Among all known birth defects, congenital heart disease (CHD) is the leading cause of death in infancy. In the United States, nearly 25% of children born with CHD will require surgery or treatment within their first year of life. Tremendous advances in surgical techniques and strategies have led to a dramatic increase in survival rates, even ≤90% in complex CHDs; today, most children born with CHD will reach adulthood. Lower mortality rates in CHD have resulted in a dramatic shift in current research efforts: from short-term survival to improving functional outcome and quality of life.

It has become increasingly clear that children born with CHD have worse long-term neurological outcomes than their peers (Figure 1). Neuroimaging and psychological evaluations have identified strong associations between altered brain maturation in CHD and neurodevelopmental abnormalities associated with CHD.

Abstract: In the past 2 decades, it has become evident that individuals born with congenital heart disease (CHD) are at risk of developing life-long neurological deficits. Multifactorial risk factors contributing to neurodevelopmental abnormalities associated with CHD have been identified; however, the underlying causes remain largely unknown, and efforts to address this issue have only recently begun. There has been a dramatic shift in focus from newly acquired brain injuries associated with corrective and palliative heart surgery to antenatal and preoperative factors governing altered brain maturation in CHD. In this review, we describe key time windows of development during which the immature brain is vulnerable to injury. Special emphasis is placed on the dynamic nature of cellular events and how CHD may adversely impact the cellular units and networks necessary for proper cognitive and motor function. In addition, we describe current gaps in knowledge and offer perspectives about what can be done to improve our understanding of neurological deficits in CHD. Ultimately, a multidisciplinary approach will be essential to prevent or improve adverse neurodevelopmental outcomes in individuals surviving CHD.
Brain maturation and congenital heart disease begins in the third week of gestation and continues into early adulthood. After birth, the brain increases over 100% in volume in the first year and another 15% by the end of the second year of life. During these key developmental time windows, individuals with CHD are at risk of altered brain development and pathological insults which may result in poor neurological outcomes.

During fetal development, the brain is initially smooth—lissencephalic—and later develops gyri (Figure 2A). Neurons form networks with one another as early as midgestation; initially, more connections are made than necessary, and these connections are then refined as new demands and challenges are met throughout life. It has been proposed that the hierarchy of connections between brain regions may underlie the order of maturation in the brain: low-level processing areas such as sensory regions would need to mature first to foster high-level integration of information used by associative regions which develop later.

Higher-order cognitive function requires proper connectivity and communication between neurons locally and distally throughout the brain. The corpus callosum, for example, is the largest white matter (WM) tract in the brain and connects the left and right cerebral hemispheres enabling interhemispheric communication essential for the proper integration of motor, sensory, and cognitive information. Myelinated axons are a primary constituent of the corpus callosum, and myelin—lipid-rich membrane synthesized by oligodendrocytes—enables rapid communication of information. WM development is a protracted process, and myelination begins near midgestation in the human brain. Oligodendrocytes originate from the subventricular zone, and myelination is a multistage process involving oligodendrocyte precursor cell (OPC) expansion, migration, differentiation, and membrane expansion. Because WM dysmaturation and injury are common in CHD, it will be important to study the cellular responses to CHD-imposed brain pathologies.

In the late 80s and early 90s, postmortem neuropathological examinations detailed the developmental and acquired brain anomalies and lesions, respectively, in infants born with hypoplastic left heart syndrome (HLHS). Microcephaly, immature cortical mantle, agenesis of the corpus callosum, and holoprosencephaly were among the identified developmental abnormalities. Intracranial hemorrhages and hypoxic–ischemic lesions, such as periventricular leukomalacia (PVL), cerebral, and brain stem necrosis, were identifiable acquired lesions. A little over a decade ago, PVL or diffuse WM gliosis was found to be the most significant types of lesions in CHD infants dying after cardiac surgery. Moreover, infants <1 month of age who had an incidence of acute PVL after cardiopulmonary bypass (CPB) with deep hypothermic circulatory arrest (DHCA) died at significantly younger ages compared with younger infants. These findings suggest an inverse correlation between brain maturation and WM vulnerability to hypoxic–ischemic brain injury.

Brain injuries during development can result in debilitating cognitive, behavioral, and psychological outcomes that may not be evident until many years later. Advances in corrective and palliative pediatric cardiac surgery over the past 60 years have substantially improved the survival rate of babies born with CHD; however, these children remain susceptible to a spectrum of neurological impairments. Initial insights into
the underlying causes of such neurological deficits were heavily limited by the lack of appropriate imaging tools required to monitor brain development noninvasively. Prospective and retrospective imaging studies have been mounted to carefully document primary structural abnormalities during development and secondary brain injuries after corrective and palliative surgery.

**Neuroimaging in CHD**

**Neuromonitoring**

Several imaging modalities have been developed and used to identify associations between physiological or anatomic alterations and neurodevelopmental outcome in CHD. These include optical imaging technologies, such as pulse oximetry and near-infrared spectroscopy (NIRS), as well as electroencephalogram (EEG), Doppler ultrasound, and head ultrasound (HUS). Several clinical and research studies have used these tools to gain insights into potential pre-, intra-, peri-, and postoperative predictors of neurodevelopmental outcomes in CHD.

NIRS has been used as a monitoring tool during the pre-, intra-, and postoperative period. Preoperative cerebral oxygenation is significantly reduced in certain cases of complex CHD, depending on the type of cardiac anomaly and arterial saturation. In addition, decreases in preoperative cerebral oxygen extraction have been reported in cases of patent ductus arteriosus and HLHS. In the early 2000s, the use of intraoperative NIRS monitoring in predicting neurological outcomes was assessed in swine. NIRS was shown to be a useful predictor of neurological recovery and injury after surgery. A few years later, diminished cerebral oxygen delivery determined by NIRS in the perioperative period in infants undergoing cardiac surgery was shown to be associated with lower Psychomotor Developmental Index scores at 1 year of age. In a recent study using NIRS in HLHS patients, reduced preoperative—but not postoperative—cerebral oxygenation was associated with worse neurodevelopmental outcomes assessed in early childhood.

Reduced systemic venous oxygen delivery, measured by fiberoptic oximetry, was significantly associated with neurodevelopmental abnormalities in 4-year olds who underwent the Norwood procedure for palliation of HLHS. In addition, longer durations of systemic venous oxygen levels <40% correlated with higher risks of abnormal neurodevelopmental outcomes. Early postoperative monitoring—within 48 hours after surgery—of cerebral oxygen saturation by NIRS was also shown to be predictive of adverse outcomes in HLHS patients who underwent the Norwood procedure. Mean cerebral oxygen saturation values <56% were predictive of worse outcomes, including longer intensive care unit stay and longer need for extracorporeal membrane oxygenation. Cerebral hypoxia determined by NIRS during the early postoperative period was reported to be associated with worse neurodevelopmental performance, particularly low visual-motor integration, when assessed in children at 4 to 5 years of age.

Although clinical studies indicate that NIRS may be a useful predictor of neurodevelopmental outcomes in CHD, the underlying causes of such neurological deficits were heavily limited by the lack of appropriate imaging tools required to monitor brain development noninvasively. Prospective and retrospective imaging studies have been mounted to carefully document primary structural abnormalities during development and secondary brain injuries after corrective and palliative surgery.

**Figure 2.** A. Cortical gyrification increases throughout fetal and perinatal brain development. Legend demarks the 4 lobes of the cortex. B. Gyrification indices and (C) cortical surface areas of fetuses with hypoplastic left heart syndrome (HLHS) compared with normal fetuses. Adapted from Dubois and Dehaene-Lambertz (A) and Clouchoux et al (B and C) with permission of the publishers. Copyrights © 2015, 2013, Elsevier and Oxford University Press, respectively. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
use of NIRS during surgery remains controversial. Currently, the evidence that NIRS is associated with improved neurodevelopmental outcomes is limited and reflective of outcomes in small cohorts, additional studies will be necessary to determine whether NIRS is a valid predictor of neurodevelopmental deficits in addition to its neuromonitoring use. Such future studies should consider the fact that cerebral oxygen levels assessed by NIRS are usually generated from probes covering the frontal lobe; acquiring measurements from other regions of the brain associated with behavioral function assessed during formal testing such as visuospatial skills will be fruitful and may provide more meaningful associations between cerebral oxygenation and specific neurodevelopmental modalities.

A recent longitudinal study assessed neurodevelopmental outcomes in a large cohort of mixed CHD patients (178 patients) after surgery. Narrower arterial-cerebral and arterial-somatic saturation by NIRS was associated with better outcomes. Higher arterial saturation values were associated with improved motor performance scores when key neuronal connections (synapses) are forming. In addition, higher arterial saturation values were associated with improved motor performance scores with age. Future studies will be essential to reach a consensus on the risks and benefits of optical imaging technologies as a management tools to improve neurodevelopmental outcomes in CHD.

Subclinical perioperative seizures are common, can be identified by EEG, and have been shown to be predictive of worse neurodevelopmental outcomes in CHD in an age-dependent manner. Continuous EEG monitoring of neonates and infants during the first 48 hours after surgery for complex CHD was not predictive of neurodevelopmental outcomes at 1 year of age. At 4 years of age, EEG seizure occurrence was associated with worse executive function and deficits in social interactions and restricted behavior; seizures did not correlate with worse cognitive, motor, and language skills nor attention or impulsivity performance. Although EEG has long since been an excellent tool for the detection of seizures, these findings indicate that it can also be used as a predictive measure of neurodevelopmental outcomes later in life and affords opportunities for intervention and management strategies.

Doppler ultrasound is also a valuable tool for identifying associations between cerebral flow with neurodevelopmental abnormalities. This technique has primarily been used in the fetal period and typically has not been used to assess cerebral blood flow in the neonatal period. Doppler evaluation during fetal echocardiography examination has been used to assess systolic, diastolic, and mean blood flow velocity in the middle cerebral artery and umbilical arteries to determine the cerebroplacental resistance ratio. Alterations in cerebrovascular resistance (CVR) and fetal cerebrovascular blood flow distribution between the brain and placenta vary depending on the type of CHD. Early investigations of fetuses with single ventricle (SV) defects have suggested that these flow alterations may have an impact on early neurodevelopmental outcomes. Future studies will be necessary to determine whether fetal Doppler ultrasound can be widely used as a tool to predict neurodevelopmental outcomes.

Historically, HUS acquisitions were most routinely performed and predate magnetic resonance imaging (MRI); however, HUS does not provide the resolution necessary to detect a majority of subtle structural alterations. A study comparing HUS and MRI demonstrated preoperative brain injury by MRI in 26% of infants with CHD, whereas HUS only detected brain injury in 3% of infants—80% of which were false positives. A recent study concluded that preoperative HUS in CHD infants undergoing surgery with CPB was not predictive of neurodevelopmental outcomes at 1 year of age. Detectable alterations in WM identified by HUS are modest in number, whereas studies using MRI have reported WM injury in ≤50% of CHD cohorts. Thus, the need for routine brain scans with more modern and standardized techniques is becoming increasingly recognized; in fact, some hospitals, including our own, have been performing routine brain MRI scans on all neonates undergoing cardiac surgery for several years.

**Magnetic Resonance Imaging**

Advances in MRI technology have enabled identification of a high frequency of immature brain development and injury in CHD patients. Before these sophisticated technologies, many brain pathologies in CHD went unnoticed without postmortem examination. With state-of-the-art techniques, we are now able to visualize the brain with high fidelity, quantify specific microstructural alterations, and identify predictive neurodevelopmental measures and potential treatment/interventional windows even before birth.

Magnetic resonance spectroscopy is particularly useful for assessing the metabolic integrity of neurons in specific brain regions. Neuronal abnormalities have been suggested in CHD fetuses by magnetic resonance spectroscopy (eg, lower N-acetylaspartic acid [NAA]:Cho ratio) during the third trimester when key neuronal connections (synapses) are forming. In addition, newborns with d-transposition of the great arteries and SV physiology displayed a 10% reduction in the adjusted mean NAA:Cho ratio before cardiac surgery. It was recently demonstrated that reduced NAA:Cho ratios in periventricular WM are predictive of worse motor outcomes in preterm infants without CHD. Hence, it will be of great importance to determine whether alterations in metabolites are also predictive of neurodevelopmental outcomes in CHD populations.

Advances in fetal neuroimaging have provided great insights into the developing brain; before these advances, the anatomic condition of the brain was unknown until birth. A groundbreaking and well-designed study demonstrated a drop-off in global brain volume by MRI during the third trimester in fetuses with HLHS. In addition, this group reported a significant reduction in cortical volume, gyrencephaly, cortical surface area, and WM volume during the later stages of gestation (Figure 2B). Interestingly, preterm newborns were recently reported to have a higher gyriﬁcation index compared with their term fetal counterparts during this time window; these ﬁndings suggest that alterations in blood ﬂow during fetal CHD development may have more deleterious effects on specific aspects of brain development, such as gyriﬁcation. A recent study using antenatal MRI demonstrated a reduction in total brain volume, gray matter volume, and subcortical brain...
Brain development and mature brain injury during the neonatal period may be associated with neurodevelopmental abnormalities. Early brain injuries, such as PVL, can alter white matter (WM) development and injury by impairing water displacement which increases in numeric value as WM matures and becomes more organized. Studies using DTI have demonstrated a high incidence of WM dysmaturation and injury in CHD patients, including adolescents who underwent surgery in infancy. Preoperative DTI studies have identified several underdeveloped WM structures in CHD from infancy to adolescence. A reduction in fractional anisotropy in key WM tracts, such as the corpus callosum, was reported in a mixed cohort of newborns with CHD. In addition, 2 studies reported microstructural abnormalities and immaturity in the splenium of the corpus callosum in preterm neonates born with CHD. Postoperative WM injury in ≤18 WM regions has also been reported in adolescents after corrective surgery; it is important to note that these injuries were barely detectible by conventional MRI.

The functional impact of WM developmental abnormalities and injury on network connectivity and neurocognitive impairments in CHD has recently come into focus. There is now evidence that perturbations in structural and microstructural brain development can result in alterations in neuronal network connectivity in neonates with CHD. Graphic analysis to acquire DTI data recently revealed that differences in the connectome mediate poor neurocognitive function seen in adolescents with d-transposition of the great arteries repaired during infancy. Reduced network integration and increased network segregation mediated differences in intelligence quotient (IQ), academic achievement, executive function, learning and memory, and visuospatial abilities between adolescents born with d-transposition of the great arteries and peers. Such advances are laying a foundation for understanding the interplay between macro/microstructural alterations, neuronal connectivity, and cognitive outcomes in CHD.

Research aimed at developing and using noninvasive imaging technologies, such as the BRAIN initiative (Brain Research Through Advancing Innovative Neurotechnologies) at the NIH, is now providing a dynamic picture of human brain development and pathologies. Implementing the use of these innovations in the CHD population will provide fruitful information about the complex nature of brain development and likely identify a wide range of developmental delays and impairments associated with CHD.

### Similarities and Differences to the Preterm Population

There are many similarities in brain alterations and neurodevelopmental outcomes associated with premature birth versus CHD. Surviving infants born prematurely at a very low birth weight are at a high risk of developing impairments in motor, cognitive, behavioral, attention, language, executive, and social abilities. Children born prematurely often display reductions in cortical gray and WM volumes as seen in CHD. There are also regional vulnerabilities to brain dysmaturation; for example, the frontotemporal lobes and hippocampi are highly vulnerable. Historically, cystic PVL and intraventricular hemorrhage were the most common forms of brain injury seen in preterm patients; however, the incidence of PVL has declined, and diffuse WM injury is now more commonly present. Importantly, as seen in CHD, WM injury identified with neuroimaging techniques is associated with, and predictive of, worse motor and cognitive outcomes after premature birth.

CHD fetuses are at a high risk of spontaneous preterm birth. Preterm birth in CHD affects mortality, morbidity, and neurodevelopmental outcomes. Infants with CHD who were born late preterm (gestational age of 34–36 weeks) were at an increased risk of hospital death, with a nearly 37% mortality rate for those born at a gestational age of 36 weeks. Using DTI, a recent clinical study reported similarities in the cerebral microstructures between preterm neonates with and without CHD; however, a microstructural abnormality in the splenium of the corpus callosum—a region associated with visuospatial abilities—was identified in all CHD neonates born preterm in this study, indicating a regional vulnerability to preterm birth in CHD. Preterm infants born at an extremely low birth weight with CHD were shown to have a higher risk of death or neurodevelopmental impairments compared with infants born without birth defects.
It is important to note that unlike the premature birth population, cases of complex CHD require complicated surgical interventions which expose the individual to a wide spectrum of complex and cumulative risk factors associated with neurodevelopmental abnormalities (Figure 3). For example, a strong association between systemic infection/inflammation and brain abnormalities in premature birth has been established.\(^44,45\) Infants born with complex CHD often require surgical interventions using CPB which can elicit an inflammatory response because of blood contact with foreign surfaces. Significant increases in the levels of inflammatory cytokines (8 out of 11) were recently reported after CPB surgery in CHD neonates; however, there was a weak correlation between WM injury determined by conventional MRI and inflammatory markers with interleukin-1β on postoperative day 1.\(^46,47\)

Because adverse neurodevelopmental outcomes have been well recognized and defined in the preterm population and have only recently received attention in CHD, it is important to consider converging causes of brain pathologies between these populations. In contrast with preterm birth, almost nothing is known about CHD-induced brain injury at a cellular level in humans. Some common spatiotemporal mechanisms of cell-specific vulnerabilities are likely; therefore, comparative studies extrapolating and expanding on findings in the preterm birth population will be fruitful in expediting the development of treatment strategies to target specific cells and molecular pathways to improve neurodevelopmental outcomes in CHD.

**Risk Factors Associated With CHD**

Several clinical and research studies have identified multifactorial causes associated with neurological impairments in CHD. These encompass intrinsic genetic and acquired risks, including pre-, peri-, and postoperative factors (Figure 3).

**Genetic Alterations in Heart and Brain Development**

Clinical genotyping is a powerful tool of great prognostic value and can be performed before the brain is even formed. Many of the mutations in histone-modifying genes which can affect many genes involved in development.\(^45\) Eight of the mutated genes in CHD have known roles in production, removal, or reading of methylation of histone H3 lysine 4 (H3K4me); H3K4me is associated with poor neurological outcomes in CHD patients after CPB. APOE is a key regulator of cholesterol metabolism and lipid transport in the brain and is involved in neuronal resiliency. Patients undergoing CPB before 6 months of age had significantly lower neurological scores assessed by the Psychomotor Developmental Index of the Bayley Scales of Infant development at 1 year of age; importantly, these poor neurological outcomes were independent of the type of cardiac defect, use of DHCA, ethnicity, or socioeconomic status (SES).\(^88\) In addition, anthropometric growth measurements were obtained, and it was shown that the APOE ε2 allele was predictive of reduced weight and head circumference growth at 4 years of age in CHD patients whom underwent surgery in infancy.\(^89\) A follow-up study that assessed Child Behavior Checklist indices during preschool ages (4–5 years old) revealed a higher prevalence of impaired social interactions, problems internalizing, and behavior problems compared with non-APOE ε2 allele carriers.\(^90\) These findings were recently validated in a new combined cohort of patients with SV after surgery.\(^91\) The APOE ε2 allele was associated with lower scores on the Psychomotor Development Index at 14 months of age independent of operative factors.\(^91\) Together, these studies have provided a predictive measure of neurodevelopmental outcome after surgery and underscore the need for genotyping and risk stratification for those undergoing surgery carrying the APOE ε2 allele.

Since the human genome was first sequenced in 2003, there have been tremendous advances in human genetic studies. We can now readily identify small genetic variations associated with particular diseases throughout the genome on a case-by-case basis. These advances have spurred ongoing genome-wide association studies aimed to identify genetic variations that indicate the potential risk of developing a disease. Recently, 2 studies identified de novo mutations in CHD patients enrolled in the Pediatric Cardiac Genomics Consortium.\(^92,93\)

Exome sequence analysis of parent–offspring trios revealed de novo point mutations in hundreds of genes contributing to ~10% of complex CHD; of particular interest were the mutations in histone-modifying genes which can affect many genes involved in development.\(^92\) Eight of the mutated genes in CHD have known roles in production, removal, or reading of methylation of histone H3 lysine 4 (H3K4me); H3K4me is associated with poor neurological outcomes in CHD patients after CPB. APOE is a key regulator of cholesterol metabolism and lipid transport in the brain and is involved in neuronal resiliency. Patients undergoing CPB before 6 months of age had significantly lower neurological scores assessed by the Psychomotor Developmental Index of the Bayley Scales of Infant development at 1 year of age; importantly, these poor neurological outcomes were independent of the type of cardiac defect, use of DHCA, ethnicity, or socioeconomic status (SES).\(^88\) In addition, anthropometric growth measurements were obtained, and it was shown that the APOE ε2 allele was predictive of reduced weight and head circumference growth at 4 years of age in CHD patients whom underwent surgery in infancy.\(^89\) A follow-up study that assessed Child Behavior Checklist indices during preschool ages (4–5 years old) revealed a higher prevalence of impaired social interactions, problems internalizing, and behavior problems compared with non-APOE ε2 allele carriers.\(^90\) These findings were recently validated in a new combined cohort of patients with SV after surgery.\(^91\) The APOE ε2 allele was associated with lower scores on the Psychomotor Development Index at 14 months of age independent of operative factors.\(^91\) Together, these studies have provided a predictive measure of neurodevelopmental outcome after surgery and underscore the need for genotyping and risk stratification for those undergoing surgery carrying the APOE ε2 allele.

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important for the activation of key genes involved in development. In addition, 2 mutations were identified in a gene that regulates H3K27 methylation, which is known for inactivating gene transcription.92

Using exome sequencing, a recent study identified new (de novo) genetic mutations in patients with CHD with neurodevelopmental disabilities.93 Genomic DNA was sequenced from blood collected from >1200 CHD patients from 10 centers located in the United States and United Kingdom. De novo mutations were identified and compared between those with CHD, those with CHD and a coexisting neurological disability, those with CHD and an extracardiac congenital anomaly, or both. In addition to identifying genes known to be associated with CHD, several protein-damaging mutations were identified, including mutations in genes involved in mRNA splicing, chromatin modification, transcriptional regulation, anatomic structure morphogenesis, cardiovascular system development, and neurodevelopmental abnormalities. One such gene, RBFOX2, encodes a RNA-binding protein essential for epithelial to mesenchymal cell transitions. Disruption of these transitions is thought to result in HLHS. These findings indicate that protein-damaging de novo gene mutations are strong predictors of neurodevelopmental anomalies in CHD and highlight the use of clinical genotyping to identify those who may have neurodevelopmental deficits later in life. A key finding in this study was that CHD patients who had neurodevelopmental disabilities were enriched for mutations in particular genes expressed in both heart and brain; hence, these shared genetic contributions for CHD and neurodevelopmental disabilities point to a common genetic cause in that the same genes that cause CHD may also cause the brain anomalies in these patients. Follow-up studies with animal models will be essential in determining how genetic alterations identified in CHD patients with neurodevelopmental abnormalities affect the molecular and cellular programs underlying heart and brain development.

Preoperative Factors
Disturbance of Fetal Cerebral Blood Flow
Normal fetal brain development requires maternal and placental circulation in order for the heart to preferentially direct highly oxygenated blood along with substrates to the brain. In addition, intrinsic autoregulatory mechanisms are in place to adjust CVR to increase cerebral oxygen delivery during fetal brain development. In many cases of complex CHD, these beneficial systems regulating cerebral blood flow are altered in utero and persist after birth.94,95 In utero hemodynamics in CHD have been assessed as early as midgestation with cerebral and umbilical artery Doppler ultrasound.38 The cerebroplacental resistance ratio—a metric of vascular resistance and the distribution of blood flow—was significantly lower (cerebroplacental resistance ratio<1) in fetuses with CHD, especially in those with HLHS. A reduction in the cerebral/umbilical resistance index was also seen in CHD fetuses. Alterations in fetal CVR have been shown to be associated with neurodevelopmental outcomes in CHD. Reduced fetal CVR was initially shown to be associated with lower cognitive scores at 18 months of age in a small cohort of mixed complex CHD.96 However, 2 studies with larger and more homogeneous populations of univentricular fetuses demonstrated that decreased CVR, determined by the middle cerebral artery pulsatility index, is associated with higher neurodevelopmental scores at 14 months of age determined by the Psychomotor Development Index.40,97 Together, these clinical studies indicate that hemodynamics should be carefully monitored during fetal development and that optimizing cerebral oxygen delivery may improve neurodevelopmental outcomes in specific CHDs.

Three recent studies have shed light on cerebral perfusion and oxygen consumption in fetuses and neonates with CHD. Fetal hemodynamics and brain size were measured in 30 CHD fetuses in late gestation.98 This group demonstrated that reduced fetal cerebral oxygen delivery and consumption is associated with smaller brain volumes in CHD. In addition, fetal brain size correlated with ascending aortic oxygen saturation and cerebral oxygen consumption. Using a combination of MRI and diffuse optical and correlation spectroscopies, Jain et al99 demonstrated reduced values in cerebral blood flow and oxygen metabolism measurements in CHD neonates compared with reported values in normal neonates. An arterial spin labeling MRI study performed by Nagaraj et al100 showed a significant reduction in global cerebral blood flow in newborns with complex CHD; these findings are similar to those previous study with a small cohort of term infants with CHD.101 In addition, this group identified specific brain regions with less mean cerebral blood flow in cyanotic compared with acyanotic CHD: occipital WM, basal ganglia, and thalami. Future studies aimed at improving fetal cerebral oxygenation in CHD, perhaps via maternal oxygen therapies, may foster healthy brain development, reduce brain injuries, and yield improved neurodevelopmental outcomes.

Perioperative Factors
Cardiopulmonary Bypass
Compared with other neurodevelopmental diseases, CHD requires major surgical procedures to correct the cardiac anomaly. These surgical procedures, such as CPB, introduce many critical factors that can impose a broad range of specific pathologies to the developing brain; such factors include, but are not limited to, dynamic changes in temperature, hemodilution, oxygenation, nonpulsatile blood flow, and low-flow perfusion or circulatory arrest under deep hypothermia (near 15°C). Therefore, early clinical trials and laboratory research studies, including our own, were focused on newly acquired brain damage during or after CPB and corrective and palliative CHD surgeries. Successful clinical trials have greatly improved our understanding of brain injuries acquired from surgical procedures and have resulted in implementation of different strategies to mitigate surgery-induced brain injuries.

In the 1980s to 1990s, infants with d-transposition of the great arteries undergoing open heart surgery with DHCA displayed larger brain perturbations shortly after surgery when compared with those who underwent low-flow cerebral perfusion.102 Follow-up studies reported that infants assigned to the DHCA strategy were at a higher risk of neurological abnormalities at 1 and 4 years of age102,103; poorer
outcomes were associated with longer durations of DHCA. Neurodevelopmental outcomes were later assessed at 8 years of age, and it was determined that a DHCA duration time of >41 minutes resulted in worse neurodevelopmental outcomes and that the relationship is nonlinear. At 16 years of age, the effects of DHCA on neurological scores were modest; however, both treatment groups typically performed lower than the normative population. These studies indicate that infants undergoing CHD surgery are at risk of neurodevelopmental problems and should be continuously monitored to determine the need for early intervention and individual education plans during school age years.

Another clinical trial addressed the effects of blood gas management—α-stat versus pH-stat—strategies implemented on a heterogeneous cohort of infants with CHD during DHCA. This study found that the pH-stat strategy was associated with better brain recovery time along with a reduction in postoperative morbidity; however, neither strategy was shown to have an impact of neurodevelopmental outcomes assessed by formal testing. Hematocrit levels during CPB are also of great importance. A series of clinical trials found that hemodilution to hematocrit levels <24% during neonatal CHD surgery are associated with worse Psychomotor Development Index scores at 1 year of age. The successful trials described above have greatly improved CPB management worldwide to minimize brain injury acquired during surgery in infants with CHD.

Neurodevelopmental outcomes in CHD have also been assessed in clinical trials comparing perioperative glycemic control and perfusion strategies. A recent clinical trial reported that tight glycemic control in infants who underwent CPB surgery was shown to have no impact on neurodevelopmental outcomes at 1 year of age compared with standard care. In addition, tight glycemic control did not significantly alter mortality or infection rates nor length of hospital stay; however, infection rates were significantly reduced in infants older than 60 days of age compared with those in the standard care group.

Infants with SV anatomy who underwent the Norwood operation with either regional cerebral perfusion or DHCA were found to have no significant differences in mental or psychomotor development scores during their 1-year follow-up. Neonates undergoing CPB for aortic arch repair with either antegrade cerebral perfusion or DHCA had similar cognitive and motor outcomes around 2 years of age. MRI was performed preoperatively and 1 week after surgery and revealed no significant differences in perioperative cerebral injury between perfusion strategies; however, focal infarctions within the basal ganglia or thalamus occurred exclusively in neonates who underwent CPB with antegrade cerebral perfusion. Preoperative brain injury was identified in half of the patients, and new WM injury was evident in over 60% postoperatively (72% of DHCA and 50% of antegrade cerebral perfusion). In addition, lower postoperative mean arterial PCO2 values were associated with newly acquired WM injury regardless of perfusion strategy.

Results from recent clinical outcome studies demonstrate that with the use of modern, improved CPB strategies, patient-related factors are better predictors of neurodevelopmental disabilities than intraoperative factors such as duration of DHCA or shunt type at 1, 3, and 16 years of age. Patient-related risk factors identified at 14 months of age after cardiac surgery include lower weight at birth, male sex, and extracardiac anomalies. Importantly, adjustments made for these factors led to improved psychomotor and mental developmental scores by a modest degree over time. Clinical studies have also demonstrated that newly acquired postoperative brain injury is commonly found in the immature brain with CHD. Therefore, new approaches need to be developed to protect underdeveloped brains that are uniquely susceptible to peri- and postoperative brain insults.

Environmental factors introduced during CPB that can be harmful to the brain still include the inflammatory response, reoxygenation, reperfusion, and rapid changes in body temperature. Future studies in animal models aimed at determining the cellular response to such factors during and after surgery will be invaluable in understanding peri- and postoperative brain injury and subsequent neurodevelopmental sequelae. Understanding the molecular and cellular responses to cardiac surgery in a highly vulnerable, underdeveloped brain will aid in further refinement of CPB strategies/management in pediatric cardiac surgery and will likely result in improved neurodevelopmental outcomes.

Anesthesia

The potential deleterious effects of anesthesia exposure on the developing brain have recently come into focus. Palliative or corrective surgeries performed early in life are typically not optional, and infants born with complex CHD often require major surgery before their sixth month of life. Neuroimaging studies have identified the risk of newly acquired brain injuries after cardiac surgery, and anesthesia may be a contributing factor to such brain injuries. Although the effects of exposure to small amounts of anesthesia on early brain development remain controversial, certain surgical procedures for complex CHD require an extensive quantity of anesthetics for several hours or even days—if required during the recovery period.

A recent clinical study demonstrated a strong link between anesthetic exposure and neurodevelopmental outcomes: patients with HLHS who underwent a higher cumulative exposure to volatile anesthetics had lower full-scale and verbal IQ scores at 4 to 5 years of age. Another study identified an association between high levels of volatile anesthetics during complex neonatal cardiac surgery and worse neurodevelopmental outcomes at 1 year of age. Although it may not be possible to control for every variable, future prospective and retrospective clinical trials are necessary to elucidate the effects of each anesthetic agent on brain development and subsequent neurological outcomes.

A majority of the evidence that suggests repeated or chronic exposure to general anesthetics can result in permanent brain damage comes from several animal studies across many different species. For example, rhesus monkeys undergoing a 5-hour exposure to isoflurane at 6 days of life displayed a 13-fold increase in neuronal cell death, particularly in the cerebral cortex; in addition, oligodendrocytes engaged in myelination were also vulnerable to anesthetic exposure.
A recent study demonstrated ketamine-induced learning deficits in GCaMP (high-affinity Ca2+) probe composed of a single GFP [green fluorescent protein] mice.124 These mice express a calcium indicator (GCaMP) in cortical neurons enabling live monitoring of neuronal activity in awake mice. Early postnatal mice received 3 doses of anesthesia (eg, ketamine), and neuronal activity was greatly decreased in the motor cortex during the recovery period. At 1 and 2 months of age, animals that had received anesthesia performed significantly worse on motor learning tasks. These deleterious effects were mitigated by administration of a drug that increases AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor activity during the postanesthesia recovery period; the AMPA receptor binds glutamate and is important in neuronal communication. Future translational studies defining the cellular/molecular mechanisms underlying the adverse effects of anesthetic agents on early brain development will be extremely useful in developing pharmacological treatment strategies for neonatal cardiac surgery.

Postoperative Factors

Low Cardiac Output and Length of Hospital Stay

Of the multiple postoperative factors associated with worsened neurodevelopmental outcomes, the impact of low cardiac output has been well studied. Postoperative brain injury correlates with low blood pressure and low cardiac output early during the recovery period. Low mean blood pressure is associated with newly acquired WM injury during the first postoperative day.8 Within 2 weeks after surgery, diastolic hypotension was associated with PVL in neonates who underwent CPB.55 In addition, low postoperative cerebral oximetry for a prolonged period (>3 hours) is associated with a high incidence of newly acquired or worsened focal ischemic lesions in infants who underwent the Norwood procedure.125 In a small cohort of children born with HLHS who underwent neonatal cardiac surgery, neurodevelopmental abnormalities were associated with reduced systemic venous oxygen saturation postoperatively.79 Postoperative low cardiac output after heart surgery for CHD in infancy has also been shown to be associated with a worse quality of life at 4 years of age.126 Low cardiac output is often associated with a more complicated postoperative course, and prolonged hospital stay and postoperative mechanical ventilation are independent risk factors related to adverse neurodevelopmental outcomes.

 Longer stays in intensive care units have been associated with (1) lower psychomotor and mental scores, (2) gross and fine motor delays, and (3) lower full-scale and verbal IQs in children who underwent corrective or palliative surgery for CHD.127–129 Increased hospital stay is necessary to stabilize hemodynamics and often involves lengthy exposure to sedatives which may also further impact neurodevelopment.130 A recent study assessed operative and postoperative factors associated with poor neurodevelopmental outcomes at 13 months of age after cardiac surgery in infancy.131 Both a longer operative support time and postoperative length of stay were associated with adverse neurological outcomes.131 Altogether, these observations suggest a cumulative impact of intraoperative factors predisposing the brain to newly acquired postoperative injuries.

Protracted hospital stay also exacerbates maternal and family stress and can result in an unhealthy parental–child relationship after hospital discharge because the family experiences challenges accommodating their child’s medical needs and neurodevelopmental deficits; in addition, maternal angst has a negative impact on child behavior which in turn increases maternal stress.130

Socioeconomic Status

SES, which encompasses parental income, education and care, and environmental stimuli, is highly predictive of language processing and executive function abilities throughout an individual’s lifespan.132 Children and adolescents raised in a low SES household develop a spectrum of neurodevelopmental deficits which positively correlate with the duration of the imposed hardships. In addition, pregnant women with a low SES are more likely to be stressed and malnourished and have a higher risk of perturbed fetal growth and premature delivery.133 Clinical studies have documented an association between lower SES and worse short- and long-term neurodevelopmental outcomes after corrective surgery for CHD.5,114,134 It is well known that SES is associated with neurological outcomes in CHD. Social class is typically adjusted for in statistical analyses.

Although several modifiable and unmodifiable risk factors associated with worse neurodevelopmental outcomes in CHD have been identified, almost nothing is known about their direct impact on individual cell populations which are critical for brain growth and function. To understand the cellular and molecular response to these risk factors, it is essential to develop and use suitable animal models.

Animal Models in CHD

Several animal models have been developed to understand the cause of structural cardiac anomalies seen in CHD. These models vary in cost, amenability to genetic and molecular tools, and translational use. To date, animal models of CHD have primarily focused on determining the genetic, environmental, and perioperative factors underlying abnormal heart development and function. Because little attention has been given to how these congenital cardiac anomalies impact brain development and neurological outcome, we will mainly focus our discussion on such models.

Zebrafish and mouse models of CHD have provided substantial insights into the genetic programs involved in heart development.135 Although zebrafish have a 2-chambered heart which is not ideal for studying many cases of CHD, CHD associated with heterotaxy, which is often seen in complex CHD, can be easily assayed. The zebrafish is a powerful model organism for several reasons: (1) transparent embryos develop quickly ex utero facilitating longitudinal live imaging with microscopy throughout heart development, (2) inexpensive and large numbers of subjects, and (3) ease of high-throughput screening paired with sophisticated genetic approaches. Zebrafish as a model of brain development have rapidly increased in popularity over the past 2 decades and are now being used in a wide variety of studies modeling
cognitive disorders, emotional and behavioral motivation, myelination, regeneration, autism, and neuropharmacological screening.136–139

Recently, it was shown that the deletion of a specific gene (DNAH6), which was recovered from CHD patients with heterotaxy, results in heterotaxy and ciliary dysfunction in zebrafish; zebrafish without this gene also developed abnormal cardiac looping.140 Primary cilia are important for normal brain development.141 Studies such as this will facilitate our understanding of the interactions between genes associated with abnormal heart development and neurodevelopmental disorders. The effects of alterations of specific conserved genes on heart and brain development in zebrafish can be quickly determined and translated to mammalian models such as mice, which is an essential next step because of notable dissimilarities between zebrafish and humans, including structural anatomy as well as brain and heart circulation.

A large-scale recessive forward genetic screen was recently conducted on mouse fetuses to recover mutations causing CHD.142 More than 87,000 chemically mutagenized mouse fetuses were scanned with ultrasound, and over 200 CHD mouse models were recovered. Of the 61 mutated genes identified, more than half were cilia related; in addition, identified pathways showed overlap with candidate genes recovered in CHD patients. Interestingly, several of the recovered genes have known roles in key neural development processes; axon pathfinding was the top pathway identified in gene enrichment analysis. Hence, alterations in genes involved in both heart and brain development are a likely cause of CHD and neurodevelopmental abnormalities.

Mice possess a 4-chambered heart, and mouse genetic models of CHD have identified many genes critical for normal heart development.59 This strategy, however, often results in severe disturbances of vital organs and embryonic lethality. Because WM development occurs postnatally in rodents,143,144 a majority of the CHD genetic mouse models are not suitable for studying the structural-functional effects of congenital cardiac anomalies on brain development. To understand how alterations in fetal and perinatal blood flow affect normal brain development, a genetic animal model of CHD with embryonic/postnatal viability will be instrumental; however, models of complex CHD will preclude long-term postnatal survival because of technical challenges in palliative surgery on such small mammals. On the other hand, many essential stages of cortical development occur before birth in mice; therefore, future studies in such genetic models in which embryonic lethality occurs closer to term birth should be considered in this context.

Although rodent models of CHD have several advantages and offer the promise of determining effects of preoperative factors, such as altered cerebral blood flow, they are not an ideal model for studying operative factors associated with neurodevelopmental outcomes in CHD because of their small size. In addition, there are several structural and cellular dissimilarities between the rodent and human brain. Large animals, including ovine and swine, share similar brain developmental profiles to humans and are large enough to undergo surgical strategies routinely used in infants with CHD145–150; hence, these animal models are suitable for determining preoperative and perioperative factors underlying neurodevelopmental abnormalities commonly seen in CHD. However, these animal models are expensive and require a skilled team of surgeons to perform complicated surgery and special postoperative care and housing.

The fetal sheep bilateral carotid artery occlusion model involves in utero cerebral hypoperfusion and has been used to induce cerebral ischemia.151,152 Bilateral carotid artery occlusion, paired with anastomoses of the vertebral arteries, midgestation has been demonstrated to result in perturbations in postnatal cortical growth.152 Lambs that underwent this procedure displayed reduced cortical volume, similar neuronal cell densities, an increase in total number of neurons, and reduced neuronal dendritic arborization and synapse formation in the cortex which correlated with microstructural alterations observed by DTI.153 In addition, another study demonstrated arrested preoligodendrocyte—oligodendrocytes at a stage between OPC and differentiated oligodendrocyte—maturation and reduced numbers of preoligodendrocytes which strongly correlated with their MRI findings of WM injury in an ovine model of cerebral ischemia.154 These fetal/preoperative findings are of great importance because they recapitulate certain brain injuries seen by neuroimaging in CHD patients and provide information on the cellular response to such insults.

Swine have served as a standard model system for open heart surgery for many years but have only recently been used as a model for brain development. In addition to their docility making them easy to manage, swine are metabolically similar to humans and display a similar area-dependent WM maturation profile with their human counterpart.145 Environmental events during CPB include the inflammatory response, reoxygenation, and rapid changes in temperature. We recently assessed the intraoperative effects on WM injury in neonatal piglets undergoing CPB during early stages of brain development.145 Piglets that underwent CPB surgery had significantly worse neurological outcomes. Immature oligodendrocytes (eg, preoligodendrocytes) were particularly vulnerable to ischemia–reperfusion and reoxygenation injury during the CPB procedure (Figure 4A through 4E).146 OPCs have the capacity to replace lost oligodendrocytes and displayed remarkable resilience to CPB; however, delayed myelination and an arrest of oligodendrocyte maturation were seen 1 month after CPB (Figure 4F, 4G, 4J, and 4K). In addition, evidence of inflammation was seen as there was a significant increase in the number of microglia within the WM shortly after CPB (Figure 4H and 4L).155 Maintaining high levels of oxygen and reducing inflammation were found to be protective of WM injury;145 importantly, these perioperative factors are easily modifiable. Future studies in which the compound effects of open heart surgery alone and paired with previous cerebral hypoxic exposure should be undertaken in swine to better assess the impact of pre- and perioperative brain injury seen in CHD.

A series of rodent studies using a brain slice model were recently published and document the impact of in utero hypoxia and CPB on WM.156,157 During the developmental equivalent of the third trimester through term birth in humans, mice were reared in a hypoxic environment with a foster mother. A perfusion system was developed to culture brain slices in
Cerebrospinal fluid with and without oxygen–glucose deprivation (OGD) to simulate cardiac arrest during CPB. As seen in the swine CPB model, OPCs were resistant, whereas preoligodendrocytes were vulnerable to OGD in brains from mice raised in normal conditions. However, hypoxic exposure before OGD resulted in a temperature-specific shift in WM injury; mature oligodendrocytes and OPCs, as opposed to preoligodendrocytes, were most vulnerable to OGD.154 Interestingly, a reduction in temperature was specifically protective of OPCs, suggesting that deep hypothermia during bypass surgery may be beneficial for certain cell populations in the oligodendrocyte lineage.154 A recent follow-up study...
using this model system demonstrated an astrocyte response to OGD in mice reared under normoxic conditions (Figure 4I and 4M). In addition, lack of an astrocyte response was seen in WM in brains that underwent previous hypoxic exposure, suggesting that preoperative cerebral hypoxia may dampen the neuroprotective potential of astrocytes in CPB.\textsuperscript{156}

The relationship between OPCs and vasculature during brain development has recently been addressed. With live imaging microscopy, OPCs were shown to migrate along blood vessels in mice during normal embryonic cortical development; in addition, the researchers demonstrated that OPCs have a similar association with blood vessels during their migration to the cortex in human brains during the 14th week of gestation.\textsuperscript{157} The authors propose that the interaction between OPCs and vascular endothelium allows for the signaling required to coordinate and guide OPC migration to their appropriate cortical destinations. In addition, it was recently demonstrated in mice that OPCs are critical in coordinating blood vessel growth in the developing WM via hypoxia-inducible factor signaling.\textsuperscript{158} Endogenous hypoxia-inducible factor signaling in OPCs promoted angiogenesis while preventing OPC differentiation before myelination. In the presence of oxygen provided by newly formed blood vessels in the WM, hypoxia-inducible factor activity is inhibited and promoted oligodendrocyte maturation and myelination. Membrane expansion during the process of myelination is a metabolically taxing process, and direct access to vital sources of oxygen and substrates delivered by blood vessels seems to be essential. Because complex CHD often alters cerebral blood supply, it will be important to determine the integrity of the vasculature and the interactions between blood vessels and OPCs during brain development.

Rodent studies modeling diffuse WM brain injury in premature birth have identified cellular and molecular mechanisms underlying lineage-specific vulnerabilities of oligodendrocytes and their regenerative response after chronic neonatal hypoxia. The subventricular zone and WM itself represent 2 major sources of OPCs with regenerative potential. Oligodendrocyte death, delayed oligodendrocyte maturation, and transient hypomyelination in the subcortical WM were seen within 2 weeks in mice after chronic neonatal hypoxic exposure.\textsuperscript{159} In addition, an endogenous regenerative response was seen as OPCs persistently generated new oligodendrocytes for ≤1 month after hypoxic exposure which ultimately resulted in restoration of myelin proteins.\textsuperscript{160} These studies also identified alterations in OPC cell cycle proteins in the WM after hypoxia and in postmortem analyses of the corpus callosum of human infants dying from hypoxic/ischemic encephalopathy.\textsuperscript{159} Many mitogenic proteins involved in the OPC regenerative response, such as epidermal growth factor and apotransferrin, can be genetically altered or stimulated pharmacologically to potentiate OPC survival, proliferation, and maturation to promote recovery of WM injury.

One promising approach that can easily be implemented in the hospital/clinical setting is intranasal drug administration. Repeated intranasal delivery of heparin-binding epidermal growth factor immediately after hypoxic exposure led to reduced oligodendrocyte death, enhanced oligodendrocyte genesis, and improved behavioral recovery in mice.\textsuperscript{160} Intranasal administration of human apotransferrin before hypoxia–ischemia resulted in less WM injury and an increase in OPC survival and proliferation within the corpus callosum and subventricular zone in mice.\textsuperscript{161} Together, these studies provide insights into the dynamic cellular and molecular mechanisms involved in WM injury and recovery imposed by chronic hypoxic exposure and hypoxia–ischemia and offer therapeutic avenues which should be explored in models of CHD-induced WM injury.

Technical and ethical limitations have restricted our abilities to determine the cellular and molecular mechanisms governing altered brain maturation and neurological sequelae in CHD. Because of the high prevalence of cortical and WM dysmaturation seen in complex CHD, it is essential to develop animal models that recapitulate the neurological outcomes and the structural alterations of the developing brain to identify cell-specific vulnerabilities and regenerative potential in response to CHD-induced brain pathologies. The bidirectionality between clinical and animal studies will be fruitful in testing novel treatment strategies to protect the immature developing brain and improve neurological outcomes in the growing CHD population.

**Ongoing Clinical Trials and Recent Advances in Technology**

Several clinical trials have identified modifiable intra- and perioperative risk factors associated with neurodevelopmental outcomes in CHD. These findings have led to refined surgical strategies resulting in decreased morbidity and mortality. However, few studies have assessed potential pharmacological interventions. Recent results were documented from a clinical trial testing the neuroprotective effects of erythropoietin in neonatal cardiac surgery.\textsuperscript{162} Although the doses of erythropoietin administered were found to be safe, there were no significant improvements in neurodevelopmental outcomes, including cognitive, language, and motor skills at 1 year of life.\textsuperscript{162} However, this study was on a small cohort of patients and will require a larger sample size to determine the impact of erythropoietin on neurodevelopmental outcomes in CHD after neonatal corrective surgery.

Three interventional clinical trials—pre-, peri-, and postoperative—were recently launched and may result in improved neurodevelopmental outcomes. An interventional clinical trial has been initiated to test the potential neuroprotective effects of maternal progesterone therapy during fetal development (https://clinicaltrials.gov/ct2/show/NCT02133573). A phase I drug safety study has been initiated to determine the safety of the anesthetic agent dexmedetomidine during CPB (https://clinicaltrials.gov/ct2/show/NCT01915277). An interventional clinical pilot study was recently launched aimed at improving executive dysfunction in adolescents born with cyanotic CHD and surviving corrective surgery within the first year of life (https://clinicaltrials.gov/ct2/show/NCT02759263).

Innovative approaches developed by surgeons and cardiologists along with devoted patients and families have led to a significant reduction in surgical mortality of complex CHD cases. It is important to consider the difficulty in acquiring
large homogeneous sample sizes and controlling for individual patient variables when conducting a clinical trial. Therefore, even if results do not reach significance, trends toward improvements should be noted. These trends can be greatly informative and may merit further investigation in animal models where it is feasible to control for confounding variables.

Recent advances in stem cell technology have opened new opportunities to model human brain development in vitro on an individual basis. Cerebral organoids are 3-dimensional brain structures that can be generated from inducible pluripotent stem cells to understand brain development and disease. Cerebral organoids have been shown to display key features of human cortical development; in addition, this approach has been used to grow microcephalic brains in vitro from inducible pluripotent stem cells derived from human patients with microcephaly. This powerful technique can be used to understand altered cortical development in CHD in a high-throughput manner by generating and studying organoids from fetuses or neonates; it is important to note that this in vitro approach is highly amenable to several genetic and molecular techniques that are not feasible in living humans.

**Future Prospects**

Owing to advances in surgical strategies and hospital care, adults currently represent the majority of the CHD population, and there is evidence of a shift from neurodevelopmental delays to cognitive decline or dementia later in adult life. Clinical and research studies have identified many risk factors associated with worse neurodevelopmental outcomes in CHD; however, direct links between single-specific factors and neurological deficits remain to be established. This is partly because of the technical and ethical limitations imposed on human studies and the lack of animal models that completely recapitulate the neurodevelopmental outcomes in CHD.

It is important to note that clinical trials often include a heterogeneous cohort with various types of congenital cardiac anomalies. The Boston Circulatory Arrest trial is a stellar example of a well-designed study, with a homogenous CHD population and nearly 3 decades of neurodevelopmental follow-up assessments. It will be interesting to see the neurodevelopmental status of these patients as they age. Future prospective clinical studies should make efforts to enroll patients with the same type of CHD and include careful, longitudinal surveillance of neurodevelopmental sequelae as performed in the SV reconstruction trial.

Several genetic studies in zebrafish and rodents have identified genes involved in structural congenital cardiac anomalies but have not thoroughly assessed these effects on brain development and function. Because there are hundreds of known genes associated with CHD, it will be essential to generate conditional genetic mouse models in which certain genes are removed in specific cell types at defined ages. Ideally, such studies would also incorporate tools and metrics used in the clinic, such as MRI/DTI and neurological evaluation, to validate the neuropathological brain signatures and neurocognitive impairments documented in CHD patients.

Animals such as pigs and sheep are amenable to the surgical procedures used on human neonates and infants with CHD; in addition, their brains share many anatomic similarities with humans, such as WM developmental profile and a gyrencephalic cortex. Because of metabolic and physiological similarities they can also serve as valuable intermediaries to pharmacological treatments before potential phase I clinical trials. In addition, recent advances in genetics offer new tools, such as CRISPR/Cas9, capable of modifying genes in large animals.

Integrative approaches involving genetics, cell biology, and molecular biology to model brain development in CHD will unarguably play a key role in defining the underlying causes of brain dysmaturation and optimal windows for treatment to improve neurodevelopmental outcomes in CHD. Ultimately, a strong synergy between researchers and clinicians will enhance the bidirectional nature of animal and human studies and facilitate developing translational platforms/programs to improve neurological outcomes in children with CHD.

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