Are isolated heart defects really isolated? A prenatal view on submicroscopic genetics and brain development

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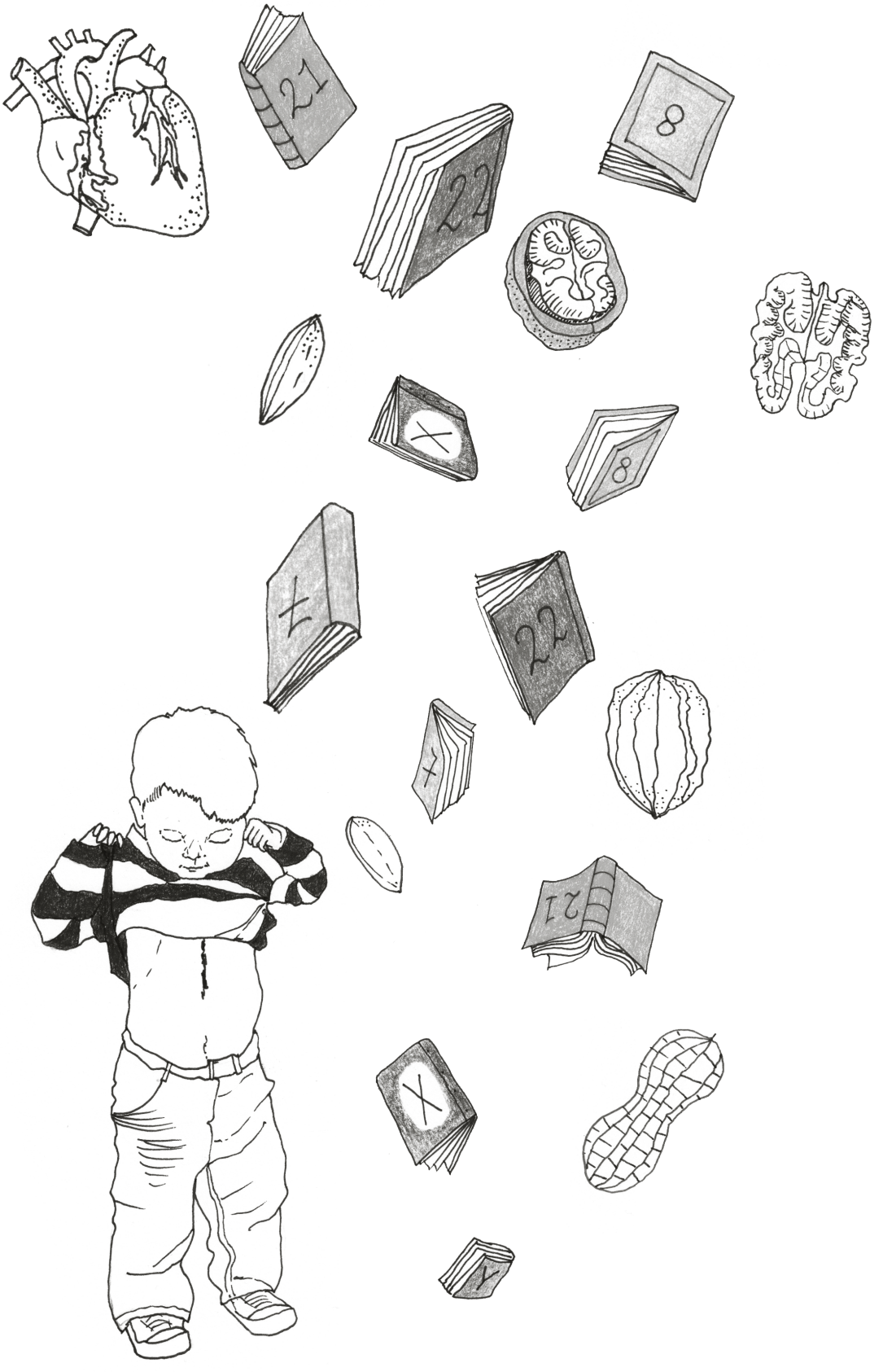
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CHAPTER 1

Introduction and outline of this thesis

Congenital heart defects (CHD) are the most common of all congenital defects and affect approximately 7-9 per 1000 live born children[1]. The collective term CHD is used for a combined group of different cardiac lesions that can be anatomically heterogeneous. CHD contribute significantly to neonatal mortality, causing a higher infant death rate than chromosomal aberrations, sudden infant death syndrome or accidents. In the Netherlands, approximately half to 75% of all CHD are detected prenatally, as shown in a large cohort from 2002-2012[2;3]. Future parents are increasingly confronted with a (suspected) fetal CHD, since the 20 weeks anomaly scan was introduced in 2007. The four-chamber view and outflow tracts of the fetal heart are systematically evaluated in this scan to detect abnormalities. The defects that are most frequently detected prenatally are *severe* CHD; severe is internationally defined as requiring percutaneous intervention or surgery in the first year of life. Examples are hypoplastic left heart syndrome (HLHS; figure A), transposition of the great arteries (TGA; figure B), double outlet right ventricle (DORV) and Tetralogy of Fallot (TOF; figure C), but the spectrum of (severe) CHD is very wide. In general, a prenatal diagnosis of CHD means that future parents can anticipate, but more importantly, that adequate medical measures can be taken once the baby is born. Especially in cases of TGA and HLHS it has been proven that prenatal detection is associated with lower postnatal mortality and morbidity[4]. A prenatal diagnosis of severe CHD might also prevent neurological injury due to acute severe hypoxia after birth in undetected cases[5].

COUNSELING

In the prenatal setting, the estimation of the severity and impact of the CHD is based on the nature of the defect and the presence of associated extracardiac and/or chromosomal abnormalities. Future parents receive comprehensive tailored counseling, including what can be expected postnatally with respect to surgery, survival chances, admission to the neonatal intensive care unit, administered medication and the possible necessity of emergency interventions like balloon atrial septostomy. Of course, there are levels of uncertainty in this prognosis, as the postnatal course can vary from case to case. In some cases parents opt for termination of pregnancy, which is chosen in approximately 25% of prenatally detected *severe* CHD. Termination of pregnancy is performed most frequently when there is a suspicion of a coinciding syndromic abnormality or when the CHD is in the severest spectrum such as HLHS and other univentricular heart defects. Approximately 5% of CHD will lead to intrauterine death, which is sometimes the result of cardiac decompensation with hydrops, but it can also occur unexpectedly[2]. Depending on the severity of the CHD, postnatal death in the first year of life occurs in approximately 6-20% of severe CHD[6]. The mortality rates depend on the type of CHD;

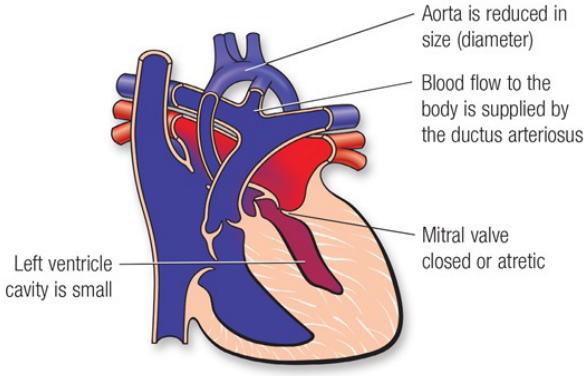

Figure A: hypoplastic left heart syndrome

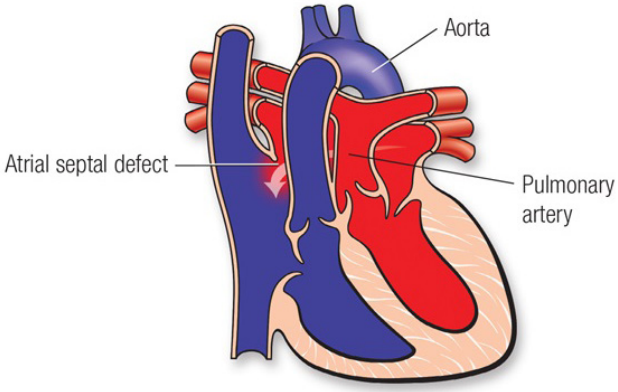

Figure B: transposition of the great arteries

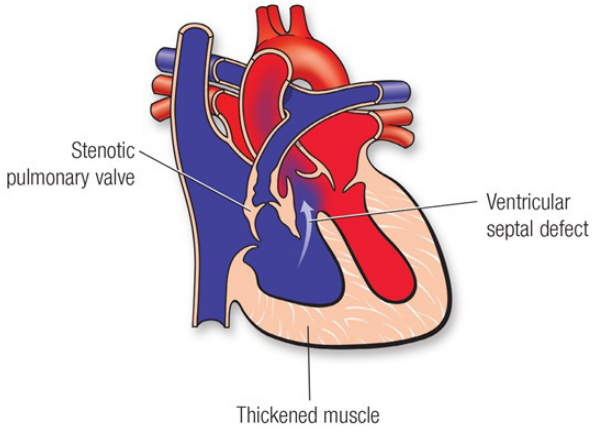

Figure C: tetralogy of Fallot

Figures A-C are reprinted from the American Heart Association

in HLHS the highest mortality occurs. When a child with HLHS survives pregnancy and the first week of life to undergo the first stage of a three-step surgical repair process, approximately 70% will be alive at the age of 1 year old[7]. The long-term outcome of CHD is partly uncertain, because cardio-surgical techniques continue to evolve. In the last decades there has been a trend of improving survival of children with severe CHD. The focus for innovation in the care for infants with CHD is therefore shifting to quality of life, neurodevelopment and (cardiovascular) complications at adolescence or older age.

ISOLATED VERSUS SYNDROMIC

Based on postnatal studies it is know that in approximately 30% the CHD is part of a genetic syndrome[8]. The most common genetic abnormalities associated with CHD are Down syndrome (trisomy 21) and Di George syndrome (22q11 microdeletion). However, many more genetic abnormalities are associated with CHD, such as other aneuploidies like Turner syndrome (monosomy X), microdeletion/-duplication syndromes like Williams-Beuren, and monogenetic syndromes like Noonan, Kabuki, Holt-Oram and CHARGE. To illustrate the magnitude and the diversity of the subject, the scientific statement "the genetic basis of CHD" issued by the American Heart Association and updated in 2018 comprises as many as 60 pages. In cases with genetic syndromes usually extracardiac abnormalities are present, such as renal dysplasia, hemivertebra, abnormal extremities, dysmorphic facial features and neurodevelopmental (ND) delay. If a fetal CHD is accompanied by a genetic abnormality, an additional extracardiac abnormality is seen on prenatal ultrasound in approximately 60-65% of cases[2]. In CHD children without genetic abnormality, however, extracardiac abnormalities can also be present, as Egbe reported 11% additional abnormalities in children with CHD born without a genetic syndrome[9]. In the antenatal phase, the presence of additional extracardiac abnormalities is therefore highly suspicious for genetic syndromes, but not pathognomic. It is important to realize that prenatal ultrasound cannot detect (mild) dysmorphic facial features or neurological developmental disorders, and genetic syndromes can still be present in the absence of extracardiac abnormalities.

The presence of a genetic syndrome influences the postnatal prognosis. For example, in children with 22q11 microdeletion syndrome perioperative complication, such as airway problems and infections, are more common and long-term survival is lower[10;11]. Thus, genetic testing is important in the prenatal phase, to be able to provide proper risk estimation to the parents.

There are several diagnostic genetic tests available, with varying resolutions and detection rates. The traditional karyogram is suitable to detect aneuploidies (trisomy/monosomy) and large deletions or duplications (size 5-10.000.000 base pairs). Until a few years back, karyotyping was the only option for prenatal genetic analysis. However, the molecular cytogenetic tests available nowadays enable us to study the genome in a higher resolution: array comparative genomic hybridization (array CGH, also known as micro-array) has evolved from bacterial artificial chromosome (BAC) array (resolution 1.000.000 base pairs), to oligonucleotide (resolution 30-50 base pairs) to even single nucleotide polymorphisms (SNP) array and next generation, whole exome or whole genome sequencing (NGS/WES/WGS) (resolution 1 base pair). These new tests can yield many copy number variants (CNVs) or point mutations, enabling accurate and comprehensive diagnosis of known syndromes and diseases such as Di George or Noonan syndrome. However, these more detailed genetic examinations have certain disadvantages as well. CNVs or smaller mutations also frequently occur in apparently healthy individuals, and some are considered 'variants of unknown significance' (VUS), indicating that its pathogenicity is unknown. This complicates the interpretation of the found genetic profile and makes counseling difficult, especially in the prenatal phase in which the phenotype can be incomplete. Counseling by a clinical geneticist is therefore of great importance in the event of an abnormal genetic profile - or any suspected syndromic disorder based on extracardiac abnormalities - because different syndromes may exhibit different penetrance, phenotypes and neurological development.

Currently, most prenatal centers in the Netherlands offer micro-array with a (reported) resolution up to 150.000 base pair. Whole exome sequencing (WES) is possible and performed incidentally, because the time window to generate and interpret the results has been reduced drastically the last few years, but it is not routinely performed prenatally yet. This is changing rapidly with the evolution of new laboratory techniques and the accelerating amount of knowledge and experience that has been acquired. Targeted testing for point mutations such as Noonan syndrome or CHARGE is possible, but rarely done because specific signs of these syndromes often lack prenatally.

NEUROLOGICAL OUTCOME

With the improving survival of children with CHD, the focus of research has shifted to the long-term and neurodevelopmental (ND) outcome. Even in the absence of chromosomal or syndromic abnormalities, ND delay may occur, especially in severe CHD cases. Large follow-up studies show that global ND delay occurs in 23% of children

with severe 'isolated' CHD, and 25% exhibit some kind of behavioral problem[12]. These ND impairments in CHD children are considered the result of cerebral injury, mostly sustained in the perioperative period. ND delay is associated with the cardiopulmonary bypass time, the type of surgery and the method of anesthesia. Two causative mechanism contributing to the cerebral injury are hypoxia and thrombo-embolic events in the brain[13]. The risk for ND delay can therefore roughly be predicted by the severity of the CHD and the complexity and number of the operation(s). Additional research, however, has shown that in certain patients some level of ND delay and cerebral 'damage' is already detectable *before* surgery, indicating that the ND impairments might have their origin in the prenatal or perinatal period[14]. This theory is supported by reports of small head circumference at birth (below the 10th percentile) in 25-36% of neonates with severe CHD. It was found that 40-55% of neonates show abnormalities at neurological examination (such as abnormal tonus, absent sucking reflex) prior to surgery[15]. Imaging studies also reported abnormalities before surgery, such as cerebral atrophy on ultrasound in 27% of the infants, and ischemic lesions on MRI in 21-41% of cases. These studies unfortunately rarely report whether the CHD was detected antenatally or not, and no studies are performed in *prenatally* detected CHD only. Nonetheless, one of the postulated theories is that altered blood flow in the heart and vessels in fetuses with severe CHD, results in a reduced amount of oxygen-rich blood in the fetal brain, resulting in chronic brain hypoxia[16]. See figure D for illustration. The assumption is that in the normal fetal circulation (left) the oxygen-rich blood from the inferior caval vein is shunted to the left side to reach the brain first. Subsequently, fetuses with TGA (second from the left) are hypothesized to receive blood with the lowest level of oxygenation in the brain, as result from the abnormal connection to the ventricles (the shunted blood reaches the pulmonary system first). Fetuses with univentricular heart defects (such as HLHS, third from the left)) or large ventricular defects (such as DORV and TOF, right) are considered to receive mixed oxygenated blood in the brain due to ventricular mixing of the shunted blood with the low-oxygen blood. In cases of reversed aortic arch flow (left ventricle obstruction such as HLHS) the restriction of flow to the carotid arteries would additionally lead to cerebral oxygen deficiency. As a result of this 'altered hemodynamics theory', it is hypothesized that the brain development and growth is restricted in CHD, which results in prenatally altered development of the brain or reduced head size, in some cases even fetal microcephaly.

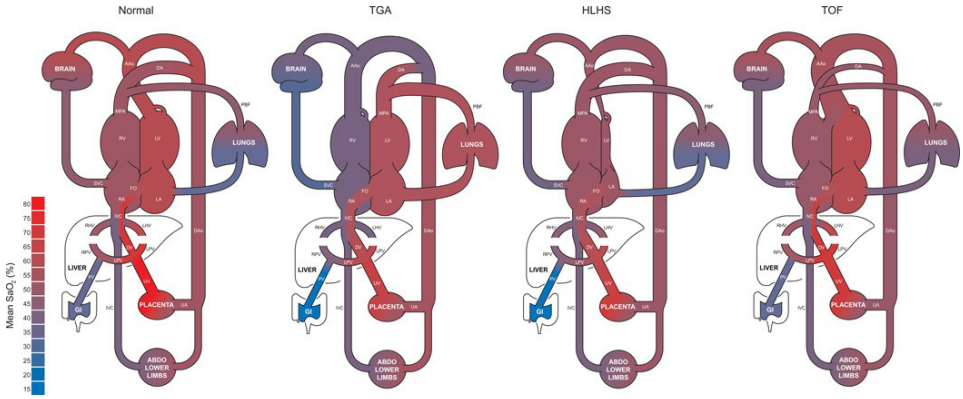

Figure D: Illustration of the altered hemodynamics theory in a normal heart, TGA, HLHS and TOF. Reprinted from Sun et al.[17]

Research in this area - altered cerebral development in fetal CHD - seems to be biased towards the severest types of CHD, consisting of inhomogeneous groups and varying definitions of heart defects, which makes comparison difficult. Also, the association of prenatal cerebral abnormalities and variations with long-term ND outcome is difficult to investigate and has rarely been reported on. ND outcome can only be assessed reliably once the child is 2-4 years old and is majorly influenced by perioperative factors. Also, there are many confounders like parental socio-economic status, need for additional cardiac interventions, etcetera, and cohorts would need to be immense to correct for this. Thus, in the absence of genetic syndromes, there are no known prenatal predictors for postnatal ND outcome.

It is known that parents of children with CHD worry about the (neurological) development of their child, and a vast amount of these children receive additional care such as remedial teaching, physical or logopedic therapy[12]. Future parents also worry about this, and usually ask the perinatologist and fetal cardiologist how his/her child will do in later life. Currently, the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG) advices to counsel future parents on this topic as follows[18]: `...the majority of fetuses/neonates with isolated CHD do well. However, there is evidence that some have a degree of ND delay, which cannot be predicted antenatally. The severity of this impairment varies from individual to individual, and the likely incidence varies with the type of CHD, being highest (up to 40–45% in some studies) in lesions with univentricular heart hemodynamics such as HLHS. We advise genetic investigations, including array-CGH to rule out associated and syndromic forms of CHD.'

OUTLINE OF THIS THESIS

CHD are associated with chromosomal and syndromic abnormalities, as well as ND delay. In the prenatal phase however, the prevalence of genetic syndromes or risks of being affected by cerebral maldevelopment is still largely unknown. In many cases, when a CHD *appears* to be isolated, it is often assumed to *be* isolated. The aim of this thesis was to explore whether prenatally *appearing* isolated CHD are *really* isolated – with*out* a genetic syndrome or a maldeveloped brain.

Part I explores the additional value of a array CGH and WES, two methods of genetic analysis with notable higher resolution than conventional karyotyping. In *chapter 2*, a systematic review and meta-analysis of the literature on array CGH in fetal CHD is presented. In *chapter 3* the additional value of array CGH is assessed in a subgroup of CHD, left sided CHD, historically assumed to have a low prevalence of syndromic anomalies - when Turner syndrome is excluded. *Chapter 4* describes an unusual case of a fetus with a small ventricular septal defect (VSD) and additional abnormalities. VSDs occur frequently and are usually innocent. However, in this case, additional abnormalities were found and indicated an underlying mitochondrial disease – identified with WES.

The second part of this thesis assesses prenatal and early postnatal brain development in CHD. In *chapter 5*, a systematic review and meta-analysis of prenatal cerebral development and cerebral variations in CHD is presented. *Chapter 6* shows the results of a retrospective analysis of head circumference growth in a large cohort of fetal CHD, with a referee commentary on our study by the reviewing editor of the journal in which the paper was published. *Chapters 7 and 8* describe analyses of a prospective cohort of isolated CHD cases compared to healthy controls, in which we performed extensive monthly neurosonography (the Heart And NeuroDevelopment (HAND) study). In *chapter 7* the results of the volume measurements of the brain between 18 and 32 weeks of gestation are shown, exploring the prenatal cerebral growth and evolution of the extracerebral fluid compartments. C*hapter 8* describes early postnatal cranial ultrasound findings and measurements, comparing prenatally detected CHD cases with healthy controls.

Chapter 9 presents a general discussion of the combined results of these studies, and *chapter 10* is a general summary.

Additional remarks on ethical and legal aspects

In the Netherlands, there are several laws and guidelines regulating medical research; the most important being the WMO ('law on medical research'). When fetal samples are taken - to analyze the genetic material for example - there might be left over material. This left over material can be used in medical research exempt from the WMO. Patients can object to the use in medical research when the sample is gathered, but they have to actively opt out themselves. This implicates that there is no need to request permission from the individual patients, as long as the patient has not objected, outcomes are not retraceable to individual patient data, and the additional analyses are performed on anonymized material. This was the case in *chapter 3*.

Observational studies with fetuses are prohibited by the 'embryowet' (2002). Therefore all fetal data analyzed in this thesis (*chapters 6, 7, 8*) were collected in the routine care for fetuses with CHD, and were therefore exempt from the WMO. The prospective inclusion of the healthy control group (*chapters 7, 8*) was only possible after a slight liberalization of the 'embryowet' in 2014, making it possible to perform observational studies on healthy fetuses after informed consent by the parents.

REFERENCES

1. Khoshnood, B., et al., *Recent decrease in the prevalence of congenital heart defects in Europe.* J Pediatr, 2013. **162**(1): p. 108-13 e2.
2. van Velzen, C.L., et al., *Prenatal detection of congenital heart disease--results of a national screening programme.* BJOG, 2016. **123**(3): p. 400-7.
3. Everwijn, S.M.P., et al., *The effect of the introduction of the three-vessel view on the detection rate of transposition of the great arteries and tetralogy of Fallot.* Prenat Diagn, 2018 Nov;**38**(12):951-957.
4. Van Velzen, C.L., et al., *Prenatal detection of transposition of the great arteries reduces mortality and morbidity.* Ultrasound Obstet. Gynecol, 2015 Mar;**45**(3):320-5
5. Peyvandi, S., et al., *Association of Prenatal Diagnosis of Critical Congenital Heart Disease With Postnatal Brain Development and the Risk of Brain Injury.* JAMA Pediatr, 2016. **170**(4): p. e154450.
6. Oster, M.E., et al., *Temporal trends in survival among infants with critical congenital heart defects.* Pediatrics, 2013. **131**(5): p. e1502-8.
7. Ohye, R.G., et al., *Comparison of shunt types in the Norwood procedure for single-ventricle lesions.* N Engl J Med, 2010. **362**(21): p. 1980-92.
8. Eskedal, L., et al., *A population-based study of extra-cardiac anomalies in children with congenital cardiac malformations.* Cardiol Young, 2004. **14**(6): p. 600-7.
9. Egbe, A., et al., *Prevalence of congenital anomalies in newborns with congenital heart disease diagnosis.* Ann Pediatr Cardiol, 2014. **7**(2): p. 86-91.
10. Simsic, J.M., et al., *Do neonates with genetic abnormalities have an increased morbidity and mortality following cardiac surgery?* Congenit. Heart Dis, 2009. **4**(3): p. 160-165.
11. Michielon, G., et al., *Impact of DEL22q11, trisomy 21, and other genetic syndromes on surgical outcome of conotruncal heart defects.* J. Thorac. Cardiovasc. Surg, 2009. **138**(3): p. 565-570.
12. Marino, B.S., et al., *Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.* Circulation, 2012. **126**(9): p. 1143-1172.
13. Andropoulos, D.B., et al., *Neurological monitoring for congenital heart surgery.* Anesth. Analg, 2004. **99**(5): p. 1365-1375.
14. Majnemer, A., et al., *A new look at outcomes of infants with congenital heart disease.* Pediatr. Neurol, 2009. **40**(3): p. 197-204.
15. Owen, M., et al., *Abnormal brain structure and function in newborns with complex congenital heart defects before open heart surgery: A review of the evidence.* Journal of Child Neurology, 2011. **26**(6): p. 743-755.
16. McQuillen, P.S., D.A. Goff, and D.J. Licht, *Effects of congenital heart disease on brain development.* Prog Pediatr Cardiol, 2010. **29**(2): p. 79-85.
17. Sun,L., et al., *Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.* Circulation. 2015 Apr 14. **131**(15): 1313–1323.
18. Paladini, D., et al., *ISUOG consensus statement on current understanding of the association of neurodevelopmental delay and congenital*

heart disease: impact on prenatal counseling. Ultrasound Obstet Gynecol, 2017. **49**(2): p. 287-288.