of the great arteries, HTX: heterotaxy, IAA: interrupted aortic arch, MAPCA: multiple aortopulmonary collateral arteries; PA: pulmonary atresia, PDA: patent ductus arteriosus; PFO: patent foramen ovale; TAPVR: total anomalous pulmonary venous return; TOF: Tetralogy of Fallot, VSD: ventricular septal defect;

^{††} Nml = normal; §

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Table 2

(on-line) : Incidence of Heart Lesions Among Patients with Abnormal CM

<u>Heart Lesions</u>	Ciliary Motion			
	Normal CM (%)	Abnormal CM (%)	P-value (Raw)	P-value (FDR)
Single Ventricle	60	83.3	0.1579	0.3158
Aortic Arch Obstruction	64	91.6	0.0759	0.2277
Conotruncal	50	83.3	0.0623	0.2277
Cyanotic	76.9	91.6	0.3807	0.5711
Aortic Valve Flow Obstruction	23.0	33.3	0.9437	0.9708
Heterotaxy	7.6	8.3	0.9708	0.9708

Table 3

Abnormal Ciliary Motion Effects on Brain Measurements in Infants with CHD

Brain measurements*	Imaging Modality [†]	Abnormal Ciliary Motion ^{††}			
		p-val (raw)	p-val (FDR)	R ²	Change
Extra-axial CSF	CUS	<.0001	0.0001	0.5296	Up
Extra-axial CSF	MRI	0.0005	0.0022	0.4525	Up
Ventriculomegaly	MRI	0.6313	0.6313	0.0361	Down
Choroid Plexus Increase Thickness	CUS	0.0004	0.0022	0.4013	Up
Choroid Plexus Nodular/Thickened	MRI	0.0132	0.031	0.2928	Up
Olfactory Abnormalities	MRI	0.0248	0.0341	0.2559	Up
Hippocampal Dysplasia/Hypoplasia	MRI	0.0024	0.0078	0.3835	Up
Corpus callosum Dysplasia	MRI	0.0248	0.0341	0.2559	Up
Corpus callosum Hypoplasia/Thinning	MRI	0.5385	0.5834	0.0483	Up
Brainstem Dysplasia	MRI	0.0143	0.031	0.2899	Up
Average Cortical Maturation Score	MRI	0.0236	0.0341	0.2887	Down
Myelination Score	MRI	0.0262	0.0341	0.2818	Down

* CUS analysis of choroid plexus comprises quantitative measurement at level of trigone MRI analysis of extra-axial CSF conducted qualitatively at supratentorial region.

Composite olfactory bulb assessment: aplasia or hypoplasia, olfactory sulcus aplasia or hypoplasia

[†] CUS: cranial ultrasound; MRI: magnetic resonance imaging

^{††} p-value determined by analysis of covariance (ANCOVA) comparing normal vs. abnormal CM groups, with pre- and post- surgical states as a covariate (corrected for before and after surgery); FDR- False Discovery Rate correction applied for multiple comparisons.

Table 4

(on-line) Comparison of Inter-Reader Reliability

BRAIN ANOMALY	κ-score
Cortical Maturation	0.888
Myelination	0.8333
Extra Axial Fluid Abnormality	0.3510
Choroid Plexus Abnormality	0.7225 *
Ventriculomegaly	0.5385
Corpus Callosum Dysplasia	0.8880
Hippocampal Abnormalities	0.9102
Olfactory Abnormalities	0.8685
Composite Brain Injury	0.9340

* Based on k-score after using cranial ultrasound pattern and better post-hoc delineation of nodular and/or thickened choroid plexus patterns: Initial kappa score using MRI was .3210

Table 5

Incidence of Brain Dysplasias in Cohort with CHD

Brain Abnormality	% Incidence
Cerebellar Hemispheres Hypoplasia	3.6
Cerebellar Hemispheres Dysplasia	10.7
Cerebellar Vermis Hypoplasia	14.3
Cerebellar Vermis Dysplasia	10.7
Supratentorial Extra-axial fluid	42.9
Right Olfactory Bulb Dysmorphometry *	21.4
Left Olfactory Bulb Dysmorphometry *	14.3
Right Olfactory Sulci Dysmorphometry **	10.7
Left Olfactory Sulci Dysmorphometry **	10.7
Hippocampal Abnormalities ***	25.0
Corpus callosum Abnormalities ****	21.4
Choroid plexus abnormality *****	42.9
Brainstem Dysplasia	25.0

* Olfactory blub abnormalities included both hypoplasia and aplasia;

** Olfactory sulcus abnormality included both hypoplasia and aplasia;

*** Hippocampal abnormalities include both hippocampal dysplasia (malrotation) and hypoplasia findings;

**** Corpus Callosum abnormalities include both dyplasia and hypoplasia findings;

***** Choroid Plexus abnormalities included both nodularity and hypertrophy of the choroid plexus at the level of the caudothalamic groove and the atria of the lateral ventricle

Table 6

(on-line): Abnormal Ciliary Motion and Brain Injury in Infants with CHD

Brain Measurements	Imaging Modality *	Abnormal Ciliary Motion			
		p-val ^{*†} (raw)	p-val ^{††} (FDR)	R ²	Change
Composite Brain Injury Score	MRI	0.1141	0.3423	0.1594	down
Hemorrhage	MRI	0.4797	0.5276	0.0571	up
Focal Infarct	MRI	0.2309	0.3785	0.1106	down
Watershed Infarct Hypoxic-Ischemic	MRI	0.2523	0.3785	0.1084	down
Punctate White Matter Lesion	MRI	0.5276	0.5276	0.0499	down
Ischemic Injury Subscore [#]	MRI	0.0749	0.3423	0.1872	down

* CUS: cranial ultrasound; MRI:magnetic resonance imaging

^{*†} p-value for significant correlation of finding with abnormal CM group as Determined by analysis of covariance (ANCOVA) comparing each metric in normal and abnormal CM groups or normal and low nNO groups, with pre- and post- surgical states as a covariate (corrected for before and after surgery);

^{††} FDR- False Discovery Rate correction applied for multiple comparisons.

[#] Ischemic Injury Subscore reflects a summation of Focal infarct, Watershed infarct, Punctate White Matter Lesions.