Imaging the prenatal brain in congenital heart defects

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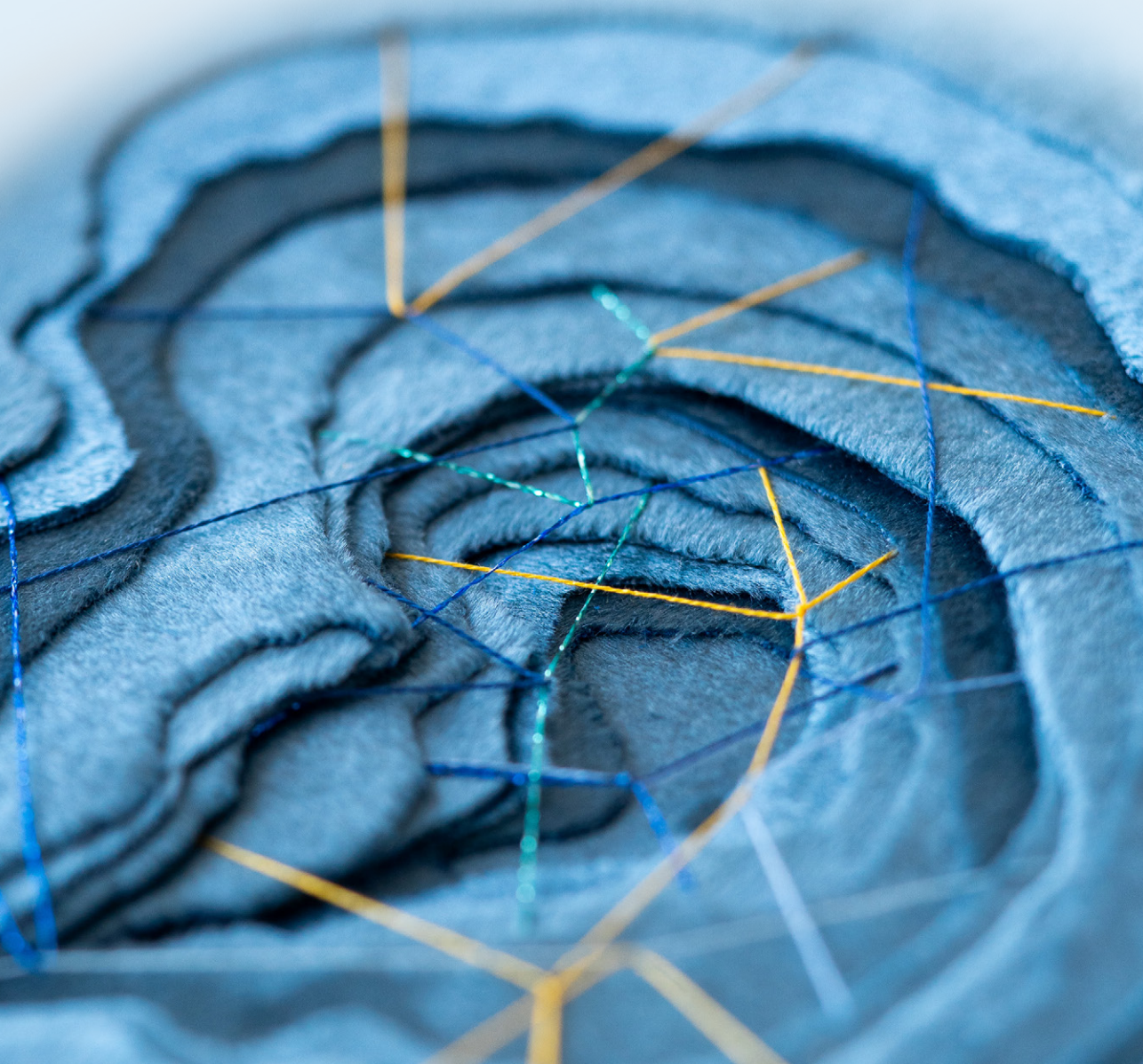
Voor mijn geliefden

Imaging the prenatal brain in congenital heart defects

CHAPTER

1

Introduction and outline



INTRODUCTION

Congenital heart defects (CHD) are the most prevalent congenital abnormalities. CHD has been defined as 'a gross structural abnormality of the heart or intra-thoracic great vessels that is actually or possibly of functional significance'[1]. About 25-30% of children that are diagnosed with CHD require surgery in the first year of life[2]. A prenatal diagnosis of CHD leads to optimized neonatal care, and is thought to be correlated to improved long-term outcome.[3] For some defects, interventions are required directly after birth, for example the administration of prostaglandins to keep the ductus arteriosus open, or a Rashkind procedure to sustain flow through the oval foramen.

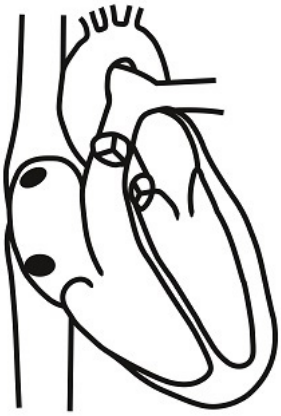
Thus, the aim of a screening program is to strive for high detection rates to optimize care for children with (cardiac) congenital defects. Previous studies in the Netherlands have shown a detection rate of 95% in defects that present with an abnormal aspect of the four-chamber view[4]. Defects that affect the out-flow tracts of the heart (conotruncal abnormalities) have a significantly lower detection percentages[5]. Detection rates differ significantly, even in developed countries. However, the detection rates in the Netherlands were already relatively good, and are rising in other countries as well[6].

PART I
Prenatal detection of CHD

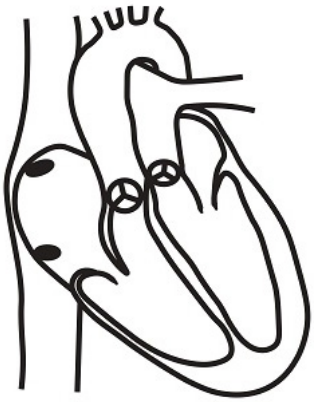
A prenatal diagnosis affects survival in neonates with critical CHD. For example, prenatally detected neonates with transposition of the great arteries (TGA) showed no mortality as compared to 12% preoperative mortality in undiagnosed cases[7]. Also, a timely diagnosis reduces morbidity such as brain and kidney damage, as a child can receive optimal peripartum care[8]. In non-critical CHD, like the majority of tetralogy of Fallot (TOF)) cases, a prenatal diagnosis does not prevent immediate postnatal deterioration, but gives the opportunity to diagnose genetic syndromes before birth. Defects like TOF carry a relatively high risk for a genetic syndrome, which may significantly alter the long-term outcome of the child. If diagnosed in time, this may enable reproductive choices for the parents.

Both TGA and Fallot are examples of CHD that usually present with a normal four-chamber view on ultrasound and detection of these abnormalities is perceived as more difficult by ultrasonographers. Recently, a study by van Nisselrooij et al[9] showed no significant differences in patient-related factors such as fetal position, obstetric history, maternal age or gestational age at examination when images of missed CHD cases were compared to detected cases. Furthermore, this study

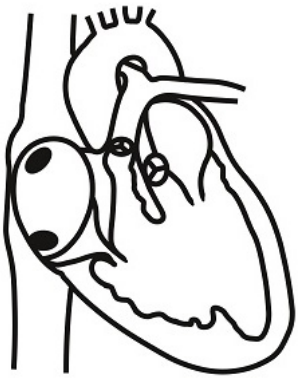
showed that cardiac examinations appeared of better quality when performed by sonographers who carried out a greater number of SAS per year. Sonographers that missed diagnoses had all passed quality assessment, however, 25% of sonographers did not obtain or save all cardiac planes, indicating that they either did not obtain and save all cardiac planes in a structured manner or accepted technically incorrect planes. A diagnostic clue in CHDs with a normal four-chamber view is the inability to produce a normal three vessel view (3vv) in these cases. In this thesis, the effect of adding this additional plane to the screening program on the detection of TGA and Fallot is examined. As the 3vv was added in 2012, we could compare the detection rates of TGA and Fallot before and after the introduction of this plane.
Part II of this thesis covers the prenatal neurodevelopment in CHD. Since long term neurodevelopmental outcome is strongly correlated to prenatal detection, this thesis starts with an article on improving prenatal detection.



Normal heart



Transposition of the great arteries



Tetralogy of Fallot

PART II
Neurodevelopment in congenital heart defects

The first open heart surgery for a congenital heart defect was performed at Johns Hopkins University, United States, in 1944. As children who underwent cardiac surgery increasingly survive into adulthood (the oldest living persons with an arterial switch operation are now in their forties), this allows for long-term follow-up studies. Adolescents living with CHD are known to have a high morbidity rate, with problems such as neurocognitive impairment, despite advances in (peri) operative care. Behavioral problems like ADHD, lower IQ and impaired executive functions are more common in this group. Until recent years these problems were attributed

to the detrimental effects of cardiopulmonary bypass and low cardiac output and low oxygen saturations in the perioperative period. Logically, children with cardiac defects in the severe end of the spectrum, such as hypoplastic left heart syndrome, are prone to worse neurodevelopmental outcome, since these children undergo more complex and often multiple operations. A study by Marino et al. published in Circulation in 2012, showed that 23 percent of children with severe CHD had behavioral and cognitive impairments and around 5% had problems in everyday life[10]. More recently, in addition to other etiological factors, a prenatal origin of delays in neurodevelopmental has been suggested. Studies performed in neonates with transposition of the great arteries and single ventricle pathology showed significantly higher rates of gray and white matter brain damage on pre ***and*** postoperative MRI's[11]. Mild microcephaly prior to surgery was seen in 25-36% of neonates with CHD, and 40-55% present with signs of neurological impairment (abnormal tonus, absent suck-reflex)[12]. Prenatal studies performed in fetuses with CHD showed signs of significant delayed head circumference (HC) growth and a lower resistance in the cerebral artery, known as brainsparing, without evident growth restriction[13, 14]. Studies addressing prenatal cortical development in this CHD fetuses using ultrasound and MRI showed severe delays up to 3 weeks compared to control fetuses. The hypothesis that neurodevelopmental impairment originates in fetal life, does not completely fit with the long term outcome of these children as most children present with minor behavioral problems. This thesis elaborates on the question: Do fetuses with CHD really show signs of delayed cortical maturation on ultrasound, and if so, what is the extend of the delay and are some types of CHD more prone to delayed maturation than others?

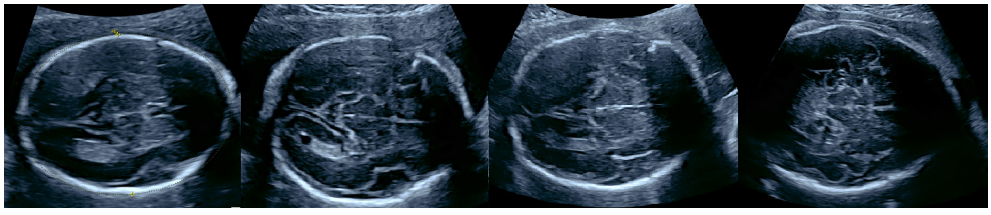


Figure 1 *The Sylvian fissure progresses from a shallow indentation (left picture, 20 weeks), to an angular shape (second picture, 24 weeks), to <50 opercularisation (third picture, 28 weeks) and ≥50% opercularisation (right picture, 32 weeks).*

Comparing cortical development in congenital heart defect cases and controls using ultrasound and advanced imaging analysis.

Fetal Cerebral Cortex

The maturation of the cerebral cortex is very rapid in fetal life and can be easily visualized with ultrasound and MRI, thus, making it a suitable parameter to study development. Moreover, it is known from studies in asphyctic neonates that periods of hypoxia can damage the cerebral cortex, leading to poor neurodevelopment later in life[15]. This has led to the concept that the hemodynamics in fetal CHD may result in altered flow/oxygenation of the fetal brain which in turn lead to different maturation patterns. Previously mentioned studies in prenatal cortical development present however, with forms of selection bias: for example, severe, non-isolated CHD cases (for example HLHS) are included more often, and only a single MRI acquisition in the third trimester was obtained. These results do not reflect cortical development in the whole group of CHD, and therefore extrapolation is difficult. The actual effect of different types of CHD on prenatal cortical development, and the extent of the delay in brain maturation is currently not known. To answer this question, a number of fetuses and healthy controls was sequentially examined with ultrasound and the maturation of the cortex was scored blinded afterwards. Since manual scoring of images is a time-consuming method and is also prone to human error, we have sought the aid of novel imaging analysis techniques. The algorithm that is presented in this thesis is a form of automated image analysis that was used to automatically analyse our imaging data.

Automated image analysis

Artificial intelligence (AI) is the technology that uses data and algorithms and has the ability to learn and adapt from input and observations. This type of AI is called deep machine learning, which means that an algorithm is trained with images that are labelled 'normal' and 'abnormal' and can subsequently recognize abnormal situations. AI is already used in daily life, for example in face and speech recognition on our smartphones. In healthcare an enormous amount of digital data is produced and daily practice relies heavily on human observations for triage, diagnosis and management. Thus, the implementation of AI, especially for specialties that produce medical imaging, seems to be a logical next step to aid physicians. A recent publication by Drukker et al.[16], describes different applications in obstetric medicine that are currently being researched, such as prediction of fetal lung maturity, pregnancy dating in advanced gestation or the prediction of shoulder dystocia. Most of these innovations use deep learning to detect patterns in observations, which were also used to program the algorithm that was applied in this thesis. It uses graytones to asses cortical development and is thereafter expressed in gestational age. This algorithm has been originally designed for developing countries, to estimate gestational age in pregnancies that did not receive accurate pregnancy dating. In

our study cohort, the algorithm is applied to CHD and control fetuses to assess brain maturation and thus conclude on prenatal delays in neurodevelopment.

OUTLINE OF THIS THESIS

Widespread use of ultrasonography has greatly evolved prenatal medicine over the past decades. Both the prenatal detection of cardiac defects and the diagnosis of prenatal brain development have significantly improved. In this thesis, the impact of screening protocols are studied on the prenatal detection of cardiac defects. Furthermore, to explore the hypothesis of altered neurodevelopment, prenatal brain development was studied in a large number of congenital heart defect cases, and analysed both manually and by using automated imaging analysis.

Part one describes the effect of the introduction of the 3vv in the standard anomaly scan protocol in 2012. The three-vessel is an instrument to increase the detection of outflow tract anomalies, which are known to have lower detection rates than CHD that present with an abnormal four-chamber view. The detection rates of transposition of the great arteries and tetralogy of Fallot were compared before and after the introduction of the three-vessel view.

In **part two** of this thesis, the prenatal neurodevelopment in congenital heart defect cases is assessed with two and three-dimensional ultrasound. Artificial intelligence has been used to estimate the magnitude of the difference in brain development.
Chapter 3 describes the results of a systematic review and meta-analysis of prenatal brain development in CHD cases. We have included all studies concerning US and MRI in isolated CHD published between January 1990 - October 2015. Multiple parameters such as HC-growth, cerebral and umbilical blood-flows, structural brain anomalies and neurological follow-up were compared to each other. In **chapter 4,** we assessed the feasibility of advanced neurosonography in a limited time frame, we have scored the visibility of cerebral structures in CHD and control cases.
In **chapter 5,** CHD-cases are compared to control fetuses to assess differences in cortical development using manual, blinded scoring of the cerebral cortex using the scoring scheme as published by Pistorius et al[17].
A novel approach to examining 3D ultrasound volumes using a brain-age estimation algorithm is presented in **chapter 6 and 7**. **Chapter 6** presents the brain maturation scores of all isolated CHD-cases compared to controls. In **chapter 7,** the brain age of CHD cases pooled according to their anatomic features are presented.
In **Chapter 8** a general discussion of the combined results is presented. Finally, **Chapter 9** shows a general summary.

Ethical aspects

The HAND-study has included both CHD-fetuses and healthy control fetuses. The local ethics committee has approved the HAND-study protocol on March 17, 2014. The CHD-fetuses were exempt from WMO, this allowed CHD fetuses to be scanned under routine care circumstances. Permission for the HAND control group of healthy fetuses was obtained by parents informed consent.

References

1 Mitchell SC, Sellmann AH, Westphal MC, et al. **Etiologic correlates in a study of congenital heart disease in 56,109 births.** The American journal of cardiology 1971;286:653-7.

2 Pasupathy D, Denbow ML, Rutherford MA, et al. **The Combined Use of Ultrasound and Fetal Magnetic Resonance Imaging for a Comprehensive Fetal Neurological Assessment in Fetal Congenital Cardiac Defects**: Scientific Impact Paper No. 60. BJOG 2019;1267:e142-e51.

3 Bonnet D. **Impacts of prenatal diagnosis of congenital heart diseases on outcomes.** Transl Pediatr 2021;108:2241-9.

4 van Velzen CL, Ket JCF, van de Ven PM, et al. S**ystematic review and meta-analysis of the performance of second-trimester screening for prenatal detection of congenital heart defects.** Int J Gynaecol Obstet 2018;1402:137-45.

5 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. P**renatal detection of congenital heart disease--results of a national screening programme.** BJOG 2016;1233:400-7.

6 Aldridge N, Pandya P, Rankin J, et al. **Detection rates of a national fetal anomaly screening programme: A national cohort study.** BJOG 2022.

7 van Velzen CL, Haak MC, Reijnders G, et al. **Prenatal detection of transposition of the great arteries reduces mortality and morbidity.** Ultrasound Obstet Gynecol 2015;453:320-5.

8 Calderon J, Angeard N, Moutier S, et al. **Impact of prenatal diagnosis on neurocognitive outcomes in children with transposition of the great arteries.** The Journal of pediatrics 2012;1611:94-8 e1.

9 van Nisselrooij AEL, Teunissen AKK, Clur SA, et al. **Why are congenital heart defects being missed?** Ultrasound Obstet Gynecol 2019.

10 Marino BS, Lipkin PH, Newburger JW, et al. **Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.** Circulation 2012;1269:1143-72.

11 Peyvandi S, Donofrio MT. **Circulatory Changes and Cerebral Blood Flow and Oxygenation During Transition in Newborns With Congenital Heart Disease.** Semin Pediatr Neurol 2018;28:38-47.

12 Majnemer A, Limperopoulos C, Shevell MI, et al. **A new look at outcomes of infants with congenital heart disease.** Pediatric neurology 2009;403:197-204.

13 Modena A, Horan C, Visintine J, et al. F**etuses with congenital heart disease demonstrate signs of decreased cerebral impedance.** Am J Obstet Gynecol 2006;1953:706-10.

14 Szwast A, Tian Z, McCann M, et al. **Comparative analysis of cerebrovascular resistance in fetuses with single-ventricle congenital heart disease.** Ultrasound Obstet Gynecol 2012;401:62-7.

15 de Vries LS, Groenendaal F. **Patterns of neonatal hypoxic-ischaemic brain injury.** Neuroradiology 2010;526:555-66.

16 Drukker L, Noble JA, Papageorghiou AT. I**ntroduction to artificial intelligence in ultrasound imaging in obstetrics and gynecology.** Ultrasound Obstet Gynecol 2020;564:498-505.

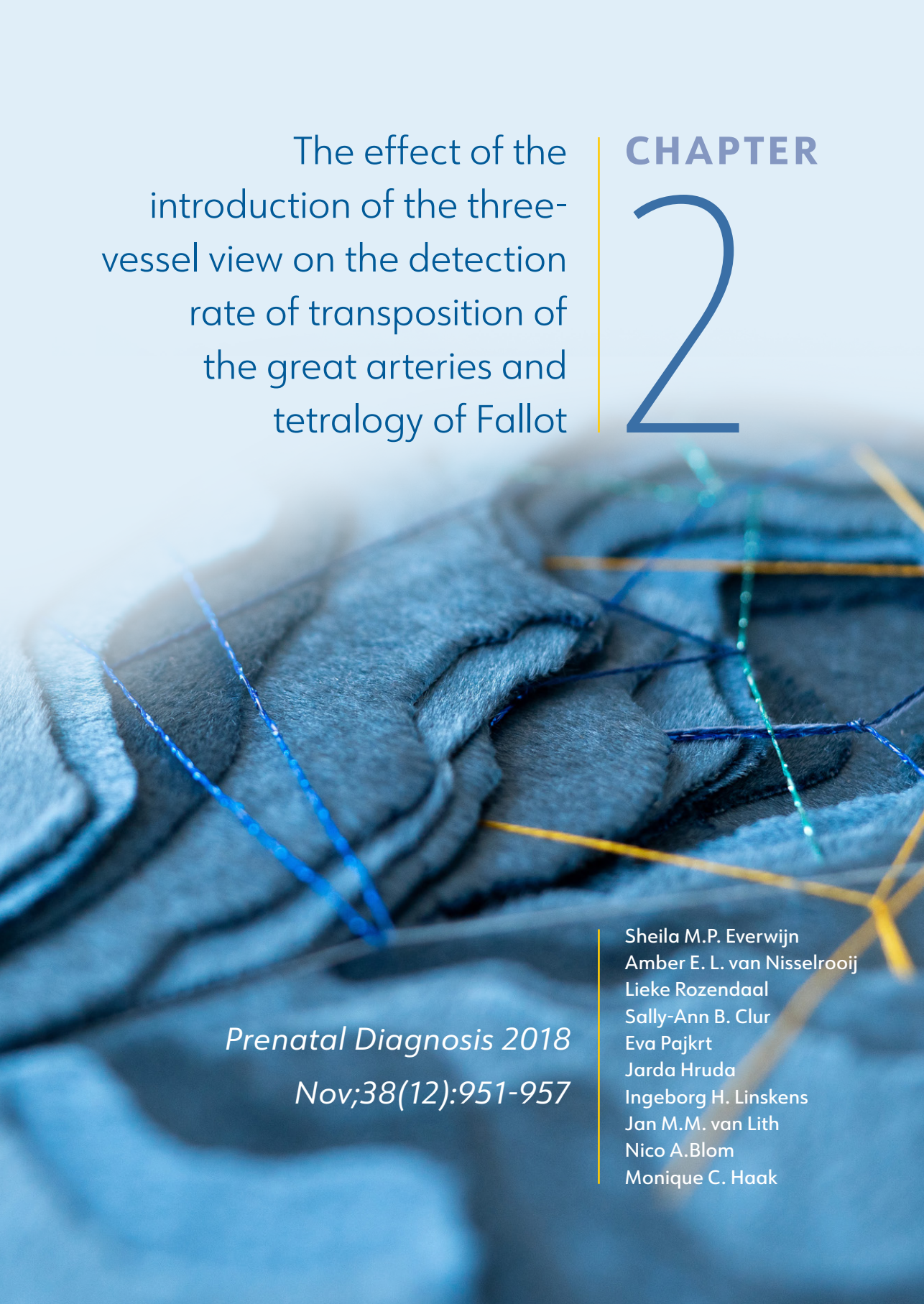
17 Pistorius LR, Stoutenbeek P, Groenendaal F, et al. **Grade and symmetry of normal fetal cortical development: a longitudinal two- and three-dimensional ultrasound study.** Ultrasound Obstet Gynecol 2010;366:700-8.

PART I

The prenatal detection of conotruncal anomalies by ultrasound

The effect of the introduction of the three-vessel view on the detection rate of transposition of the great arteries and tetralogy of Fallot

CHAPTER 2



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ABSTRACT

OBJECTIVES: The aim of this study was to analyze the annual detection rate (DR) of TGA (Transposition of the Great Arteries) and ToF (Tetrology of Fallot), after the introduction of the three-vessel view as a mandatory plane in 2012.

METHODS: All registered TGA and ToF cases were retrospectively extracted from our registry between 2007 to 2016. We compared the DR in a 10-year period: before 2011 to the DR of TGA and ToF after 2012.

RESULTS: In the period before 2012, 23 of the 52 TGA cases were prenatally detected (44.2%), compared to 42 of the 51 cases (82.4%) after 2012. For ToF, the DRs increased from 28 of 64 cases (43.8%) to 42 of 62 cases (67.7%) in the aforementioned periods. The increase in DRs for both defects was statistically significant ($p = < 0.001$ and $p = < 0.05$).

CONCLUSIONS: In this nationally organized prenatal screening program with a quality monitoring system and a uniform protocol, detection rates of 82.4% for TGA and 67.7% for ToF were reached after the introduction of the three-vessel view as a mandatory item. The three-vessel view significantly contributes to the detection of these conotruncal anomalies.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC:

- Prenatal detection of transposition of the great arteries and tetralogy of Fallot leads to decreased mortality and morbidity of affected neonates.
- Screening for congenital heart defects has improved significantly over the past decade, but has, however, not reached detection rates of 100%.

WHAT DOES THIS STUDY ADD

- Protocol changes like adding additional planes to echocardiographic evaluation, leads to increased detection of transposition of the great arteries and tetralogy of Fallot.

INTRODUCTION

Transposition of the great arteries (TGA) and tetralogy of Fallot (ToF) are common conotruncal abnormalities. Despite the relatively high prevalence, the detection rates for ToF and TGA in prenatal screening programs need improvement, especially if studies based on regional cohorts are taken into account[1]. Both TGA and ToF generally present with a normal four-chamber view, which may explain the low Detection Rate (DR) of these defects. The three-vessel view(3VV) has been shown to improve detection of ToF and TGA[2,3], but the effect on detection rates has only been sparsely investigated on institutional basis[4,5]. The effect of guideline changes in obstetrical ultrasound on detection rates in regional cohorts is only reported once and only for TGA[6]. Prenatal detection of TGA is important, as it reduces neonatal mortality and morbidity[7-11]. Prenatal detection of ToF has less effect on the postnatal presurgical mortality of these infants[12]. However, since ToF is associated with a significant risk of genetic syndromes[1], timely prenatal diagnosis provides the opportunity to perform genetic analysis during pregnancy, if desired by the parents.

The screening program in the Netherlands is centrally organized, with a uniform protocol and regulations regarding training and quality monitoring of the ultrasonographers. Compared to other regional cohort studies[13,14], the DRs for congenital heart defects in the Netherlands are high (59,6% for al Congenital Heart Defects (CHD) combined)[1,15]. From the 1st of January in 2012 the 3VV became mandatory in the national guideline of the standard anomaly scan. The aim of this study was to explore the effect of the introduction of the 3VV on the prenatal detection of TGA and ToF.

Methods

The cases in this study were collected from the PRECOR registry, an encrypted database in which all fetuses and neonates with a congenital heart defect (CHD) are entered since 2002. PRECOR is a registry of three university medical centers (Leiden University Medical Center, Academic Medical Center Amsterdam and VU University Medical Center) in the North-West region of the Netherlands, collaborating in the care for children with cardiac defects as CAHAL (Dutch: Centrum voor Aangeboren Hartafwijkingen Amsterdam Leiden), which are the only centers for fetal medicine in this region. All fetuses and infants with a CHD born in this region are entered in this registry. Until 2015 the fetal cases were entered prospectively in the fetal database with a retrospective collection of cases that were missed and presented postnatally. The methodology and data collection of the PRECOR registry has previously been published[1]. In PRECOR the prevalence of severe CHD, defined as heart defects needing surgery or a therapeutic intervention in the first year of life, is 2.3/1000 live births, which corresponds with earlier reported prevalence of CHD. The encountered prevalence in the registry is a strong indicator of the completeness of this registry[1]. From 2015 onwards, both prenatal diagnosed cases, as postnatal diagnosed cases are entered prospectively in PRECOR.

All neonates born between May 1st 2007 and April 30th 2017 with the diagnosis TGA or ToF were extracted from PRECOR. This time period was chosen as it corresponds with a standard anomaly scan performed between January 2007 to December 2016. Data on timing of diagnosis (prenatal or postnatal) were retrieved. Prenatal cases were entered in PRECOR by the fetal medicine specialists at the time of diagnosis by echocardiographic examination. Cases with a prenatal diagnosis that opted for pregnancy termination or pregnancies that ended in intra uterine fetal death (IUFD) were included. Postnatal cases, in which the diagnosis was missed at the standard anomaly scan, were entered in PRECOR after birth at the time of diagnosis by the pediatric cardiologists. These included cases referred through secondary pediatric units, general practitioners and first aid units. We cross-checked the data with neonatal mortality registries, heart catheterization registries and emergency admission charts, to prevent missing any CHD cases.

The medical and ethical committee based in the Leiden University Medical center has decided informed consent was not required for studies with a retrospective character without patient identifiers.

Between 19 and 22 gestational weeks a standard anomaly scan (SAS) is performed in the Netherlands. The SAS is mainly performed by midwives with an additional

ultrasound training. The training is uniform and regulated on a national level. The national screening program is continuously monitored by audits including biannual image analysis of the ultrasonographers. The uptake for the SAS is 95%, with the remaining proportion of women receiving an anomaly scan in a center for prenatal diagnosis because of an increased risk for fetal anomalies. Until 2012 the following cardiac planes were obligatory: four chamber view, left and right outflow tract. In January 2012, the 3VV was added as a mandatory item to protocol. The 3VV is obtained in a transverse plane, cranial to the four-chamber view. This plane is consists of the pulmonary artery/ductus arteriosus, with a transverse section through the aorta and superior caval vein, right sided of the pulmonary artery (figure 1a). No other mandatory planes were added or other significant changes were made to the SAS protocol during the studied period.

Screening for aneuploid fetuses was performed with the combined test at 11-13 weeks with an uptake of 25% until April 2017, when the Non-Invasive Prenatal Test became available as a first-tier test for all pregnant women.

We included the diagnosis of ToF and Double Outlet Right Ventricle Fallot-type with normal four-chamber view, DORV-ToF), TGA with or without a ventricular septum defect (VSD). Complex TGA cases (TGA with valvulopathies, aortic arch anomalies etc.) were excluded, as these cases often present with an abnormal four-chamber view.

To assess the effect of the introduction of the 3VV, we differentiated the ToF cases in isolated and non-isolated cases of ToF. As non-isolated cases have a higher chance of being detected. In TGA this was not done, as only 3 cases were in TGA were non-isolated CHD, hampering statistical analysis of subgroups. Isolated CHD was defined as a fetus with the CHD as only prenatal abnormality present.

Data Analysis

Frequencies and percentages were used to describe categorical variables per year. Values were divided in two groups corresponding with the period before and after the 3VV became mandatory (2007-2011 and 2012-2016). The Chi-square test was performed to test between categorical variables. Independent samples T-test was performed to compare means. We considered $p < 0.05$ to be statistically significant. Data analysis was performed with the SPSS software package version 24 (SPSS inc., Chicago, IL, USA).

Results

Between 2007 and 2016 we identified 103 cases of TGA as isolated CHD in the registry. Of these, 26 (25%) had a VSD (table 1). In the first period (2007-2011) a prenatal diagnosis of TGA was identified in 23/52 (44.2%) cases, as was reported before[7]. In the second period (2012-2016), after the introduction of the 3VV, TGA was diagnosed in 42/51 cases (82.4%). The increase of 38.2% (Table 1) was statistically significant (*p= 0.000057*).

Between 2007 and 2016 we identified 126 cases of ToF/DORV-ToF. Of these, 95 (75.4%) were ToF cases and 31 (24.6%) were DORV-ToF cases (table 2). In the first period (2007-2011), a prenatal diagnosis of ToF/DORV-ToF cases was found in 28/64 (43.9%). In the second period (2012-2016), after the introduction of the 3VV, ToF/DORV-ToF was prenatally diagnosed in 42/62 cases (67.7%). The increase of 23.8% was statistically significant (p = 0.00673) (Table 2). The baseline characteristics are shown in table 3 (TGA) and table 4 (ToF), showing that the two time periods are comparable. In the ToF/DORV-ToF cases, the DRs remained stable in de non-isolated group (82 vs 92%, p = 0.62), but the isolated group (cases without additional ultrasound anomalies) showed a significant increase (30 vs 62%, p = 0.001), illustrating that these cases are detected due to the use of the 3VV.
Mortality rates <1jr did not significantly decrease for both TGA and ToF. In ToF this is not surprising, as this CHD is not duct dependent, in the majority of cases. In TGA the relatively high mortality in the second period was caused by one death in the detected group due to abnormal coronary artery anatomy, and a relatively high mortality rate in the undetected group (33%). The causes of mortality in TGA is described in table 5 to show the possible yield of prenatal detection. Assuming that these cases would have received immediate postnatal care, to maintain mixing of deoxygenated and oxygenated blood, at least 4 of the 8 deaths could have been preventable with a prenatal diagnosis.

Discussion

The addition of the 3VV as a mandatory plane in the standard anomaly scan, has led to a significant increase in detection of TGA and ToF. In this centrally organized screening program the mean DR for TGA was 82% and 68% for ToF after the 3VV was added. To our knowledge, our study is the first to investigate the effect of the non-gradual implementation of a single plane to the screening protocol on detection rates.

Previously described screening programs looking at regional cohorts, show persistently low DRs of 40-50% for all CHD combined, with very low DRs for conotruncal anomalies like TGA and ToF in the majority of programs[1]. Although the prenatal screening program in the Netherlands started relatively late, it is uniformly managed and monitored, which provides the opportunity to introduce improvements. The introduction of the 3VV as a mandatory plane is an example of a successful change with a direct effect. Recently Ravi et al[6] reported comparable DR for TGA only, with a gradual improvement following guideline changes. Our study differs from Ravi et al. as our data show the strength of the 3VV in itself on TGA detection and the fact that the DRs for TGA change immediately after the introduction of 3VV in a centrally organized screening program. Furthermore, we show that the 3VV, as a mandatory plane, is capable of increasing DRs for ToF significantly, especially in isolated ToF. The prenatal detection rates for these defects (82% for TGA, 68% for ToF, 62% in isolated ToF) are higher than previously reported in the setting of a geographical cohort evaluation[16].

The increase of the detection rate of ToF/DORV-ToF after 2011 was more gradual compared to the TGA cases, in which the increase was abrupt. Furthermore, the magnitude of the increase in ToF/DORV-ToF was less profound, with 38% increase for TGA compared to 23% increase for ToF. A possible explanation could be that the encountered abnormalities in the 3VV can be subtle in ToF (figure 1c). The configuration of the vessels is relatively normal in ToF and the size of the pulmonary trunk in fetal life can be normal to only slightly decreased[17]. The key to the diagnosis in ToF cases with a relatively normal sized pulmonary artery, is the more anterior positioned aorta in the 3VV. This aspect of the 3VV did not gain much attention in the past, probably because most training programs and studies were focused on gaining higher DRs for TGA[18-20]. We decided to train our region on this aspect in the last three years of the studied period and called the normal spatial relationship and positioning of pulmonary artery, aorta and vena cava superior in the 3VV, the 'Leiden line'[21]. The presented data of this study are, however, far too small to prove the help of this training tool.

The absence of an effect on mortality in TGA cases in this cohort was unexpected. This can be explained by the relative small numbers of cases with mortality and the relatively high mortality in the undetected cases in the second period of the cohort. As table 5 shows, cases without a prenatal diagnosis encounter periods of severe hypoxia. Although prenatal detection did not result in lower mortality in this cohort due to lack of statistical power, other studies showed a positive effect on mortality[7] and even more importantly, a beneficial effect on neurological outcome[11],

underlining the importance of a prenatal diagnosis of TGA. In our opinion, in ToF the advantage of a prenatal diagnosis is not the prevention of mortality, but the opportunity to perform genetic analysis in pregnancy, if desired by the parents. This study shows that especially the ultrasonographic isolated ToF cases, benefit from increased detection due to the introduction of the 3VV, providing the opportunity to offer this.

The assessment of the fetal heart is perceived as the most difficult part of the standard anomaly scan[22]. The complex geometric anatomy with its crossing outflow tracts could explain the difficulty in the examination. Despite the effort of the ultrasonographers, detection rates in a screening setting are unlikely to equal those of tertiary referral centers, which may be explained by a lack of advanced knowledge of normal cardiac anatomy and most importantly, low exposure to pathology. Future research on this subject could explore if measurements of the valves, adding additional views, like the three-vessel trachea view, and improving knowledge on the details of normality of existing views, as described above, could help to further improve DRs of these important and frequently occurring heart defects.

Our DR of 82% in TGA is amongst the highest reported in literature, but 18% were still missed with considerable morbidity and mortality in these cases. In an attempt to achieve complete detection, novel techniques like automated imaging interpretation should be studied in a large screening setting[23,24].

Limitations

A limitation of this study is the observational retrospective character. A study comparing geographical regions utilizing different screening protocols, would be more ideal. The described effect in this study could therefore be attributed to increased experience as well. The sudden effect on TGA-DRs, however, opposes to that hypothesis in our opinion.

Conclusion

This study shows the increased detection rate of two common conotruncal abnormalities during the standard anomaly scan, after the introduction of the 3VV. The introduction of the 3VV in screening programs in addition to the four-chamber view, could lead to a prenatal detection of 82% of TGA and 68% of ToF.

References

1 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. **Prenatal detection of congenital heart disease--results of a national screening programme.** BJOG : an international journal of obstetrics and gynaecology. 2016;123(3):400-7.

2 Gardiner H, Chaoui R. **The fetal three-vessel and tracheal view revisited.** Semin Fetal Neonatal Med. 2013;18(5):261-8.

3 Yagel S, Arbel R, Anteby EY, et al. **The three vessels and trachea view (3VT) in fetal cardiac scanning.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2002;20(4):340-5.

4 De Robertis V, Rembouskos G, Fanelli T, et al. **The three-vessel and trachea view (3VTV) in the first trimester of pregnancy: an additional tool in screening for congenital heart defects (CHD) in an unselected population.** Prenatal diagnosis. 2017;37(7):693-8.

5 Del Bianco A, Russo S, Lacerenza N, et al. **Four chamber view plus three-vessel and trachea view for a complete evaluation of the fetal heart during the second trimester.** J Perinat Med. 2006;34(4):309-12.

6 Ravi P, Mills L, Fruitman D, et al. **Population Trends in Prenatal Detection of Transposition of Great Arteries: Impact of Obstetrical Screening Ultrasound Guidelines.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017.

7 van Velzen CL, Haak MC, Reijnders G, et al. **Prenatal detection of transposition of the great arteries reduces mortality and morbidity.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015;45(3):320-5.

8 Bonnet D, Coltri A, Butera G, et al. **Detection of transposition of the great arteries in fetuses reduces neonatal morbidity and mortality.** Circulation. 1999;99(7):916-8.

9 Khoshnood B, De Vigan C, Vodovar V, et al. **Trends in prenatal diagnosis, pregnancy termination, and perinatal mortality of newborns with congenital heart disease in France, 1983-2000: A population-based evaluation.** Pediatrics. 2005;115(1):95-101.

10 Blyth M, Howe D, Gnanapragasam J, et al. **The hidden mortality of transposition of the great arteries and survival advantage provided by prenatal diagnosis.** BJOG : an international journal of obstetrics and gynaecology. 2008;115(9):1096-100.

11 Calderon J, Angeard N, Moutier S, et al. **Impact of prenatal diagnosis on neurocognitive outcomes in children with transposition of the great arteries.** The Journal of pediatrics. 2012;161(1):94-8 e1.

12 Kaguelidou F, Fermont L, Boudjemline Y, et al. **Foetal echocardiographic assessment of tetralogy of Fallot and post-natal outcome.** Eur Heart J. 2008;29(11):1432-8.

13 Marek J, Tomek V, Skovranek J, et al. **Prenatal ultrasound screening of congenital heart disease in an unselected national population: a 21-year experience.** Heart. 2011;97(2):124-30.

14 Ramaekers P, Mannaerts D, Jacquemyn Y. Re: **Prenatal detection of congenital heart disease--results of a national screening programme.** BJOG : an international journal of obstetrics and gynaecology. 2015;122(10):1420-1.

15 Baardman ME, du Marchie Sarvaas GJ, de Walle HE, et al. **Impact of introduction of 20-week ultrasound scan on prevalence and fetal and neonatal outcomes in cases of selected severe congenital heart defects in The Netherlands.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;44(1):58-63.

16 van Velzen CL, Ket JCF, van de Ven PM, et al. **Systematic review and meta-analysis of the performance of second-trimester screening for prenatal detection of congenital heart defects.** Int J Gynaecol Obstet. 2018;140(2):137-45.

17 Rollins RC, Acherman RJ, Castillo WJ, et al. **Aorta Larger Than Pulmonary Artery in the Fetal 3 Vessel View. Journal of Ultrasound in Medicine.** 2009;28(1):9-12.

18 Escobar-Diaz MC, Freud LR, Bueno A, et al. **Prenatal diagnosis of transposition of the great arteries over a 20-year period: improved but imperfect.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015;45(6):678-82.

19 Ravi P, Mills L, Fruitman D, et al. **Population trends in prenatal detection of transposition of great arteries: impact of obstetric screening ultrasound guidelines.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2018;51(5):659-64.

20 Vinals F, Ascenzo R, Poblete P, et al. **Simple approach to prenatal diagnosis of transposition of the great arteries.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2006;28(1):22-5.

21 Haak MC. **Monique Haak on fetal heart defects** [youtube clip]. 2017 [Available from: https://www.youtube.com/watch?v=3TwLZUxMBGI&t=1s.

22 Oosterhuis JJ, Gillissen A, Snijder CA, et al. **Decision-making in the referral process of sonographers in primary care screening centers.** Prenatal diagnosis. 2016;36(6):555-60.

23 Yeo L, Romero R. **Fetal Intelligent Navigation Echocardiography (FINE): a novel method for rapid, simple, and automatic examination of the fetal heart.** Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2013;42(3):268-84.

24 Veronese P, Bogana G, Cerutti A, et al. **A Prospective Study of the Use of Fetal Intelligent Navigation Echocardiography (FINE) to Obtain Standard Fetal Echocardiography Views.** Fetal Diagnosis and Therapy. 2017;41(2):89-99.

Tables

Table 1 *Detection rate of cases diagnosed with TGA in the period 2007-2016. The three vessel view was introduced in 2012.*

TGA cases	2007-2011	2012-2016	Total	DR
	n (%)	*n (%)*	*n (%)*	
Detected total	23 (44)	42 (82)	65 (63)	***p*=0.000057**
of which TGA+VSD	*6 (26)*	*12 (29)*	*18 (28)*	
Undetected total	29 (56)	9 (18)	38 (37)	
of which TGA+VSD	*7 (24)*	*1 (11)*	*8 (21)*	
	52 (51)	**51 (49)**	**103 (100)**	

Table 2 *Detection rate 2007-2016 of cases diagnosed with ToF / DORV-ToF. The three vessel view was introduced in 2012.*

Fallot/DORV-Fallot cases	2007-2011	2012-2016	Total	DR
	n (%)	*n (%)*	*n (%)*	
Detected total	28 (44)	42 (68)	70 (56)	***p*=0.007**
of which DORV-Fallot	*9 (32)*	*17 (40)*	*26 (37)*	
Undetected total	36 (56)	20 (32)	56 (44)	
of which DORV-Fallot	*5 (14)*	*0 (0)*	*5 (9)*	
	64 (51)	**62 (49)**	**126 (100)**	

Table 3 *Baseline characteristics for transposition of the great arteries cases.*

TGA	2007-2011	2012-2016	P-value
Total no. of cases	**52**	**51**	
GA at detection weeks (SD)	21.7 (3.3)	21.1 (3.1)	0.54
DR	23/52	42/51	<0.005†
Cases detected > 24 weeks n (%)	4 (8)	2 (4)	0.41
Male gender n (%)	36 (69)	34 (67)	0.78
GA at birth weeks (SD)			
Overall	**39.6 (1.1)**	**39.3 (1.4)**	**0.24**
Detected	39.3 (0.7)	39.1 (1.3)	0.55
Undetected	39.9 (1.3)	40.3 (1.5)	0.45
Pregnancy results n(%)			
Termination of Pregnancy	0 (0)	1 (2)	0.31
IUFD	0 (0)	0 (0)	n.a.
Mortality < 1 year n/total			
Overall	**5/52**	**4/50**	**1.00**
Detected	0/23	1/41	1.00
Undetected	5/29	3/9	0.36
Neonatal Death* n/total			
Overall	**4/52**	**2/50**	**0.68**
Detected	0/23	1/41	1.00
Undetected	4/29	1/9	1.00

* Neonatal death was defined as death of an infant within the first 28 days of life.

† Statistically significant

Table 4 *Baseline characteristics for Tetralogy of Fallot cases.*

Tetralogy of Fallot	2007-2011	2012-2016	*p*-value
Total no. of cases	64	62	
GA at detection weeks (SD)	21.2 (2.8)	21.1 (2.6)	0.93
DR	28/64	42/62	0.007†
Cases detected > 24 weeks n (%)	1 (2)	2 (3)	1.00
Male gender n (%)	34 (55)	38 (61)	0.35
Genetic testing in pregnancy ‡	24/28	26/42	0.04
Pregnancy results n(%)			
Termination of Pregnancy	1 (2)	7 (11)	0.13
IUFD	1 (2)	1 (2)	1.00
GA at birth weeks (SD)			
Overall	**38.4 (2.4)**	**39 (1.5)**	**0.08**
Detected	38.1 (2.6)	39.1 (1.5)	0.06
Undetected	38.6 (2.2)	38.9 (1.4)	0.68
Mortality < 1 year			
n/total (without TOP and IUFD)			
Overall	**4/62**	**1/54**	**0.37**
Detected	3/26	1/34	0.31
Undetected	1/36	0/20	1.00
Neonatal Death*			
n/total (without TOP and IUFD)			
Overall	**2/62**	**0/54**	**0.50**
Detected	2/26	0/34	0.18
Undetected	0/36	0/20	n.a.
Non-isolated cases ¥			
Total n (%)	**17 (27)**	**12 (19)**	**0.33**
Detected n (%)	14/17 (82)	11/12 (92)	0.62
Isolated cases			
Total n (%)	**47 (73)**	**50 (81)**	**0.34**
Detected n (%)	14/47 (30)	31/50 (62)	0.001†
Isolated + no genetic abnormalities			
Total n(%)	**45 (70)**	**47 (76)**	**0.49**
Detected n(%)	12/45 (27)	28/47 (60)	0.001†

† Statistically Significant

‡ Genetic findings: XYY, 4 cases 22Q11 microdeletion syndrome, 3 cases T21, T18

¥ Additional ultrasound findings: Unilateral renal agenesis, Intra-uterine growth restriction, increased nuchal translucency, unilateral clubfoot, ventriculomegaly, hydronephrosis, Single Umbilical Artery, Dolichocephaly, hypoplastic cerebellum, short femur, intracranial cyst, echogenic bowel.

* Neonatal death was defined as death of an infant within the 28 days of life.

Table 5 *Overview of TGA cases with mortality*

Subject	Prenatal detection Yes/no	gender	GA at birth	Age at detection	Age at death	Cause of death	Preventable death
#1 2015	No	Male	41	0 days	9 days	Respiratory insufficiency 5 hours after birth. ERP. Subdural hematoma and severe ischemic brain damage. DMT.	maybe
#2 2015	No	Male	42	49 days	118 days	Presents with cyanosis and kidney failure. Two stage cardiac repair. Brain damage due to ischemia. DMT.	yes
#3 2013	Yes	Male	38	n/a	18 days	Birth via CS followed by RP. Abnormal Coronary anatomy. ASO. Myocardial infarction after ASO and MOF.	no
#4 2013	No	Female	40	7 days	35 days	Presenting with cyanosis. ERP. ECMO. MOF. DMT.	yes
#5 2011	No	Male	40	0 day	1 day	Agitation 12 hours after birth. Later pale and unresponsive, cardiac arrest. Deceased pre-operative	maybe
#6 2010	No	Male	39	14 days	15 days	Respiratory + circulatory collapse. Severe acidosis. DMT.	yes
#7 2009	No	Male	40	0 days	8 days	Cyanosis directly after birth. ERP hours after birth. Severe hypoxic brain damage. DMT.	maybe

#8 2008	No	Female	39	0 day	1 day	Grunting directly after birth. Respiratory distress. Diagnosis directly after birth. Prostin started. Prolonged resuscitation. DMT.	no
#9 2007	No	Female	40	21 days	29 days	Failure to thrive, presents with cyanosis and cardiac souffle. Two stage cardiac repair; BT-shunt + banding. Complicated post-operative period which resulted in MOF.	yes

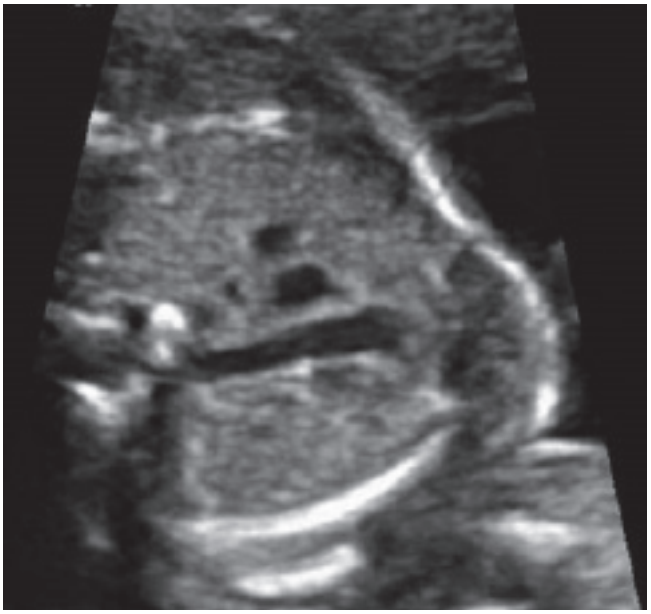

Figure 1a *An example of a three-vessel view at 20 week gestation, obtained during the structural anomaly scan.*

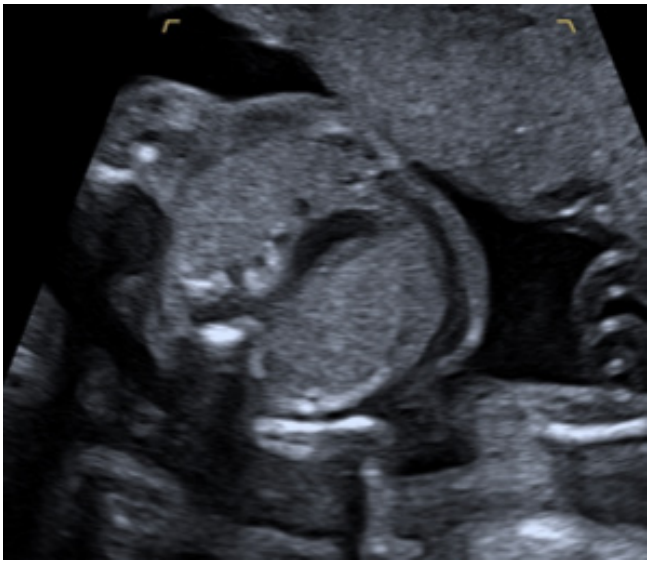

Figure 1b *In a case of transposition of the great arteries the three-vessel view is often abnormal: the aorta is displaced anteriorly and the pulmonary artery is not visible as it emerges from the heart below this plane.*

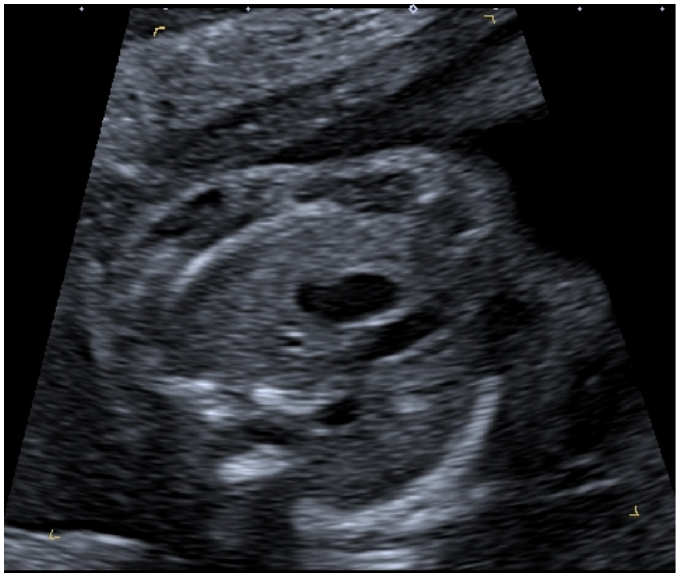

Figure 1c *Three-vessel view in Tetralogy of Fallot can mimic a normal spatial relationship, yet the aorta is displaced anteriorly.*

PART II

The HAND-study: The assessment of different techniques of brain development in fetuses with isolated congenital heart defects.

CHAPTER

3

Fetal brain imaging in isolated congenital heart defects – a systematic review and meta-analysis

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ABSTRACT

INTRODUCTION: Congenital heart defects (CHD) are associated with neurodevelopmental (ND) delay. This study aims to assess evidence for impaired prenatal brain development, in fetuses with CHD.

METHODS: A systematical search was performed and 34 studies evaluating the fetal brain (MRI or ultrasound) in isolated CHD were included (1990-2015). Data regarding cerebral abnormalities, head circumference (HC) growth and middle cerebral artery (MCA) flow were extracted.

RESULTS: natal MRI was studied in 10 articles (445 fetuses), resulting in a pooled prevalence of 18% (95%CI -6% to 42%) for combined structural and acquired cerebral abnormalities. Prenatal HC was studied in 13 articles (753 fetuses), resulting in a pooled z-score of -0.51 (95%CI -0.84;-0.18). Doppler was studied in 21 articles (1412 fetuses), resulting in a lower MCA pulsatility index (z-score -0.70 95%CI -0.99;-0.41) in left sided CHD only.

CONCLUSIONS: We conclude that prenatal MRI and ultrasound demonstrate brain abnormalities, delay in head growth and brainsparing in subgroups of CHD. However, large MRI studies are scarce and ultrasound data are biased towards severe and left-sided CHD. Long term follow-up studies correlating prenatal findings with postnatal ND outcome are limited and data is lacking to support counselling families regarding ND outcome based on prenatal findings suggestive of altered brain development.

BACKGROUND

Congenital heart disease (CHD) is the most common congenital malformation, affecting six to eight per 1000 newborns. Although the survival rates of these children have increased over the last decades, there is a significant risk for adverse neurodevelopmental (ND) outcome, even in the absence of associated chromosomal or syndromic abnormalities[1-4]. ND sequelae, like developmental delay and low IQ, are mainly encountered in children with severe CHD, who require surgery in the first year of life[5]. Until recently, these ND sequelae were assumed to be the result of perioperative conditions resulting in cerebral hypoxia and thromboembolic events[6].

Recent studies demonstrated signs of abnormal neurological development already present at birth, prior to surgery. These studies demonstrated abnormal results of early neurological examination and abnormal imaging findings such as periventricular leukomalacia, white matter injury and cerebral atrophy[7;8]. Some studies related these findings to a poor neurological development later in life[9;10]. The characteristics of certain pre-operative neurological abnormalities, such as cerebral atrophy and delayed maturation, suggest that these abnormalities originate in utero. A second finding indicating towards a fetal origin is a smaller head circumference (HC), found in neonates with severe isolated CHD. Smaller HC is mainly reported in neonates with transposition of the great arteries (TGA), tetralogy of Fallot (ToF) and hypoplastic left heart syndrome (HLHS)[11-14] and is associated with a higher risk for ND outcome[15].When a CHD is identified before birth, basic fetal ultrasound (US) can be used to identify delayed fetal head growth and abnormal cerebral flow. Dedicated fetal neurosonography or fetal brain magnetic resonance imaging (MRI) can be used to identify more subtle signs impaired fetal cerebral development[16;17;18].The aim of this study was to systematically review existing evidence for impaired brain development *in utero,* in fetuses with isolated CHD. More specifically, we aimed to objectify the presence of fetal hemodynamic brainsparing effects, delay in fetal brain growth or fetal brain abnormalities in general, in these cases. Furthermore we attempted to stratify the findings to the type of CHD.

Methods

Search strategy

A systematic search was conducted in PubMed, Embase, Web of Science and Cochrane databases in October 2015. Publications from 1 January 1990 to 28 October 2015, containing the search terms *imaging (ultrasonography or MRI), fetology, congenital heart disease and neurodevelopment* were included. The complete search string is available in Supplement 1. Studies on genetic syndromes asso-

ciated with CHD, such as Trisomy 21, Noonan syndrome and 22q11.2 microdeletion syndrome were excluded, as well as functional CHD, arrhythmias and lethal abnormalities. The extracted articles were evaluated for relevance by 3 independent researchers (FJ, SE, MH). Studies were eligible for inclusion if MRI and/or US was performed *before birth*, assessing cerebral maturation, brain volume or growth, measuring Doppler flow patterns in the middle cerebral artery (MCA), and/or measuring head biometry, in fetuses with isolated CHD. To maximize the sample size, selected articles were cross-referenced. We assessed study quality and risk of bias by rating the articles based on the Strobe criteria[19]. Disagreement was resolved by consensus. Low methodological quality was not an exclusion criterion. The consensus statement on reporting in meta-analysis of observational studies in epidemiology (MOOSE-statement), was followed when possible and appropriate[20].

Data extraction and processing

The number and type of identified cerebral abnormalities were extracted from the MRI studies. Reported abnormalities were assessed by a paediatric neurologist (CP) and subdivided into four categories: 1) structural malformations, such as callosal agenesis, 2) cystiform anomalies including arachnoid, subependymal and germinolytic cysts, 3) ventricular anomalies including asymmetrical appearing ventricles and intraventricular haemorrhage, and 4) lesions possibly caused by hemodynamic changes, such as cerebral atrophy, white matter injury and delay in maturation. The prevalence of the abnormalities was pooled per category, based on the available data.

Biometrical values of head circumference (HC) and MCA-pulsatility indices (MCA PIs) were extracted from the US studies, as mean or median z-scores, percentiles or absolute values. If z-scores or percentiles were reported, the used reference population was noted. Reported percentiles, absolute values or median PI z-scores with range intervals were transformed to mean z-scores to correct for differences in gestational age (GA) at sampling (Supplement 2). To transform the HC outcome measures into z-scores, the population parameters by Hadlock were used[21]. To transform the MCA-PI outcome measures into z-scores, the population parameters by Arduini were used[22]. Furthermore, we extracted the gestational age of assessment, the type of included CHD, the used exclusion criteria and the centres and time span of data-collection. Also, if included, the used control group was noted, as well as the method of postnatal neurodevelopmental assessment, if performed. An e-mail request was sent to authors if data were not extractable from the original article.

Meta-analysis models

The metafor package (Viechtbauer 2010, version 1.9-4) for the statistical program R (R Development Core Team 2010), was used to conduct the meta-analyses. For the HC and MCA PI measurements, meta-analysis models were constructed for all CHD combined (mixed types of CHD). For the MCA PI measurements meta-analysis models were also constructed for the following subgroups: 1) left sided obstructive lesions (LSOL) such as hypoplastic left heart syndrome and coarctation of the aorta, 2) right sided obstructive lesions (RSOL), such as tricuspid atresia, pulmonary stenosis, Ebstein's anomaly and tetralogy of Fallot, and 3) transposition of great arteries (TGA). If combined effect sizes for a mixture of CHDs were not reported in a study, missing combined effect sizes were calculated from documented subgroup effect sizes (Supplement 2).

For each meta-analysis model the following parameters were calculated using a random effect model: the estimated overall effect size with its standard error, the statistical significance of the estimated effect and several parameters describing the heterogeneity (between studies variance (tau2), Cochran's Q-test and I^2). The variance between studies was calculated with the restricted maximum likelihood method. A forest plot and a funnel plot were created additionally for each fitted model. Sensitivity analyses were performed to check whether the meta-analysis models should be corrected for two possible origins of estimation biases: bias related to overlapping cohorts between studies (duplicate/secondary publication) and missing effect sizes of control groups.

Results

The search resulted in 34 included articles (1983 fetuses, Figure 1). The study characteristics are summarized in Tables 1a (MRI studies) and 1b (US studies). Quality assessment is summarized in Figures 2a (MRI studies) and 2b (US studies). The included types of CHD per cohort are delineated in Tables 1a and 1b. CHD characteristics were not always described thoroughly. Several authors divided the cohort into LSOL, RSOL and mixed lesions. Others only included certain types of CHD (TGA, TOF, HLHS). Variation was found in the description of HLHS and LSOL: some only included those with retrograde aortic arch flow, others included cases of aortic stenosis, coarctation of the aorta and/or interrupted arch as well in the LSOL group. In this meta-analysis, all types of LSOL were combined in one group.

MAGNETIC RESONANCE IMAGING

The search resulted in ten studies (445 fetuses) addressing fetal cerebral MRI. In Table 2 the main conclusions and extracted data regarding cerebral abnormalities are summarized. We noted variation in reported cerebral data: some studies reported

fetal brain volumes or brain weights, but the methods to calculate the variables differed[23-28]. Others reported on metabolic aspects[23;28;29], cerebral maturation indices[27;28] or derived cerebral oxygenation parameters[24;26]. Most authors also described cerebral malformations or acquired cerebral lesions. Possible duplicate cohorts were identified in three publications[23;25;27]. The data of five articles (duplicate cohorts not included) were summarized to a prevalence estimate of cerebral abnormalities of 18% (95% CI -6; 42)[28-32]. Structural brain anomalies were present in 3% (95% CI -1; 8). The subdivision in the three other categories was feasible in two articles without possible overlap; the estimated prevalence is displayed in Table 2 (cystiform abnormalities 4%, ventricular abnormalities 12% and other (acquired) lesions 6%)[30;31]. The data were reported as mixed CHD group, however most authors included severe CHD only. The data did not allow further subdivision into in specific CHD.

ULTRASOUND STUDIES

Head circumference

The search resulted in 13 studies (753 fetuses) addressing fetal head biometry values. Eight studies included larger groups of mixed CHD[28;33-39] and five only included HLHS[40-44]. Eight studies calculated z-scores or percentiles to correct for the effect of gestational age, using various normal reference populations (Table 1b)[28;33-36;40-42]. One study reported absolute values at 34 weeks of gestation[37]. Data from five articles were excluded from the meta-analysis because of incomparable data (BPD[36] or head/weight ratio[38;43]), or because data were not displayed[39;44]. Four meta-analysis models were fitted, including three models for sensitivity analysis (Supplement 3). Three articles contained possible duplicates and were excluded from the final meta-analysis model[28;41;42]. The final model (Figure 3) includes the data of five articles, of which three included a control group[34;35;37]. The results were analyzed as data of a mixed CHD group, but LSOL and single ventricle defects cases are overrepresented. The HC has a pooled z-score of -0.51 (95% CI -0.84; -0.18), indicating a smaller HC in CHD fetuses of 0,5 SD below the population mean. The data did not allow further subdivision into in specific CHD, because of small numbers in each category. Funnel plots and information on statistical heterogeneity are available in Supplement 4.

Several authors reported the percentage of fetuses with "abnormal" HC values, meaning cases with a HC below the third percentile or a z-score below -2, at various gestational ages[28;41;42]. Only a few studies explored the HC/abdominal circumference or HC/fetal weight ratio as well[37;38;41;43], but the data were not suitable for pooling.

Doppler flow

Data regarding middle cerebral artery (MCA) flow were available in 21 articles (1412 fetuses). Most studies (n=14) calculated z-scores or percentiles to correct for the effect of the gestational age, using various normal reference populations (Table 1b)[28;33;35;40;45-54]. Seven studies reported absolute values at a specific gestational age[36-39;42;55;56]. Three articles were excluded from the meta-analysis because the pulsatility indices (PIs) were not available[23;36;38]. In three studies the combined effect size for a group of mixed CHDs was not reported, but could be calculated from documented subgroup effect sizes[46;52;53](Supplement 2). Sixteen meta-analysis models were fitted, including twelve models for sensitivity analyses (Supplement 3). Several studies were identified to report overlapping populations[28;39;42;52;54;56], and 13 studies included a control group. Only the largest and/or the most recent cohorts, including a control group, were used in the final meta-analyses displayed in Figures 4a-d; either as a mixed CHD group (nine articles, Figure 4a) or for specific CHD: LSOL (six articles, Figure 4b); RSOL (seven articles, Figure 4c) or TGA (four articles, Figure 4d). A significant lower MCA PI z-score was found in the mixed CHD group (-0.33; 95% CI -0.50 to -0.16) and the LSOL group (-0.70; 95% CI -0.99 to -0.41). Only two studies reported a subgroup with ToF[49;50]. These numbers were too small for a subgroup meta-analysis. Funnel plots and information on statistical heterogeneity are available in Supplement 4.

Several authors reported the percentage of fetuses with "abnormal" values. The definitions of abnormal varied (MCA PI, or RI, below the fifth or 2,3rd percentile or a z-score below -2, at least once in pregnancy or at a certain gestational age)[27;34-37;40;42;46-48;50;52;56]. Reports on the prevalence of CPR below 1.0 (labeled as "brain-sparing") varied, ranging from 11% (in the third trimester) to 56% (in the second trimester) and 44% at any time in pregnancy[36;38;49]. A higher prevalence of CPR below the fifth percentile was reported in CHD fetuses[47], and CPR z-scores below -2 were reported more frequently in HLHS fetuses[50]. These data were not suitable for pooling.

CORRELATION WITH NEURODEVELOPMENTAL OUTCOME

In eight articles (331 fetuses, Table 3), postnatal ND outcome was assessed. The correlation with fetal data was studied in seven articles[28;33;34;42;49;53;54]. One study was identified as a possible duplicate[54]. Results varied between studies. Three articles focused on correlating ND outcome to fetal cerebral flows, but had conflicting results[49;53;54]. The largest of the studies correlated brainsparing with a favourable ND outcome[54]. Three articles focused on correlating ND outcome to fetal biometry, possibly showing a trend towards worse ND outcome in fetuses with lower age-adjusted weight in general[33;34;42]. Relative small fetal HC compared to weight (small

HC/weight or HC/abdomen ratio) was not unanimously identified as a predictor for adverse ND outcome.

Discussion

This review and meta-analysis show that fetuses with CHD demonstrate signs of impaired brain development, identified either with fetal US or MRI. The most studied parameter is the middle cerebral artery (MCA), which demonstrates a slightly increased end-diastolic blood flow in CHD fetuses. This effect appears to be predominantly present in LSOL. The MCA-flow appears not to be altered in TGA or RSOL. Although one of the larger studies focusing solely on TGA showed a significant lower MCA PI[55], this effect is contradicted by the three other studies that included a TGA subgroup.

Combining all types of CHD, a smaller HC throughout gestation is encountered [21;57;58], but pre-selected CHD cases, mainly LSOL, are overrepresented in these data. In postnatal studies, neonates with HLHS, TGA and ToF have been associated with a smaller HC and subsequent adverse ND outcome[12-15]. Recently, a large population-based study reported a smaller neonatal HC in less severe CHD such as ventricular septal defects[59], but this has not been confirmed in prenatal studies yet.

Reported US data are limited to the measurement of flows or biometry at specific gestational ages. The effect of growth throughout gestation has not been investigated thoroughly, thus the timing of (ND) deterioration is difficult to determine. The small HC might already be present at midgestation in specific types of CHD[35]. The MCA PI appears to diminish with advancing GA[52].

We did not encounter any fetal US studies assessing detailed cerebral characteristics, such as cortical thickness, cerebral maturation or spinal fluid amount, even though neonatal studies report delayed brain maturation and cerebral atrophy in newborns with CHD[7;8] and dedicated neurosonography has the capacity to detect most of the reported anomalies[16]. Fetal brain volume was addressed in one ultrasound study, showing reduced cerebral volume growth in fetuses with HLHS, TGA, aortic arch hypoplasia and ToF[39]. As demonstrated in our review, ND details and cerebral metabolism are increasingly being studied in CHD using fetal MRI. The reported variables in the included MRI studies are however very heterogeneous and were assessed at different gestational ages. Normal values are not available for comparison, and methods of data reporting vary between studies, hampering meta-analysis. Studies including a control group do consistently report smaller fetal brain volumes in different types of CHD[23-25;27;28;32;60]. Reports on this topic are emerging rapidly, but the analysis of fetal cerebral volume, metabolism and maturation appears to be limited to research settings and have not permeated to daily clinical practice yet. Also, the definition of (the pathogenicity of) MRI lesions varies between studies. For example,

subependymal cysts are generally considered to be physiological variants[61], but are reported as abnormalities by some authors[23;30;60]. Furthermore, genetic abnormalities and severe structural lesions, such as holoprosencephaly, were not excluded in certain studies[26;30], resulting in an overrating of found anomalies.

Several theories were postulated to explain the correlation of CHD with prenatal cerebral findings, but exact pathophysiologic mechanisms remain unclear. A high prevalence of children small for gestational age in CHD[62-64] prompted several authors to investigate the relationship between fetal CHD and placental development[26;60;65]. Placental insufficiency and subsequent growth restriction usually presents with a relative large HC/abdomen ratio in fetuses without CHD[66]. Most of the fetal biometry reports addressing HC growth in CHD fetuses did, however, not include the abdominal growth, fetal weight or HC/abdomen ratios. Therefore, we are unable to determine a possible correlation between the found smaller HC and fetal weight. Postnatal studies have indicated that most of the neonates with CHD and smaller HC are also small for gestational age at birth; but HC/weight ratios have not been extensively investigated[11;14].

Secondly, a correlation between cerebral hemodynamics and fetal neurodevelopment in CHD has been suggested[38]. It seems plausible that the restriction of flow to the carotid arteries, in cases of reversed aortic arch flow, can induce vasodilatation in the cerebral circulatory system to facilitate and secure transport of oxygen and nutrients. This is not proven in an in-vivo setting yet. In this review, heterogeneity in the reported data resulted in an impossibility to pool the data on the cerebroplacental ratio, hampering a definite conclusion regarding a possible brainsparing effect in CHD. Moreover, research concerning the brainsparing effect has mainly been performed in placental insufficiency, in which it is considered a mechanism to prevent fetal brain hypoxia rather than a sign of impending brain damage[67]. Because of the totally different pathophysiological and hemodynamic circumstances in CHD versus growth restriction, conclusions are not interchangeable. Brainsparing is correlated with worse ND outcome in growth restricted fetuses[68]. Even though available data suggests that brainsparing correlates with altered brain development in CHD[27;28], a correlation with long-term outcome has not been established. On the contrary, it has even been suggested that brainsparing is associated with more favourable ND outcome in CHD[54].

Another explanation for the cerebral variations in neonates with CHD could be a common genetic pathway, causing fetal CHD and ND delay[69;70]. The fact that fetuses and neonates with types of CHD that do not have a significant effect on fetal hemodynamics (minor CHD), also demonstrate cerebral anomalies, a smaller HC and low birth weight[59] supports this theory. The development of the fetal brain in less severe

CHD, such as ventricular septal defects, has however not been reported separately. In future research, the inclusion of minor CHD will be necessary to investigate this. Furthermore, a thorough genetic assessment in fetal CHD studies is necessary to exclude the effect of confounding genetic factors. Prenatal genetic testing is generally limited to array CGH with a reasonable resolution[71], but it is known that smaller (point)mutations can play a role in CHD or neurodevelopment[72-74].

To our knowledge, this review and meta-analysis is the first to perform a meta-analysis of fetal cerebral flow abnormalities in fetuses with (severe) CHD. A narrative review published in 2010 and a recent systematic review on MRI findings are available[75;76]. Certain included publications overlap, but our study includes a larger sample size with regard to cerebral flow, facilitating meta-analyses and subgroup assessment. Furthermore, exclusion of certain duplicate MRI cohorts results in a lower prevalence of MRI abnormalities in our review, which did not reach statistical significance. Two reviews focusing on postnatal findings are available for comparison, reporting high rates of cerebral damage in fetuses with CHD prior to surgery[7;8]. However, these reviews probably included children with CHD that were undetected prior to birth, thus possibly including postnatally developed abnormalities, possibly due to asphyxia because of a delayed diagnosis.

In our meta-analyses we compared z-scores. A z-score is a statistical tool to compare the results from cases with a standardized population, eliminating the influence of gestational age. We have tried to maximize our sample size by transforming divergent outcome measures to mean z-scores, which also has some drawbacks. Grouped effect sizes would have been more accurate when individual measurements were corrected for individual gestational age, but these data are not available and assumptions on a higher data aggregation level are made. Another source of bias can be the various used normal values, which causes difficulty comparing the studies with each other. In the sensitivity analyses we determined that the use of population references, compared to the use of a control group, leads to a different outcome. In most studies it lead to a larger difference in effect size. To eliminate the effect of the various used standard populations we have chosen to use the studies reported control group as reference value.

Conclusion

This meta-analysis shows that fetuses with isolated, severe CHD demonstrate signs of impaired fetal cerebral development, demonstrated by fetal ultrasound or MRI. Our findings underline the importance of fetal neurological evaluation in CHD. However, reports correlating postnatal ND outcome to fetal findings remain incon-

clusive. Therefore, it is too early to conclude that aberrations revealed by neuroimaging involve a high risk of ND delay. To define the clinical meaning of prenatal cerebral variations in CHD, prospective large and long-term cohort studies are required, combining pre- and postnatal data. Such studies should include thorough genetic assessment and compare the findings to healthy controls. Parents should be counselled about the correlation of (specific types of) CHD with ND delay[77], but until a correlation with ND outcome has been ascertained, there is insufficient data to support counselling families regarding ND outcome, or as a rationale for fetal therapy, based on prenatal findings suggestive of altered brain development.

Supplemental material:
S1: complete search string
S2: transformation of extracted data to z-scores
S3: results of sensitivity analyses
S4: estimation of heterogeneity and funnel plots
These materials can be accessed online via: https://obgyn.onlinelibrary.wiley.com/journal/10970223

References

1 Bellinger DC, Jonas RA, Rappaport LA et al. **Developmental and neurologic status of children after heart surgery with hypothermic circulatory arrest or low-flow cardiopulmonary bypass.** N Engl J Med 1995;332:549-555.

2 Stieh J, Kramer HH, Harding P, Fischer G. **Gross and fine motor development is impaired in children with cyanotic congenital heart disease.** Neuropediatrics 1999;30:77-82.

3 Marino B, Digilio MC. **Congenital heart disease and genetic syndromes: specific correlation between cardiac phenotype and genotype.** Cardiovasc Pathol 2000;9:303-315.

4 Marino BS, Lipkin PH, Newburger JW et al. **Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.** Circulation 2012;126:1143-1172.

5 McQuillen PS, Miller SP. **Congenital heart disease and brain development.** Ann N Y Acad Sci 2010;1184:68-86.

6 du Plessis AJ. **Mechanisms of brain injury during infant cardiac surgery.** Semin Pediatr Neurol 1999;6:32-47.

7 Owen M, Shevell M, Majnemer A, Limperopoulos C. **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;26:743-755.

8 Khalil A, Suff N, Thilaganathan B, Hurrell A, Cooper D, Carvalho JS. **Brain abnormalities and neurodevelopmental delay in congenital heart disease: systematic review and meta-analysis.** Ultrasound Obstet Gynecol 2014;43:14-24.

9 Majnemer A, Limperopoulos C, Shevell MI, Rohlicek C, Rosenblatt B, Tchervenkov C. **A new look at outcomes of infants with congenital heart disease.** Pediatr Neurol 2009;40:197-204.

10 Donofrio MT, Duplessis AJ, Limperopoulos C. **Impact of congenital heart disease on fetal brain development and injury.** Current Opinion in Pediatrics 2011;23:502-511.

11 Rosenthal GL. **Patterns of prenatal growth among infants with cardiovascular malformations: possible fetal hemodynamic effects.** Am J Epidemiol 1996;143:505-513.

12 Manzar S, Nair AK, Pai MG, Al-Khhusaiby SM. **Head size at birth in neonates with transposition of great arteries and hypoplastic left heart syndrome.** Saudi Medical Journal 2005;26:453-456.

13 Shillingford AJ, Ittenbach RF, Marino BS et al. **Aortic morphometry and microcephaly in hypoplastic left heart syndrome.** Cardiol Young 2007;17:189-195.

14 Barbu D, Mert I, Kruger M, Bahado-Singh RO. **Evidence of fetal central nervous system injury in isolated congenital heart defects: microcephaly at birth.** Am J Obstet Gynecol 2009;201:43-47.

15 Limperopoulos C, Majnemer A, Shevell MI et al. **Predictors of developmental disabilities after open heart surgery in young children with congenital heart defects.** J Pediatr 2002;141:51-58.

16 Pistorius LR, Stoutenbeek P, Groenendaal F et al. **Grade and symmetry of normal fetal cortical development: a longitudinal two- and three-dimensional ultrasound study.** Ultrasound Obstet Gynecol 2010;36:700-708.

17 Rossi AC, Prefumo F. **Additional value of fetal magnetic resonance imaging in the prenatal diagnosis of central nervous system anomalies: a systematic review of the literature.** Ultrasound Obstet Gynecol 2014;44:388-393.

18 Frick N, Fazelnia C, Kanzian K et al. **The reliability of fetal MRI in the assessment of brain malformations.** Fetal Diagn Ther 2015;37:93-101.

19 von EE, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. **The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies.** Lancet 2007;370:1453-1457.

20 Stroup DF, Berlin JA, Morton SC et al. **Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group.** JAMA 2000;283:2008-2012.

21 Hadlock FP. **Computer-assisted, multiple-parameter assessment of fetal age and growth.** Semin Ultrasound CT MR 1989;10:383-395.

22 Arduini D, Rizzo G. **Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses.** J Perinat Med 1990;18:165-172.

23 Limperopoulos C, Tworetzky W, McElhinney DB et al. **Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy.** Circulation 2010;121:26-33.

24 Al NB, van Amerom JF, Forsey J et al. **Fetal circulation in left-sided congenital heart disease measured by cardiovascular magnetic resonance: a case-control study.** J Cardiovasc Magn Reson 2013;15:65.

25 Schellen C, Ernst S, Gruber GM et al. **Fetal MRI detects early alterations of brain development in Tetralogy of Fallot.** Am J Obstet Gynecol 2015;213:392-397.

26 Sun L, Macgowan CK, Sled JG et al. **Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.** Circulation 2015;131:1313-1323.

27 Clouchoux C, du Plessis AJ, Bouyssi-Kobar M et al. **Delayed cortical development in fetuses with complex congenital heart disease.** Cereb Cortex 2013;23:2932-2943.

28 Masoller N, Sanz-Cortes M, Crispi F et al. **Severity of Fetal Brain Abnormalities in Congenital Heart Disease in Relation to the Main Expected Pattern of in utero Brain Blood Supply.** Fetal Diagn Ther 2016; 39: 269– 78.

29 Berman JI, Hamrick SE, McQuillen PS et al. **Diffusion-weighted imaging in fetuses with severe congenital heart defects.** AJNR Am J Neuroradiol 2011;32:E21-E22.

30 Mlczoch E, Brugger P, Ulm B et al. **Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program.** Eur J Paediatr Neurol 2013; 17: 153– 60.

31 Brossard-Racine M, du Plessis AJ, Vezina G et al. **Prevalence and spectrum of in utero structural brain abnormalities in fetuses with complex congenital heart disease.** AJNR Am J Neuroradiol 2014;35:1593-1599.

32 Paladini, Alfirevic Z, Carvalho JS, et al. **Prenatal counselling for neurodevelopmental delay in congenital heart disease. The results of a worldwide survey of experts' attitudes advise caution.** Ultrasound Obstet Gynecol 2016.

33 Hahn E, Szwast A, Cnota J et al. **The association of fetal growth, cerebral blood flow, and neurodevelopmental outcome in single ventricle fetuses.** Ultrasound Obstet Gynecol 2016; 47(4): 460– 5.

34 Williams IA, Fifer WP, Andrews H. **Fetal Growth and Neurodevelopmental Outcome in Congenital Heart Disease.** Pediatr Cardiol 2015;(6): 1135– 44.

35 Masoller N, Martinez JM, Gomez O et al. **Evidence of second-trimester changes in head biometry and brain perfusion in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2014;44:182-187.

36 Itsukaichi M, Kikuchi A, Yoshihara K, Serikawa T, Takakuwa K, Tanaka K. **Changes in fetal circulation associated with congenital heart disease and their effects on fetal growth.** Fetal Diagn Ther 2011;30:219-224.

37 Arduini M, Rosati P, Caforio L et al. **Cerebral blood flow autoregulation and congenital heart disease: possible causes of abnormal prenatal neurologic development.** J Matern Fetal Neonatal Med 2011;24:1208-1211.

38 Donofrio MT, Bremer YA, Schieken RM et al. **Autoregulation of cerebral blood flow in fetuses with congenital heart disease: the brain sparing effect.** Pediatr Cardiol 2003;24:436-443.

39 Zeng S, Zhou QC, Zhou JW, Li M, Long C, Peng QH. **Volume of intracranial structures on three-dimensional ultrasound in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2015;46:174-181.

40 McElhinney DB, Benson CB, Brown DW et al. **Cerebral blood flow characteristics and biometry in fetuses undergoing prenatal intervention for aortic stenosis with evolving hypoplastic left heart syndrome.** Ultrasound Med Biol 2010;36:29-37.

41 Hinton RB, Andelfinger G, Sekar P et al. **Prenatal head growth and white matter injury in hypoplastic left heart syndrome.** Pediatr Res 2008;64:364-369.

42 Hangge PT, Cnota JF, Woo JG et al. **Microcephaly is associated with early adverse neurologic outcomes in hypoplastic left heart syndrome.** Pediatr Res 2013;74:61-67.

43 Miller TA, Joss-Moore L, Menon SC, Weng C, Puchalski MD. **Umbilical artery systolic to diastolic ratio is associated with growth and myocardial performance in infants with hypoplastic left heart syndrome.** Prenat Diagn 2014;34:128-133.

44 Cnota JF, Hangge PT, Wang Y et al. **Somatic growth trajectory in the fetus with hypoplastic left heart syndrome.** Pediatr Res 2013;74:284-289.

45 Meise C, Germer U, Gembruch U. **Arterial Doppler ultrasound in 115 second- and third-trimester fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2001;17:398-402.

46 Kaltman JR, Di H, Tian Z, Rychik J. **Impact of congenital heart disease on cerebrovascular blood flow dynamics in the fetus.** Ultrasound Obstet Gynecol 2005;25:32-36.

47 Modena A, Horan C, Visintine J, Chanthasenanont A, Wood D, Weiner S. **Fetuses with congenital heart disease demonstrate signs of decreased cerebral impedance.** Am J Obstet Gynecol 2006;195:706-710.

48 Guorong L, Shaohui L, Peng J et al. **Cerebrovascular blood flow dynamic changes in fetuses with congenital heart disease.** Fetal Diagn Ther 2009;25:167-172.

49 Williams IA, Tarullo AR, Grieve PG et al. **Fetal cerebrovascular resistance and neonatal EEG predict 18-month neurodevelopmental outcome in infants with congenital heart disease.** Ultrasound Obstet Gynecol 2012;40:304-309.

50 Berg C, Gembruch O, Gembruch U, Geipel A. **Doppler indices of the middle cerebral artery in fetuses with cardiac defects theoretically associated with impaired cerebral oxygen delivery in utero: is there a brain-sparing effect?** Ultrasound Obstet Gynecol 2009;34:666-672.

51 Yamamoto Y, Khoo NS, Brooks PA, Savard W, Hirose A, Hornberger LK. **Severe left heart obstruction with retrograde arch flow influences fetal cerebral and placental blood flow.** Ultrasound Obstet Gynecol 2013;42:294-299.

52 Szwast A, Tian Z, McCann M, Soffer D, Rychik J. **Comparative analysis of cerebrovascular resistance in fetuses with single-ventricle congenital heart disease.** Ultrasound Obstet Gynecol 2012;40:62-67.

53 Zeng S, Zhou J, Peng Q et al. **Assessment by three-dimensional power Doppler ultrasound of cerebral blood flow perfusion in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2015;45:649-656.

54 Williams IA, Fifer C, Jaeggi E, Levine JC, Michelfelder EC, Szwast AL. **The association of fetal cerebrovascular resistance with early neurodevelopment in single ventricle congenital heart disease.** Am Heart J 2013;165:544-550.

55 Jouannic JM, Benachi A, Bonnet D et al. **Middle cerebral artery Doppler in fetuses with transposition of the great arteries.** Ultrasound Obstet Gynecol 2002;20:122-124.

56 Chen Y, Lv G, Li B, Wang Z. **Cerebral vascular resistance and left ventricular myocardial performance in fetuses with Ebstein's anomaly.** Am J Perinatol 2009;26:253-258.

57 Kurmanavicius J, Wright EM, Royston P et al. **Fetal ultrasound biometry: 1. Head reference values.** Br J Obstet Gynaecol 1999;106:126-135.

58 Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. **New intrauterine growth curves based on United States data.** Pediatrics 2010;125:e214-e224.

59 Matthiesen NB, Henriksen TB, Gaynor JW et al. **Congenital Heart Defects and Indices of Fetal Cerebral Growth in a Nationwide Cohort of 924 422 Liveborn Infants.** Circulation 2016;133:566-575.

60 Andescavage N, Yarish A, Donofrio M et al. **3-D volumetric MRI evaluation of the placenta in fetuses with complex congenital heart disease.** Placenta 2015;36:1024-1030.

61 Pappalardo EM, Militello M, Rapisarda G et al. **Fetal intracranial cysts: prenatal diagnosis and outcome.** J Prenat Med 2009;3:28-30.

62 Kramer HH, Trampisch HJ, Rammos S, Giese A. **Birth weight of children with congenital heart disease.** Eur J Pediatr 1990;149:752-757.

63 Rosenthal GL, Wilson PD, Permutt T, Boughman JA, Ferencz C. **Birth weight and cardiovascular malformations: a population-based study. The Baltimore-Washington Infant Study.** Am J Epidemiol 1991;133:1273-1281.

64 Spiers PS. **Does growth retardation predispose the fetus to congenital malformation?** Lancet 1982;1:312-314.

65 Huhta J, Linask KK. **Environmental origins of congenital heart disease: the heart-placenta connection.** Semin Fetal Neonatal Med 2013;18:245-250.

66 Kramer MS, McLean FH, Olivier M, Willis DM, Usher RH. **Body proportionality and head and length 'sparing' in growth-retarded neonates: a critical reappraisal.** Pediatrics 1989;84:717-723.

67 Scherjon SA, Smolders-DeHaas H, Kok JH, Zondervan HA. **The "brain-sparing" effect: antenatal cerebral Doppler findings in relation to neurologic outcome in very preterm infants.** Am J Obstet Gynecol 1993;169:169-175.

68 Hernandez-Andrade E, Serralde JA, Cruz-Martinez R. **Can anomalies of fetal brain circulation be useful in the management of growth restricted fetuses?** Prenat Diagn 2012;32:103-112.

69 Connor JA, Hinton RB, Miller EM, Sund KL, Ruschman JG, Ware SM. **Genetic testing practices in infants with congenital heart disease.** Congenit Heart Dis 2014;9:158-167.

70 Levenson D. **Genetic testing using array comparative genomic hybridization may benefit newborns with congenital heart disease: study shows aCGH more effective at detecting potential genetic causes of CHD than other methods.** Am J Med Genet A 2014;164A:ix.

71 Wapner RJ, Martin CL, Levy B et al. **Chromosomal microarray versus karyotyping for prenatal diagnosis.** N Engl J Med 2012;367:2175-2184.

72 Fahed AC, Gelb BD, Seidman JG, Seidman CE. **Genetics of congenital heart disease: the glass half empty.** Circ Res 2013;112:707-720.

73 Hartman RJ, Rasmussen SA, Botto LD et al. **The contribution of chromosomal abnormalities to congenital heart defects: a population-based study.** Pediatr Cardiol 2011;32:1147-1157.

74 Wessels MW, Willems PJ. **Genetic factors in non-syndromic congenital heart malformations.** Clin Genet 2010;78:103-123.

75 Donofrio MT, Massaro AN. **Impact of congenital heart disease on brain development and neurodevelopmental outcome.** Int J Pediatr 2010;2010.

76 Khalil A, Bennet S, Thilaganathan B, Paladini D, Griffiths P, Carvalho JS. **Prevalence of Prenatal Brain Abnormalities in Fetuses with Congenital Heart Disease: Systematic Review.** Ultrasound Obstet Gynecol 2016.

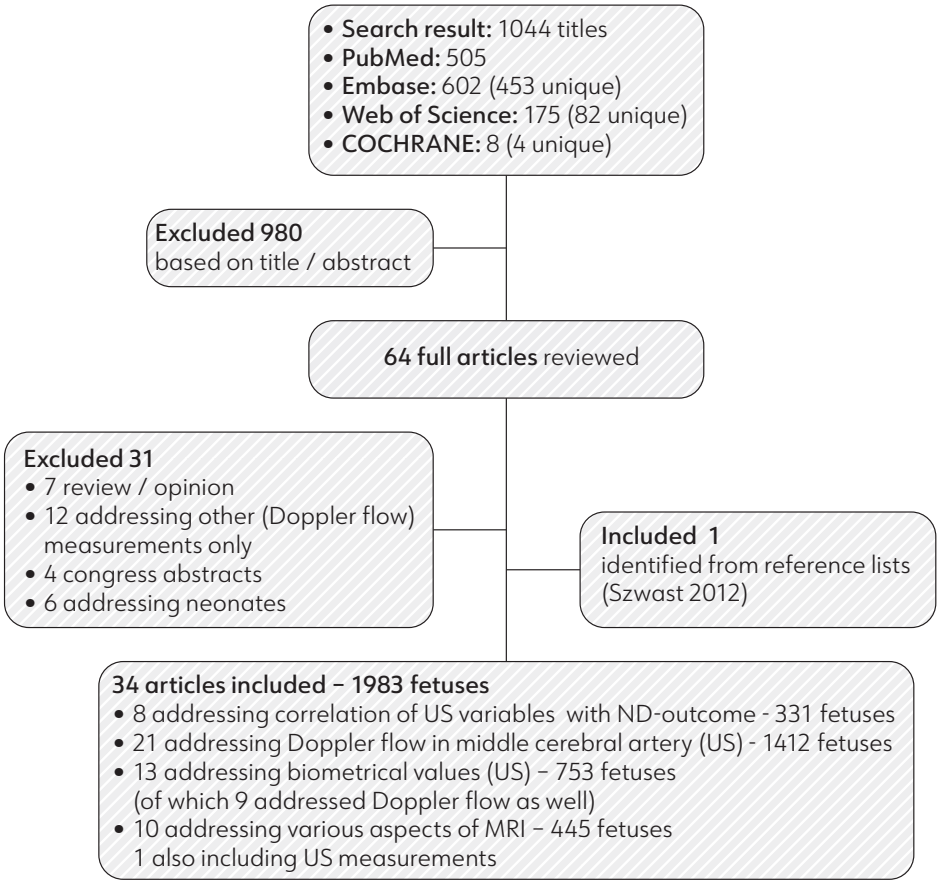


Figure 1: flow diagram of search and inclusion process

Table 1a *overview of included MRI studies*

Author (year) / country	Design*	Multi-/ single center*	Patients	Type of CHD†	GA at MRI (weeks)	Controls	Outcome measure ‡	ND assessment	Quality score*
Sun (2015) Canada	P	S	30	AS, CoA, DORV, Ebstein, TA, TGA, ToF	36	30	Fetal brain size, oxygen saturation in major blood vessels	-	*20*
Andescavage (2015) USA	P	S	38	CoA, DORV, HLHS, TGA, ToF other	29±6	94	Brain volumes, placental volumes	-	*18*
Schellen (2015) Austria	R	S	24	ToF	20-34 (mean 25,7)	24	Cerebellar, intracranial, ventricular cavity volumes	-	*17*
Masoller (2015) Spain	P	S	58	AVSD, AS, CoA, complex CHD, Ebstein, HLHS, PA, TA, TGA, ToF, truncus,	22,1±0,9	58	Brain volume, brain maturation, HC/BPD, MCA PI/CPR, (US) metabolic profile (MRI)	+	*20*
Brossard (2014) USA	P	M	144	AVSD, DORV, HLHS, PA, TGA, ToF	19-39 (mean 30,6±4,7)	194	No. of brain abnormalities	-	*17*
al Nafisi (2013) Canada	P	S	22	HLHS+AS/CoA, HLHS, HLHS+restictive FO	30-39 (mean 35)	12	Brain weight (MRI), CMR blood flow, MCA RI (US)	-	*17*
Mlczoch (2012) Austria	R	S	53	RSOL, LSOL, other(incl TGA)	20-37 (mean 24)	-	Acquired, cerebral spinal fluid spaces, no. of brain abnormalities, malformations	+	*17*
Clouchoux (2012) USA/Canada/France	P	M	18	HLHS	25-37 (mean 30.8±3,8)	30	Brain volume, cortical surface area and dept, CSF volume, gyrification index	-	*16*
Berman (2011) USA/ Canada	case reports	S	3	HLHS ,TGA,	32-35	33	diffusion weighted imaging	-	*n/a*
Limperopoulos (2010) USA/Canada	*P*	*S*	*55*	*AS, DORV, Ebstein , HLHS, PA, PS, TA, ToF, TGA, truncus other*	*25-37 (median 30)*	*50*	*Anatomical abnormalities, spectroscopy, Brain volumes: intracranial cavity, total brain, fluid.*	-	*17*

Table 1b: overview of included Ultrasound studies

Author (year) / country	Design*	Multi-/ sigle-center*	Cases	Type of CHD†	GA at US(weeks)	Con-trols	Outcome measures ‡	ND assess-ment	Quality score	*Meta-analysis***	*Used normal values*
Williams (2015) USA/ Canada	P	S	66	HLHS, TGA ,ToF	F1 (mean 23,6±2), F2 (mean30,6±1,6), F3 (mean 36,4±1)	41	BPD, HC	+	*15*	*HC*	*Hadlock 1984*
Hahn (2015) USA	R	M	133	single ventricle	20-34 (mean 27,2±5,3)	-	Biometry(n=133), MCA PI(n=119)	+	*18*	*mix; HC*	*Arduini 1990; Hadlock 1984*
Masoller (2015) Spain	P	S	58	AS, AVSD, CoA, complex CHD, Ebstein, HLHS, PA, TA, TGA, ToF, truncus	20-24 (mean 22.3±0.9)	58	BPD, CPR, composite score, correlation with MRI, HC, MCA PI,	+	*19*	*mix (n);*	*Arduini 1990; Kurmanana-vicius 1999*
										HC (n)	
Zeng 3d Flow (2015) China	P	S	112	HLHS, LSOL, RSOL, TGA	19+6-30+3 (mean 25,7±2,74)	112	3D flow , MCA PI	+	*20*	*mix; lsol; rsol; tga*	*Arduini 1990*
Zeng 3d Volume (2015) China	P	S	73	Ao hypoplasia, AVSD, HLHS, PA, PS, TA, TGA, ToF, truncus	20+4–36+4 (mean 28,2 ± 4,4)	168	cerebral volume(VO-CAL), HC, MCA PI	-	*18*	*mix (n)*	*n/a*
Miller (2014) USA	R	S	43	AS, HLHS	mean 33+5	-	Biometry	-	*19*	*n/a*	*Olsen 2010*

Masoller (2014) Spain	P	S	95	Group 1: AS, CoA, HLHS, TGA Group 2: AVSD, complex CHD, DORV, ToF, truncus arteriosus Group 3: Ebstein, PA, PS, TA	20-23+5 (mean 22+3)	95	Biometry, CPR, fractional moving blood volume, MCA PI	-	*19*	*mix; rsol; HC*	*Arduini 1990; Kurmanana-vicius 1999*
Williams (2013) USA/ Canada	*R*	*M*	*119*	*single ventricle(79% HLHS)*	*18-38 (mean 27)*	-	*MCA PI, neonatal HC*	+	*18*	*mix (n); lsol (n)*	*Arduini 1990*
Hangge (2013) USA	*R*	*S*	*38*	*HLHS*	*mean 26,6±5*	-	*Biometry, MCA PI, neonatal HC*	+	*19*	*lsol (n); HC (n)*	*Olsen 2010*
Cnota (2013) USA	*R*	*S*	*33*	*HLHS*	*19-37 (median 27)*	-	*Biometry, UA flow, neonatal HC*	-	*17*	*n/a*	*Olsen 2010+Hadlock 1984*
Yamamoto (2013) Canada	*R*	*S*	*89*	*CoA, HLHS, HLHS + CoA, PA , TGA*	*closest to term (mean 32±5)*	*89*	*CPR, MCA PI, neonatal HC*	-	*17*	*mix; lsol; rsol; tga*	*Ebbing 2007*
Szwast (2012) USA	*R*	*S*	*131*	*single ventricle (RSOL/LSOL)*	*LSOL mean 28,0±5,4; RSOL mean 24,4±4,0*	*92*	*CPR, MCA PI*	-	*15*	*mix (n); lsol; rsol*	*Arduini 1990*
Williams (2012) USA	*pilot*	*S*	*16*	*HLHS, TGA, TOF*	*18-24 (mean 22,8±2,8)*	-	*CPR, MCA PI,*	+	*16*	*mix; lsol; tga*	*Arduini 1990*
Arduini (2011) Italy	*R*	*S*	*60*	*AS, AVSD, CoA, HLHS, PA, PS, TGA, ToF, other*	*30-35 (mean 34,1±1,9)*	*65*	*Biometry, CPR , MCA PI*	-	*15*	*mix; HC*	*n/a*

Itsukaichi (2011) Japan	*R*	*S*	*44*	*CoA, DORV, Ebstein, HLHS, HRHS, PA, PS, single ventricle, TA, TGA, ToF, tumor, truncus, VSD*	*28-34*	*140*	*Biometry(AC, BPD, FL), CPR, MCA PI*	*-*	*18*	*n/a*	*n/a*
McElhinney (2010) USA	*P*	*S*	*46*	*HLHS + valvu-loplasty*	*20-31 (mean 24,3 ±3)*	*-*	*Biometry, MCA PI*	*-*	*17*	*lsol; HC*	*Arduini 1990; Kurmanana-vicius 1999*
Guorong (2009) China	*P*	*S*	*45*	*AS, CoA, completed endocardial cushion defect, DORV, Ebstein, HLHS, PA, PS, single ventricle, TA, TGA, ToF, truncus,*	*20-40*	*275*	*CPR, MCA PI*	*-*	*15*	*mix; lsol; rsol*	*not reported*
Chen (2009) China	*R*	*S*	*11*	*Ebstein*	*23-37*	*44*	*MCA PI*	*-*	*16*	*rsol (n)*	*n/a*
Berg (2009) Germany	*R*	*S*	*113*	*AS, HLHS, PA, TGA, ToF*	*19-41 'closest to term'*	*1378*	*CPR, HC postnatal, MCA PI*	*-*	*18*	*mix; lsol; rsol; tga*	*Ebbing 2007*
Hinton (2008) USA/Canada	*R*	*S*	*28*	*HLHS/AS*	*17-36*	*-*	*HC pre -and postnatal*	*-*	*15*	*HC (n)*	*Hadlock 1984*
Modena (2006) USA	*R*	*S*	*71*	*AS, AVSD, CoA, DORV, HLHS, HRHS, PA, TA, TGA, ToF, Truncus, tumor, VSD*	*'closest to mid second trimester'*	*71*	*CPR, MCA PI*	*-*	*16*	*mix*	*Arduini 1990*

Kaltman (2005) USA	*P*	*S*	*58*	*arch interruption, AS, CoA, Ebstein, PA, PS, TA, ToF*	*20-39*	*114*	*CPR, MCA PI*	*-*	*19*	*mix; lsol; rsol*	*Arduini 1990*
Donofrio (2003) USA	*P*	*M*	*36*	*HLHS, HRHS, TGA, LVOTO, ToF,*	*23-29 (mean 26,5±3,8)*	*21*	*CPR, HC, MCA RI*	*-*	*20*	*n/a*	*n/a*
Jouannic (2002) France	*P*	*S*	*23*	*TGA (+/- VSD)*	*36-38 (mean 36,7)*	*40*	*MCA PI, UA PI*	*-*	*15*	*tga*	*n/a*
Meise (2001) Germany	*P*	*S*	*115*	*AS, ASD, AVSD, CoA, DORV, Ebstein, HLHS, PA, PS, TA, ToF, truncus, VSD, other*	*19-41 (mean 30,8)*	*100*	*MCA PI, UA PI*	*-*	*14*	*mix*	*not reported*

Table 2: Overview of MRI anomalies

Author / year	other findings	total reported anomalies (CHD)	structural anomalies†	cystiform abnormalities	ventricular abnormalities	lesions/ delay possibly caused by hemodynamic changes	total reported anomalies (controls)
Sun 2015	CHD: lower umbilical vein oxygen content and lower cerebral oxygen delivery. Reduced fetal brain size correlated with these findings	1 in 30	1	n/a	n/a	n/a	n/a in 30
Andescavage 2015	CHD: smaller brain volume and cerebral volume but larger brainstem volumes, than control fetuses. Placental volumes were not associated with the differences in brain volumes.	4 in 38*	0*	1*	1*	2*	0 in 94
Schellen 2015	TOF: abnormally low total brain volumes and enlarged CSF spaces as early as 20 weeks of gestation	5 in 24**	0**	n/a	5**	n/a	0 in 24
Masoller 2015	CHD: smaller brain volumes, also decreased depths for several fissures and metabolic changes, when compared with controls	0 in 58‡	0‡	n/a	n/a	n/a	0 in 58
Brossard 2013	CHD: Brain abnormalities in 23% versus 1,5% of normal controls. Subgroup analyses comparing the type and frequency of brain abnormalities based on cardiac physiology did not reveal significant associations, suggesting that the brain abnormalities were not limited to those with the most severe CHD	33 in 144*	3*	2	13	15	3 in 194
al Nafisi 2013	CHD: 6/22 fetuses showed brain weights at or below the 5th centile for gestational age (controls 0/12). No correlation found between brain weight and flow in the SVC, AAo or CVO. No correlation found between SVC flow or brain weight with Doppler flows or aortic isthmus size or Doppler gradient.	n/a	n/a	n/a	n/a	n/a	n/a in 12
McIlzoch 2012	'Congenital brain disease' was found in 39% of fetuses with CHD	21 in 53**	7**	4	9**	1	n/a

Clouchoux 2012	HLHS fetuses demonstrate diminishing brain volumes in third trimester, as well as delay in cortical gyrification as early as 25 weeks.	5 in 18*	0*	n/a	5*	n/a	0 in 30
Berman 2011	3 fetuses with CHD demonstrated abnormally high water diffusion in the thalamus and periventricular white matter	1 in 3	1	n/a	n/a	n/a	n/a in 33
Limperopoulos 2010	Third-trimester fetuses with some forms of CHD have smaller total brain volumes than normal fetuses and display impaired neuroaxonal development and metabolism.	6 in 52*	2*	2*	4*	n/a	0 in 55
Total (without overlapping cohorts; without articles not assessing / reporting particular focus of interest)		*56 in 288*	*12 in 288*	*6 in 197*	*22 in 197*	*16 in 197*	*3 in 276*
Prevalence (95%CI)		*0,18 (-0,06; 0,42)*	*0,03 (-0,01; 0,08)*	*0,04 (-0,03; 0,10)*	*0,12 (0,04; 0,19)*	*0,06 (-0,03; 0,16)*	*0,01 (-0,01; 0,03)*

*	overlapping publications Andescavage / Brossard /Clouchoux / Limperopoulos
**	overlapping publications Mlczoch /Schellen
‡	overlapping publications Masoller 2015 and 2016
†	includes cerebellar hypoplasia; corpus callosum agenesis; holoprosencephaly; other cerebral malformations; microcephaly; macrocephaly
n/a	not applicable/or stated

Table 3: *Articles assessing neurodevelopmental outcome*

author (year) / country	type of CHD†	fetal data	ND assessment at age	n assessed of total cohort	correlation of ND outcome with fetal data
Williams (2015) USA/Canada	HLHS, TGA, ToF	Biometry	BSID-III at 18 months	46 of 68	After multivariate correction: Low BSID-III cognitive score correlated with low HC/AC ratio at midgestation. Low BSID-III language score was predicted by FL/BPD at beginning 3rd trimester
Hahn (2015) USA *	single ventricle	Biometry	BSID-II at 14 months	82 of 133	Low BSID-II mental score fetal correlated with low AC z-score at > 34 weeks. Low BSID-II psychomotor score correlated with high mean HC/AC ratio, low EFW z-score and low AC z-score at 24-29 weeks.
Hangge (2013) USA	HLHS	Biometry	Adverse neurologic outcomes included clinical seizure activity ischemia, hemorrhage, other injury	38 of 104	Early adverse ND outcome trended with fetal HC < 3rd percentile (p=0.06) .
Masoller (2015) Spain	mixed CHD, not requiring surgery in the first 6 months of life	MRI	BSID-III at 4-6 months	17 of 58	BSID-III average score correlated with fetal MRI brain volume, fissure depth and metabolic aspects. "Abnormal brain development "(MRI composite score) correlated with HC and MCA PI at midgestation
Zeng 3d Flow (2015) China	HLHS, LSOL, RSOL, TGA	Flows	BSID-II at 12 months	41 of 112	No significant correlation between MCA-PI and BSID-II score. Low BSID mental and psychomotor score fetal correlated with several aspects of low 3D intracranial flow.
Williams (2013) USA/Canada *	single ventricle(79% HLHS)	Flows	BSID-II at 14 months	72 of 119	High BSID-II psychomotor score was associated with low MCA PI z-score
Williams (2012) USA	HLHS, TGA, ToF	Flows	BSID-III at 18 months and neonatal EEG	13 of 16	Low BSID-III cognitive score correlated with low MCA PI z-score and CPR z score <-1 at midgestation (but not in a multivariate model)
Mlczoch (2012) Austria	LSOL, RSOL, other(incl TGA)		developmental status questionnaire	22 of 53	3/8 (37,5%) children with fetal brain abnormalities and 3/14 (21,4%) children with no fetal brain abnormality exhibited developmental problems. Because of the small groups, no statistics were performed.

Figure 2a *Quality of included MRI studies*

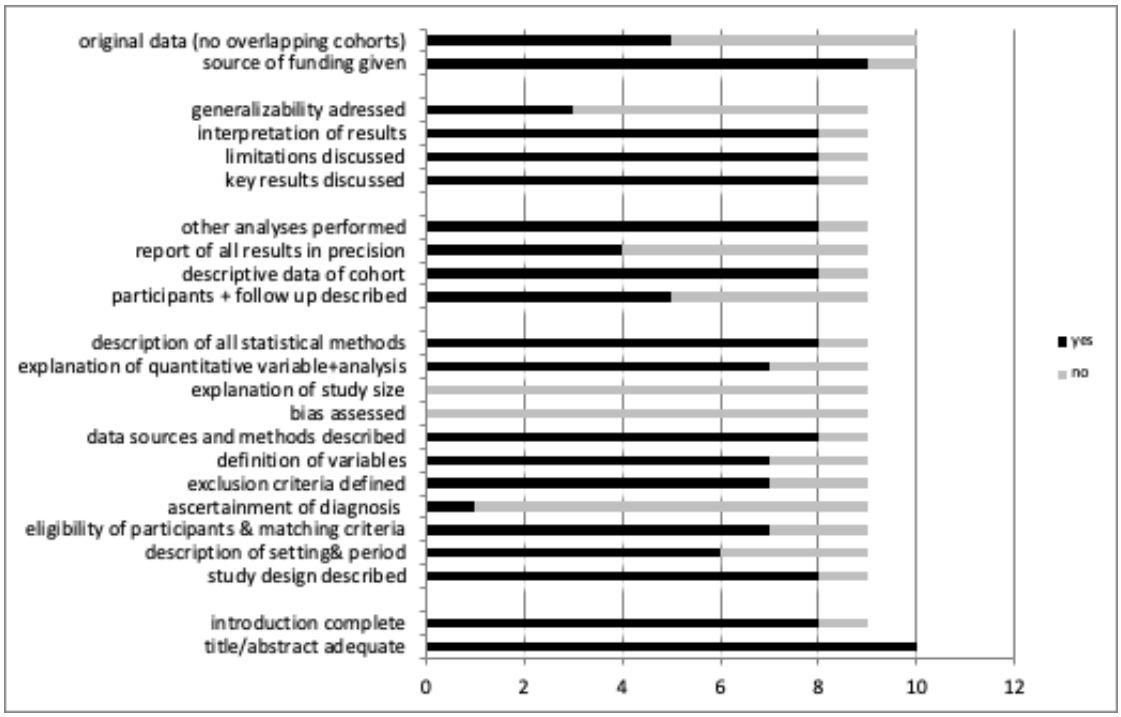


Figure 2b *Quality of included ultrasound studies*

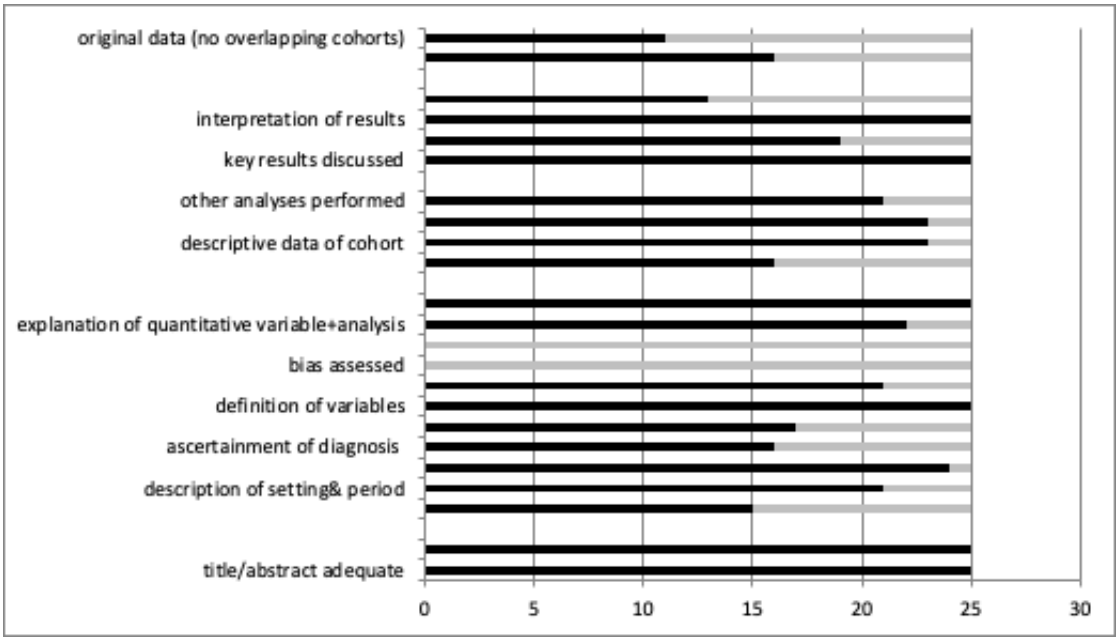


Figure 3 *Pooled head circumference z-score – fetuses with mixed types of CHD*

Suspected duplicate cohorts are excluded; the effect sizes are corrected for an included control group.

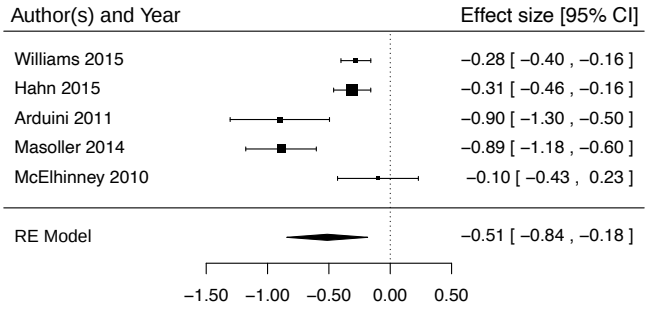


Figure 4a *Pooled middle cerebral artery pulsatility index z-score – ffetuses with mixed types of CHD*

Suspected duplicate cohorts are excluded; the effect sizes are corrected for an included control group.

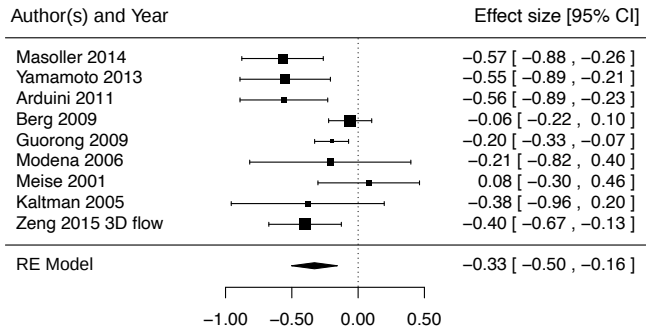


Figure 4b *Pooled middle cerebral artery pulsatility index z-score – fetuses with left sided obstructive lesions*

Suspected duplicate cohorts are excluded; the effect sizes are corrected for an included control group.

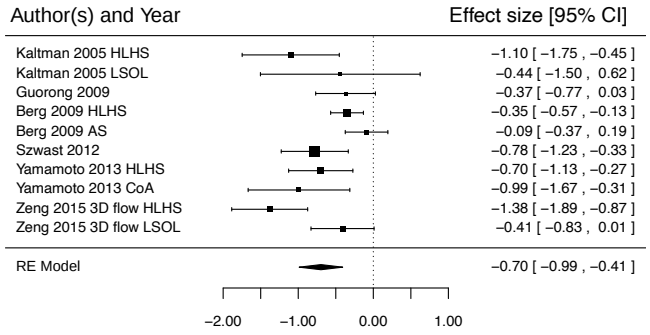


Figure 4c *Pooled middle cerebral artery pulsatility index z-score – fetuses with right sided obstructive lesions*

Suspected duplicate cohorts are excluded; the effect sizes are corrected for an included control group.

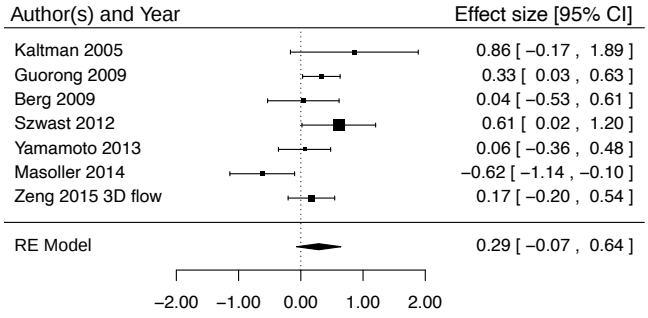
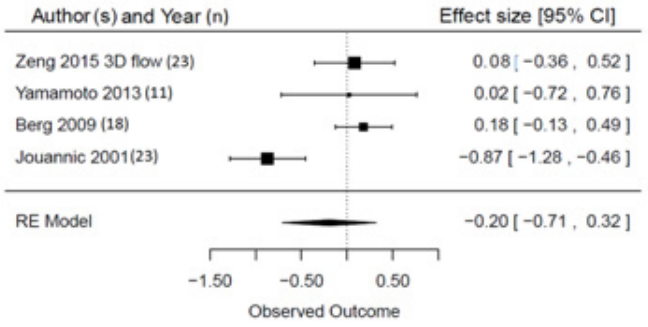


Figure 4d *Pooled middle cerebral artery pulsatility index z-score – fetuses with transposition of the great arteries*

Suspected duplicate cohorts are excluded; the effect sizes are corrected for an included control group.



Abbreviations:

*** P,** prospective;
R, retrospective;
S, single center;
M, multicenter
† Ao hypoplasia, aortic arch hypoplasia;
AS, Aorta Stenose;
AVSD, Atrioventricular Septum Defect;
CoA, Coarctatio Aortae;
DORV, Double Outlet Right Ventricle;
Ebstein, Eb-stein's anomaly;
FO, Foramen Ovale;
tumor, intracardiac tumor;
HLHS, hypoplastic left heart syndrome ;
other, other non-chromosomal extracardiac malformations;
PA, Pul-monary Atresia;
PS, Pulmonary Stenosis;
TA, Tricuspidalis atresia;
TI, Tricuspidalis Insufficiency;
TGA, Transposition of the Great Arteries;
ToF, Tetralogy of Fallot;
Truncus, Truncus Arteriosus;
RSOL, Right Sided Obstructive Lesions;

‡ AC, Abdominal Circumference;
BPD, Biparietal Diameter;
CMR, Cardiac Magnetic Resonance;
CPR, Cerebral-Placental Ratio;
CSF, Cerebro-spinal fluid;
FL, Femur Length;
HC, Head Circumference;
MCA, Middle Cerebral Artery;
PI, Pulsatility Index;
RI, Resistance Index;
UA, Umbilical Artery;
VOCAL, Virtual Organ Computer-aided AnaLysis;
n/a, not applicable

****** in which meta-analyses are data included:
mix, all/mixed types of CHD combined (MCA);
lsol, left sided obstructive defects (MCA);
rsol, rightsided obstructive defects (MCA);
tga, transposition of the great arteries (MCA);
HC, head circumference;
(n) not included in the final model, overlapping publication

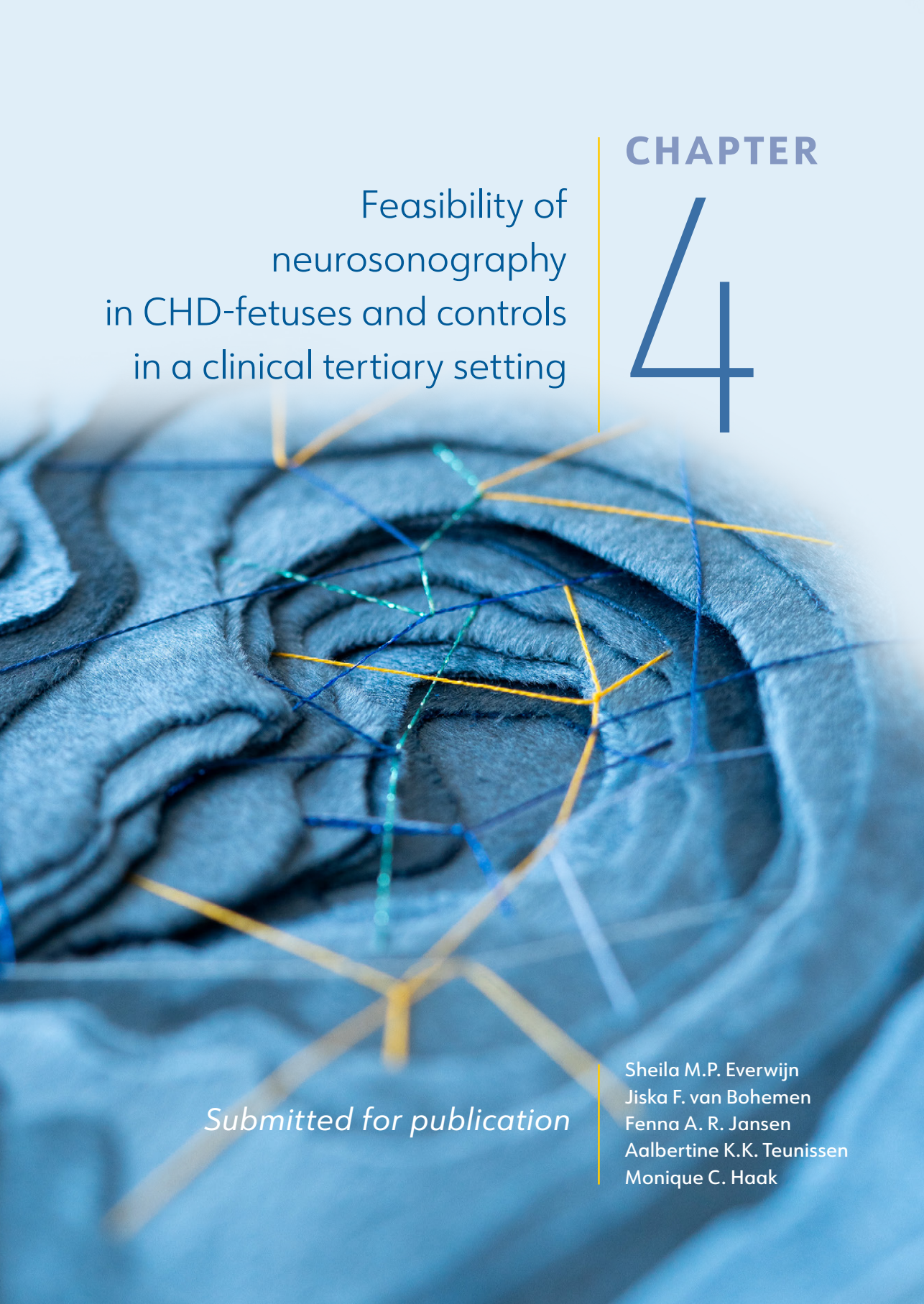
CHAPTER

3

CHAPTER

4

Feasibility of neurosonography in CHD-fetuses and controls in a clinical tertiary setting



Submitted for publication

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ABSTRACT

INTRODUCTION: Ultrasonographic examination is the first-tier test to detect abnormal development of central nervous system (CNS). In optimal conditions, neurosonography can detect all important hallmarks of neurological development. It is, however, not known how the performance of this modality is in a routine setting. We aim to evaluate the feasibility of neurosonography in a time-limited routine setting.

METHODS: We have performed basic neurosonography examination according to the guideline 'how to perform a basic screening examination of the CNS', published by the international society of ultrasound in obstetrics and gynecology. We have included a group of pregnant women carrying a fetus with an isolated congenital heart defect (CHD), and a control group of fetuses without structural anomalies. Examinations were scored off-line by researchers blinded for group allocation.

RESULTS: A total of 574 neurosonographic examinations were performed in 151 fetuses, 90 in the CHD-group and 61 in the control group. In all these examinations, 9 brain structures were scored in 3 different planes. A successful neurosonogram could be performed in 79% (234/294) of cases in a real clinical setting (CHD cases) and in 90% (253/280) of control pregnancies. Higher maternal BMI (>30), maternal age, fetal cephalic position, fetal gender and placental position did not significantly influence neurosonography scores.

CONCLUSION: In real clinical setting, basic neurosonography can successfully be performed in the majority of cases. This was not significantly influenced by maternal or fetal factors. When an abnormality is suspected in a screening setting, longer time slots for diagnostic neurosonography have to be planned, which allows for a complete examination.

INTRODUCTION

Abnormalities in the fetal central nervous system (CNS) have a prevalence of 1-2/1000 live births. The value of prenatal detection of these defects is important for expecting parents, as malformations of the CNS can have a great effect on the quality of life of a child. It may guide the decision to have an invasive genetic diagnostic procedure or, in severe cases, to terminate the pregnancy within the legal constraints of the law. Dedicated neurosonography, performed by a team of well-trained ultrasonographers with a uniform protocol, has the ability to correctly diagnose 84% of the CNS-anomalies without the use of magnetic resonance imaging (MRI)[1]. Additional pathology or a different diagnosis was found with MRI in only 1.3% of the cases . The diagnostic accuracy of CNS abnormalities improves when the examiner works in a center with a high volume of referrals, within an experienced multi-disciplinary team[2, 3]. CNS abnormalities are known to be more prevalent in fetuses and neonates with congenital heart defects (CHD), even in the absence of genetic syndromes. To explore the prevalence of CNS abnormalities in isolated CHD, we have performed neurosonography routinely to detect CNS anomalies in a group of fetuses with a broad range of CHDs and a group of controls.

The aim of this study was to evaluate the performance of two-dimensional ultrasound in a tertiary setting. We used a limited time frame, to reflect daily clinical care. We hypothesize that complete visibility of all possible structures with neurosonography, might not be entirely achievable in a daily clinical setting.

Methods

All fetal neurosonography scans were performed prior to a fetal echocardiography scan, as part of a neurological surveillance program, in the Leiden University Medical Center, a tertiary care center for prenatal diagnosis. The examinations were performed by experienced fetal ultrasonographers (SE/FJ/AT), according to the HAND (Heart And NeuroDevelopment)-study protocol every four weeks from 20 weeks onwards. We have performed the examinations according to the ISUOG guidelines for the performance of 'basic screening' and 'fetal neurosonogram'[4]. All examinations were performed with a RAB 6-D three-dimensional transducer on Voluson E8 and E10 systems (General Electric, Milwaukee, WI, USA). A group of prenatally detected CHD cases and a group of healthy control volunteers were recruited after giving informed consent. The study protocol was approved by the local ethics committee (P13.07). All cranial planes, including axial (trans-ventricular, trans-thalamic, trans-cerebellar planes), coronal (trans-caudate plane) and sagittal (mid- and para sagittal planes) were attempted. We developed a neurosonography score which was

the composite score of the visibility of nine brain structures in three different planes, resulting in a total score of 0 in case no plane was visible to nine if all structures could be retrieved. In the axial plane, cavum septum pellucidum (CSP), lateral ventricle (LV), third ventricle (3V), fourth ventricle (4V), cerebellum (CB) and Cisterna Magna (CM) were scored. In the coronal plane the frontal horns (FH), and in the sagittal plane the corpus callosum (CC) and the thalamo-occipital depth (TOD) were scored. A sufficient neurosonography score was defined as ≥7 points (>77.8%), an insufficient score as <7 (<77.8%). Additional vaginal ultrasound was added, after maternal informed consent, if abdominal planes were insufficient, but were not standard. To reflect daily clinical practice in a population in which normal findings were expected, time slots of 20 minutes were scheduled to perform all necessary planes of the ISUOG guideline for basic screening neurosonogram. Fetal echocardiography (in fetuses with (suspected) CHD) and fetal biometry were performed apart from this time slot. In the CHD-group, cases with extracardiac structural malformations or genetic syndromes were not included. If a genetic syndrome was diagnosed in the first year of life, the data were excluded from analysis. Both groups had a neonatal cerebral follow-up scan. Maternal characteristics such as BMI, maternal age, parity and diabetes were recorded. Furthermore, gestational age (GA), placental position and fetal position were recorded for each scanning session.

All fetal neurosonography examinations were stored as images and clips and were analysed offline by two researchers (SE/JvB) that were blinded for group allocation, GA and clinical outcome.
In the stored images and clips the aforementioned nine structures of brain anatomy were identified for visibility: The brain structure was scored as visible if the anatomy was clearly visible, without shadowing and in full width and length. In case of blurred vision of vague borders of the structure, the anatomic structure was scored as not visible. To avoid intra-observer variation, a set of 30 examinations were scored and compared between the two examinators. These 30 training sets were not a part of the studied data, in this initial training period, differences were agreed upon by consensus. The intra-observer variation was calculated after the training period and the method was found to have excellent intraobserver variation with an ICC of 0.97 (95% CI, 0.95-0.98).
Analysis in categorical variables were performed with Chi-square testing, and continuous variables were analysed with independent T-testing. All statistical analyses were performed using IBM SPSS statistics version 24.0.0.0 (IBM, Armonk, NY, USA). Statistical significance was set at $p \leq 0.05$. The results are presented as the visible percentage of structures of total number of scored structures.

Results

A total of 574 neurosonographic examinations were performed in 151 fetuses, 90 in the CHD-group and 61 in the control group. Baseline characteristics did not differ, except for maternal age which was slightly higher in the control group (30.2 vs 32.1 years, $p = 0.01$, Table 1).

The mean neurosonography score was 81.3% ± 11.7 in the CHD group and 85.2% ± 9.0 in the control group. Mean neurosonography score was lower for primigravidae was 78.8% ± 13.5 and for non-primigravidae 83.1% ± 9.9, $p = 0.01$ in the CHD-group. This difference was not observed in the control group. Patients with maternal diabetes (n=2), had significantly reduced neurosonography scores in the CHD-group. Mean neurosonography score for patients with maternal diabetes was 66.7% ± 16.7 and for patients without maternal diabetes 81.9% ± 11.7, $p = 0.002$. There were no patients in the control group with maternal diabetes, as they were included based on normal uncomplicated pregnancy. Maternal BMI negatively influenced neurosonography scores in CHD-cases, however, the difference was not statistically significant. Maternal age, fetal cephalic position, fetal gender and placental position did not statistically influence neurosonography scores in both CHD-cases and control groups (see Table 2).

In table 3 and 4, the evaluated brain structures are shown according to the GA in which the scan took place. In the axial plane, for both CHD-cases and controls, more than 80% of the structures are visible. The CSP, LV and CB were visible in almost all examinations (>94%) in both groups. In the coronal plane, the FH was visible in >80% cases in both groups. The structures that are only be visible in the sagittal plane were visualised in the minority of cases; the CC 14-40% and TOD >46% in both groups.

Examinations in which 85-100% of the studied brain structures could be visualised, were performed between 22 - 34 weeks gestation, defining this as the optimal GA-window for fetal neurosonography.

Discussion

This study presents a large group of ultrasound examinations of the fetal brain, that were systematically scored for visibility of well-known brain structures. We have found that fetal brain structures were best visualized between 22 and 34 weeks. The standard neurosonogram can successfully be performed within a time limit of 20 minutes, in 79% (234/294) of cases in a real clinical setting (CHD cases) and in 90% (253/280) of control pregnancies. We did not find that maternal BMI, fetal cephalic position and placental position significantly influenced the visibility of brain structures.

This study used the ISUOG practice guideline: sonographic examination of the fetal central nervous system part 1, which describes the basic planes to perform a fetal neurosonographic examination. As these fetuses were not expected to have structural brain abnormalities, we aimed to perform and complete a basic screening neurosonographic exam. A previous study by Hormazabal analysed the feasibility of neurosonography in the second and third trimester by scoring the visibility of different brain structures[5]. They found higher scores (around 95%) in the performance to visualize the different brain structures. The examinations were, however, performed in a research setting without time-restriction. Presumably, in a clinical setting with time restriction, as was presented in our study, scores higher than 90% are not achievable due to clinical demands. Another study that has analysed the feasibility of an ISUOG screening protocol, described the learning curve of experienced and non-experienced sonographers in performing a first-trimester fetal anatomy screening [6]. Although these authors conclude that complete scans were feasible in the majority of cases as was found in our study, both experienced and non-experienced sonographers were not able to reach maximum scores for each examination. We conclude, based on the results of Sripilaipong and our results that succesrates of around 90% reflect the performance of a screening neurosonogram in routine practice. If a CNS-abnormality is expected through a screening ultrasound, a broader time slot should be planned, to allow the sonographer time to produce all the necessary planes to accurately diagnose the CNS abnormality.
Although this is not the primary aim of our study, the differences between the control group and the CHD group were noteworthy. Mean neurosonography scores were lower in the CHD-groups as compared to control group. We suspect that the attitude of the sonographer towards maternal anxiety in the situation of an already diagnosed CHD could have played a role, as well as time pressure of the scheduled subsequent scan, since the neurosonography exam was planned prior to the echocardiography,

This study also provides a unique insight in the performance of fetal neurosonographic screening relating to maternal or fetal factors. Of the patient related factors, maternal BMI (although not significant) and the number of previous pregnancies, negatively influenced the neurosonography score, this finding is in line with the prenatal detection of cardiac defects[7-9]. It is noteworthy that the mentioned factors did not seem to influence the visibility of CNS structures, as we all know from clinical practice that BMI influences image quality. A possible explanation could be that with modern ultrasound equipment that was used in this study, the image quality is stable despite scanning women with higher BMI's.

A limitation of this study, is the sparse use of transvaginal ultrasound. In the minority of cephalic presenting cases, transvaginal ultrasound was added, although it is well known that transvaginal ultrasound has a significant diagnostic value in combination with abdominal US[10]. A reason for this reserved attitude towards invasive examination at that time was the absence of suspicion of a CNS abnormality combined with sufficient visualization of the CNS anatomy by abdominal US.

In conclusion, neurosonography in a tertiary center for the purpose of neurosonography surveillance is able to detect more than 80% of CNS structures in the axial and coronal planes in second and third trimester examinations. Structures in the sagittal planes are more difficult to detect. Furthermore, maternal habitus, fetal position and placenta position did not significantly influence the visibility of brain structures.

Tables

Table 1 *Baseline characteristics (n=574 ultrasounds)*

TGA cases	CHD- cases *n = 294 ultrasounds*	Controls *n = 280 ultrasounds*	*p*-value
	90 fetuses	**61 fetuses**	
Maternal age in years (Mean(SD))	30.2 (4.6)	32.1 (4.6)	0.01
Maternal Diabetes	2	0	0.24
BMI	23.6 (3.9)	24 (4.6)	0.60
Primigravidae (%)	36 (40)	20 (33)	0.49
Male gender	52 fetuses	28 fetuses	0.18
Fetal position	Cephalic: 223 Breech: 52 Transverse: 18	Cephalic: 213 Breech: 56 Transverse: 11	0.63
Placenta position	Anterior: 142 Posterior: 129 Lateral: 12 Fundus: 11	Anterior: 115 Posterior: 133 Lateral: 9 Fundus: 23	0.28
HHS n(%)	6 (6.7)		
TGA n(%)	14 (15.6)		
Ao Hypoplasia and/or aortic stenosis n(%)	17 (18.9)		
TA/PA n (%)	6 (6.7)		
Fallot of Fallot-like n(%)	14 (15.6)		
AVSD n(%)	3 (3.3)		
Other Major CHD n(%)	17 (18.9)		
Other minor CHD n(%)	9 (10)		

***** *p*-values of <0.05 are considered statistically significant

Table 2 *Influence of confounding variables on neurosonography scores*

	Percentage of visible structures % ± SD (n)		p-value
	Normal-low BMI (<30)	**High BMI**	
CHD-cases	82.2 ± 14.4 (190)	77.0 ± 15.5 (28)	*P* = 0.06
Controls	85.7 ± 12.2 (177)	81.0 ± 8.9 (17)	*P* = 0.1
	Primigravidae	**Non-primigravidae**	
CHD-cases	78.8 ± 16.7 (115)	83.1 ± 12.2 (179)	*P* = <0.01*
Controls	86.8 ± 8.9 (88)	84.5 ± 12.2 (192)	*P* = 0.1
	Cephalic position	**Non-cephalic position**	
CHD-cases	82.3 ± 14.4 (224)	81.1 ± 1.3 (70)	*P* =0.5
Controls	84.9 ± 11.1 (213)	86.4 ± 12.2 (67)	*P* = 0.4
	Anterior placenta	**Non-anterior placenta**	
CHD-cases	80.7 ± 14.4 (129)	82.0 ± 14.4 (165)	*P* = 0.5
Controls	84.8 ± 11.1 (133)	85.7 ± 12.2 (147)	*P* = 0.5

N= number of analysed examinations,

***** p-values of <0.05 are considered statistically significan

Table 3 *Evaluable brain structures in neurosonography of the CHD-group (n=294 ultrasounds)*

		AXIAL						CORONAL	SAGITTAL	
Gestational age	n	CSP n (%)	LV n (%)	3V n (%)	4V n (%)	CB n (%)	CM n (%)	*FH n (%)*	*CC n (%)*	*TOD n (%)*
19+0 – 21+6	37	36 (97.3)	37 (100)	25 (67.6)	22 (59.2)	37 (100)	37 (100)	*33 (89.2)*	*11 (29.7)*	*18 (48.6)*
22+0 – 25+6	54	54 (100)	54 (100)	46 (85.2)	47 (87)	54 (100)	53 (98.1)	*53 (98.1)*	*22 (40.7)*	*43 (79.6)*
26+0 – 29+6	65	64 (98.5)	65 (100)	56 (86.2)	57 (87.7)	65 (100)	63 (96.9)	*64 (98.5)*	*12 (18.5)*	*46 (70.8)*
30+0 – 33+6	74	72 (98.6)	73 (100)	71 (97.3)	59 (80.8)	72 (98.6)	66 (90.4)	*68 (93.2)*	*13 (17.8)*	*54 (74)*
34+0 – 37+6	64	60 (93.8)	60 (93.8)	62 (96.9)	46 (71.9)	63 (98.4)	43 (67.2)	*52 (81.3)*	*9 (14.1)*	*30 (46.9)*

Table 4 *Evaluable brain structures in neurosonography of the control-group (n=280 ultrasounds)*

		AXIAL						CORONAL	SAGITTAL	
Gestational age	n	CSP n (%)	LV n (%)	3V n (%)	4V n (%)	CB n (%)	CM n (%)	*FH n (%)*	*CC n (%)*	*TOD n (%)*
19+0 – 21+6	38	38 (100)	38 (100)	27 (71.1)	30 (78.9)	38 (100)	38 (100)	*38 (100)*	*13 (34.2)*	*28 (73.7)*
22+0 – 25+6	64	64 (100)	64 (100)	60 (93.8)	60 (93.8)	63 (98.4)	61 (95.3)	*63 (98.4)*	*21 (32.8)*	*53 (82.8)*
26+0 – 29+6	63	61 (96.8)	62 (98.4)	60 (95.2)	59 (93.7)	63 (100)	61 (96.8)	*62 (98.4)*	*13 (20.6)*	*56 (88.9)*
30+0 – 33+6	58	57 (98.3)	58 (100)	56 (96.6)	57 (98.3)	58 (100)	50 (86.2)	*57 (98.3)*	*10 (17.2)*	*50 (86.2)*
34+0 – 37+6	57	54 (94.7)	55 (96.5)	54 (94.7)	51 (89.5)	56 (98.2)	28 (49.1)	*53 (93)*	*8 (14)*	*42 (73.7)*

Table 5 *Total neurosonography-score in the control group: sum score of the 'visible' brain structures, of the* **CHD-group** *(n=294 ultrasounds)*

Gestational age	n	Insufficient score (0-77.8%) n (%)	Sufficient score (77.8-100%) n (%)
19+0 – 21+6	37	11 (29.7)	26 (70.3)
22+0 – 25+6	54	6 (11.1)	48 (88.9)
26+0 – 29+6	65	9 (13.8)	56 (86.2)
30+0 – 33+6	74	11 (14.9)	63 (85.1)
34+0 – 37+6	64	23 (35.9)	41 (64.1)

Table 6 *Total neurosonography-score in the control group: sum score of the 'visible' brain structures, of the* **control-group** *(n=280 ultrasounds)*

Gestational age	n	Insufficient score (0-77.8%) n (%)	Sufficient score (77.8-100%) n (%)
22+0 – 25+6	64	3 (4.7)	61 (95.3)
26+0 – 29+6	63	3 (4.7)	60 (95.2)
30+0 – 33+6	58	0 (0)	58 (100)
34+0 – 37+6	57	14 (24.6)	43 (75.4)

References

1 Paladini D, Quarantelli M, Sglavo G, Pastore G, Cavallaro A, D'Armiento MR, Salvatore M, Nappi C. **Accuracy of neurosonography and MRI in clinical management of fetuses referred with central nervous system abnormalities.** Ultrasound Obstet Gynecol. 2014;44(2):188-96.

2 Iruretagoyena JI, Shah D, Malinger G. **Dedicated fetal neurosonographic evaluation improves patient care and maternal fetal medicine fellow training.** The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(3):482-6.

3 Paladini D, Malinger G, Pilu G, Timor-Trisch I, Volpe P. **The MERIDIAN trial: caution is needed** Lancet. 2017;389(10084):2103.

4 **International Society of Ultrasound in O, Gynecology Education C. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram'.** Ultrasound Obstet Gynecol. 2007;29(1):109-16.

5 Hormazabal L, Correa F, Escribano D, Quiroz G, Saint-Jean C, Espinel A, Diaz L, Zambrano B, Galindo A, Vinals F. **Feasibility and agreement of including anterior-posterior complexes and landmarks of the proximal hemisphere into basic examination of the fetal brain: A prospective study.** Prenat Diagn. 2020;40(5):596-604.

6 Sripilaipong S, Panburana P, Wattanayingcharoenchai R, Tangshewinsirikul C. **Feasibility and learning curve of performing first trimester fetal anatomy screening among operators with varying experience using the protocol of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG).** The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2022;35(25):8691-7.

7 Wong SF, Chan FY, Cincotta RB, Lee-Tannock A, Ward C. **Factors influencing the prenatal detection of structural congenital heart diseases.** Ultrasound Obstet Gynecol. 2003;21(1):19-25.

8 Pinto NM, Keenan HT, Minich LL, Puchalski MD, Heywood M, Botto LD. **Barriers to prenatal detection of congenital heart disease: a population-based study.** Ultrasound Obstet Gynecol. 2012;40(4):418-25.

9 Vavolizza RD, Dar P, Suskin B, Moore RM, Stern KWD. **Clinical yield of fetal echocardiography for suboptimal cardiac visualization on obstetric ultrasound.** Congenit Heart Dis. 2018;13(3):407-12.

10 Birnbaum R, Barzilay R, Brusilov M, Acharya P, Malinger G, Krajden Haratz K. E**arly second-trimester three-dimensional transvaginal neurosonography of fetal midbrain and hindbrain: normative data and technical aspects.** Ultrasound Obstet Gynecol. 2022;59(3):317-24.

CHAPTER

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CHAPTER

5

Serial neurosonography in fetuses with congenital heart defects shows mild delays in cortical development

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ABSTRACT

INTRODUCTION: Neurodevelopmental delay is more common in children born with congenital heart defects (CHD), even with optimal perinatal and peri-operative care. It is hypothesized that fetuses with CHD are prone to neurological impairment in utero due to their cardiac defect, possibly leading to delayed cortical development.

METHODS: Cerebral cortical maturation was assessed with advanced neurosonographic examinations every four weeks in fetuses with CHD and compared to control fetuses. 5 different primary fissures and 4 areas were scored (ranging 0-5) by blinded examiners using a cortical maturation scheme.

RESULTS: Cortical staging was assessed in 574 ultrasound examinations in 85 CHD fetuses and 61 controls. Small differences in grading were seen in Sylvian and cingulate fissures. (Sylvian fissure: -0.12 grade, 95% CI (-0.23; -0.01) p = 0.05, cingulate fissure: -0.24 grade, 95% CI (-0.38; -.10) p = <0.001. Other cortical areas showed normal maturation as compared to control fetuses.

CONCLUSION: Small differences were seen in two of the nine analysed cortical areas in CHD fetuses, in contrast to previous reports on progressive third-trimester delay. The clinical implications of the small differences however, remain unknown.

INTRODUCTION

Congenital heart defects (CHD) are known to be associated with impaired neurodevelopmental outcome in children and adolescents, even in the absence of genetic syndromes[1, 2]. Behavioral, executive function problems and lower scores on IQ tests are found to be more prevalent in these children, even in cases with optimal perinatal and perioperative care[3, 4]. Hypoxemia and hemodynamic changes during fetal life are known to influence the development of the cerebral cortex in growth restricted fetuses[5]. In fetuses with CHD it is hypothesized that the altered cardiac anatomy results in reduced blood flow or oxygen delivery in the brain, comparable to IUGR fetuses, which would cause a delay in cerebral development. Studies in fetuses with CHD show a smaller head circumference and a smaller cerebral volume assessed by ultrasound[6] and MRI[7]. Furthermore, a delay in the development of sulcation was seen in neonates with transposition of the great arteries and hypoplastic left heart syndrome prior to surgery [8, 9], suggesting a fetal origin of neurodevel opmental delay as well. Studies have shown that severe CHD that are expected to have worse cerebral oxygenation, are more prone to altered neurodevelopment[10]. However, the exact mechanisms are not known yet and a genetic, epigenetic and placental origin have to be considered as well[11]. The aim of this study is to assess cortical development in fetuses with a CHD. We hypothesized that cortical development in fetuses with various isolated CHD is delayed compared to control fetuses.

Methods

Consecutive cases of isolated congenital heart defects were prospectively included in the ongoing heart and neurodevelopment (HAND) study from September 2013 onwards. A group of healthy volunteers with normally developing pregnancies was recruited to create a control group. Both groups were included after informed consent. All subjects underwent fetal neurosonography according to the ISUOG guidelines[12] every four weeks from 20 weeks onwards (around 20-24-28-32 and 36 weeks), by experienced sonographers (SE/FJ/AK). Fetuses were scanned in all cranial planes (axial, sagittal, coronal and parasagittal) using Voluson E8 and E10 systems (General Electric, Milwaukee, WI, USA) with a RAB 6-D three-dimensional transducer. Non-isolated cases (defined as cases with multiple abnormalities or positive results with genetic testing during pregnancy or in the first year of life), multiple pregnancies, referred cases >32 weeks and cases with postnatal normal cardiac anatomy, were excluded from participation or analysis. The sample size was calculated based on two MRI studies [13, 14] , that showed a two-week delay in brain development compared to control cases. The current presented study analyses cortical grading as a proxy for brain development. We have calculated both the case and control groups

to consist of at least 60 subjects, to have a 90% power to detect a mean difference of 2 weeks (SD 1 week), evaluated with a two sided level of significance.
The obtained images and clips were analysed offline by three researchers (SE/JvB/FJ) who were blinded for group allocation, gestational age (GA) and outcome. The grading system proposed by Pistorius[15] was used to score the development of the fetal brain during pregnancy. Nine cerebral cortical fissures and cortical areas that are representative of cerebral development were scored: Sylvian Fissure, parieto-occipital sulcus, central sulcus, cingulate sulcus and the calcarine sulcus and the frontal, parietal, temporal and occipital areas. The grading system is shown in Figures 1-3, and progresses from 0 (no visible development) to 5 (end-stage sulcation). All three researchers underwent a training period of 30 subjects not included in this study, in which differences in scores were agreed upon by consensus. After this initial training period, the intra-observer agreement was calculated on the included subjects. In cases with a cephalic position, transvaginal ultrasound could be included to optimize visibility after maternal approval.

Statistical analysis
The intraclass correlation coefficient was calculated with a two-way random model, to quantify intra-observer agreement on the included subjects (thus not using the cases scored during the initial training period). The nine different brain fissures and areas were analysed to compare differences in cortical development stage between CHD-cases and controls. To account for multiple assessments of cortical development within the same fetus over the course of pregnancy, linear mixed model regression was applied. For each of the five brain fissures and the four areas, a linear mixed model was used with the grades as outcome to analyse the differences in cortical development between CHD-cases and controls during pregnancy. We chose the variance/covariance structure according to the best model fit assessed by the Akaike information Criterion. The included covariates were GA (grouped into five age categories), the group (CHD-cases and controls) and the interaction between GA and group. Two effects were described for all nine different brain fissures and areas. Main group differences were reported as an overall mean difference with confidence interval. The interaction effect was calculated to determine differences in development speed with advancing GA. These effects were also calculated after adding the following five potentially confounding variables to the model: maternal age, BMI, diabetes, parity and fetal gender. Results from this adjusted model were primarily used for presentation of results. All statistical analyses were performed using IBM SPSS statistics version 24.0.0.0 (IBM, Armonk, NY, USA). Statistical significance was set at $p \leq 0.05$.

Results

In the study period, 97 cases of isolated CHD were included in this study (Table 1). 12 cases were excluded, encompassing 4 cases with a normal heart after birth (all suspected of coarctation of the aorta), and 8 cases with postnatal diagnosis of a genetic syndrome (2 cases with Kabuki syndrome, 4 cases with CHARGE syndrome, 2 cases with clear dysmorphic traits after birth, with no genetic diagnosis following whole exome sequencing (all patients died)). Of the 85 cases eligible for inclusion, 81 cases resulted in a live-born neonate, in which the prenatal diagnosis was confirmed with a postnatal echocardiogram. Of the 85 included cases, 4 decided to terminate the pregnancy. In these cases, no consent was given for postmortem examination, thus the prenatal diagnosis was adopted as the final diagnosis, as discrepancy rates are very low in our unit16. Secondly, 61 controls were included in this study following the same study protocol. Also, controls were examined postnatally with a cranial ultrasound without showing any abnormalities and displayed normal neurodevelopment up to one year of age.

In total, 608 ultrasound examinations were performed in 85 CHD-cases and 61 controls. 574 ultrasound examinations comprised of advanced neurosonography examinations, 280 examinations in the control group (mean 4.56 examinations per control), 294 exams in the CHD-cases (mean 3.47 examinations per case). In 34 examinations (3 from control group, 31 from CHD-cases), ultrasonographers were not able to produce all planes according to ISUOG guidelines due to fetal position or poor ultrasound quality, these examinations were excluded from analyses (5.6%). For all the analysed primary sulci and areas, the mean grade and standard deviation was calculated per age category and displayed in figures 4 a-e and 5 a-d. The method of scoring gyri and sulci was found to have excellent intraobserver variation with an ICC (95% CI) of 0.97 (95% CI, 0.95-0.98).

Of the analysed fissures, statistically significant delayed development was found in Sylvian and cingulate fissures as compared to controls: average difference (range 0-5) was for Sylvian fissure: adjusted difference -0.12, 95%CI (-0.23; -0.01) p = 0.05 and for cingulate fissure: adjusted difference -0.24, 95% CI (-0.38; -.10) p = <0.001. These two fissures showed no significant difference in speed of development with advancing GA. The remaining three fissures (parieto-occipital, calcarine and central) showed no significant differences in the adjusted models as compared to controls (table 2). The parieto-occipital did show significant difference in speed of development: p = 0.02, indicating that the difference between cases and controls decreased as GA progressed (as is visible in figure 4b). None of the analysed cortical areas showed significant differences in cortical development as compared to controls.

Discussion

This study describes the largest number of extended neurosonography examinations performed in a consecutive cohort of fetuses with isolated congenital heart defect cases. The Sylvian and cingulate fissures were found to be significantly delayed in CHD fetuses as compared to controls. In line with previous work from our group, the differences are, however, very small, less than 0.25 grade point per fissure. It remains the question, whether this finding is clinically relevant and explains the reported neurodevelopment delay in CHD children.

Mild delays in maturation were observed in the Sylvian and cingulate fissures in our cohort. The magnitude of delay did not change during the course of pregnancy. In a study describing newborns prior to cardiac surgery, sulcal depth differences were found in the peri-Sylvian region[8]. Two ultrasound studies in fetuses with a comparable design as ours, but with a smaller sample size[17, 18], showed results that were not in agreement with each other. Peng et al. showed reduced Sylvian fissure depth comparable to our results, but Koning et al. only found reduced insular depth. In contrast to our study in which we found normal development of the parieto-occipital fissure, both Peng and Koning found this fissure to be delayed in development. The differences between our results and Peng and Koning may be explained by the fact that they performed measurements of fissure depth whereas we used a grading system with excellent intra-observer variation, that allows complete and more accurate assessment of a fissure. Furthermore, our study describes a much larger cohort than Koning[17] and Peng[18], which allows more robust conclusions.

Magnetic Resonance Imaging (MRI) has also been performed to study cortical development in fetuses. In addition to sulcal depth, MRI is able to express the extent of delay by assessment of cortical development, white matter aspect and gyrification index. These studies showed statistically significant delays compared to control fetuses for the Sylvian fissure and all other visible sulci [19-22]. The extent of the delays found in these MRI-studies are much more profound than in the ultrasound studies, which suggests this to be a more precise modality to detect changes in fissure depth. The generalizability of these MRI studies towards all CHD fetuses is, however, questionable, as these concerned a single measurement in a broad range of gestational ages and included only cases at the severe end of the spectrum[1, 22].
The advantage of the assessment of brain development by ultrasound -in the hands of experienced sonographers- is the low cost and the availability of ultrasound technology, providing the possibility to perform serial neurosonography scans to assess cortical development during the course of pregnancy to all cases that present in a fetal cardiology unit, which prevents selection bias

The exact mechanisms behind the described delays in neurodevelopment are, however, not fully understood. Theoretically, the brain of a fetus with severe CHD resides in a state of relative hypoxia due to the cardiac lesion[23], and it is thus conceivable that the effect on cortical maturation is similar to studies in lamb fetuses in hypoxia[24]. Several studies, that categorized their cases according to their effect on oxygenation towards the fetal brain, have shown that fetuses with hypoxic cardiac lesions show delayed cortical development, increased frequency of brain lesions and smaller head circumferences[6, 25, 26]. However, long-term neurodevelopmental outcome in these fetuses, is generally good, with normal IQ's[27], if we take TGA children as an example (with prenatal detection, and optimal peripartum and perioperative care). A second indication that hemodynamic effects are not the sole explanation for alterations in the fetal brain is the fact that reduced head circumference is found in fetuses with non-hypoxic CHD's as well[11]. Therefore, we have chosen to analyse all types of CHD together, to prevent selection bias as we included a consecutive cohort of fetuses.

Many authors have studied the insular region of the fetal brain, as altered development of the insular region is correlated to decreased Sylvian fissure depth because of the close relation of both regions. Since the insula is known to have connections to many other parts of the brain, reduced development of this structure is associated with cognitive and behavioral deficits, the insular region is correlated with speech, emotion-regulation and social interaction[28, 29]. Reduced insular cortical thickness in growth-restricted fetuses correlates with altered neurobehavior [5]. As CHD children display deficits on multiple domains including attention deficit disorder, the found delay in both the Sylvian fissure as well as the cingulate fissure, which is close to the insula as well, might be associated with these problems later in life. As CHD-children have been known to display deficits on multiple cognitive domains[4, 30], the delays found in the Sylvian fissure in our study might play a role in these restrictions. Moreover, alterations in the cingulate fissure area, as were found in our study, are associated with attention deficit disorder[31, 32], which is significantly more prevalent in CHD children[33]. Therefore, it is possible the delay in maturation found in our study in the Sylvian and cingulate fissures are associated with altered cognitive and behavioral problems in later life.

Alternative pathways that could explain cognitive and behavioral impairment in CHD-children have to be considered. Multiple factors, like genetics, epigenetics, the absence of a prenatal detection of the defect, peripartum setting and complications around cardiothoracic procedures play a role in the long-term neurodevelopment in these children.

A strength of the current study is the prospective inclusion of a large number of consecutive cases with congenital heart defects, and the thorough scrutiny of included cases, resulting in the exclusion of cases with additional abnormalities and suspected or proven genetic syndromes.
Another strength of the methodology is the use of the grading system, which allows to view the morphology of a fissure instead of depth alone, to express the development numerically. We feel that measuring a sulcus could potentially overestimate the development of a sulcus, and therefore lead to incorrect conclusions.
As previously discussed, the findings in our large US study in isolated CHD cases underline cortical delays found in previous MRI studies. The found delays are, however, much smaller, making us question the extent of delay mentioned in previous studies. The modest differences in CHD-cases compared to controls (uncorrected for multiple testing) found in our study may also mean that these differences have little clinical implications.
In the current study, transabdominal scanning of sufficient quality (only 5.6% of scans were excluded due to suboptimal quality) was obtained. Since we have scanned both groups with the same protocol and baseline characteristics are similar, both groups are considered comparable to each other. In our ongoing cohort of CHD cases, we are including transvaginal sequences in addition to transabdominal imaging, as transvaginal scanning in vertex presenting fetuses can produce superior image quality[34].
The absence of the possibility to correlate of our findings with postnatal neurodevelopment at this stage is a limitation of the current study, since small differences in fetal life could theoretically lead to cumulative delays in childhood.

Conclusion

Minor changes in cortical development were observed in fetuses with congenital heart defects, in the areas that are associated with cognitive and behavioral deficits detected with serial ultrasonography. However, these changes were less profound than previously reported, and mostly stable throughout pregnancy, which limits firm conclusion on prenatal decelerated maturation in CHD.

References

1 Majnemer A, Limperopoulos C, Shevell MI, Rohlicek C, Rosenblatt B, Tchervenkov C. **A new look at outcomes of infants with congenital heart disease.** Pediatric neurology. 2009;40(3):197-204.

2 Jansen FA, Everwijn SM, Scheepjens R, et al. **Fetal brain imaging in isolated congenital heart defects - a systematic review and meta-analysis.** Prenat Diagn. 2016;36(7):601-13.

3 Bellinger DC, Wypij D, Duplessis AJ, et al. **Neurodevelopmental status at eight years in children with dextro-transposition of the great arteries: the Boston Circulatory Arrest Trial.** J Thorac Cardiovasc Surg. 2003;126(5):1385-96.

4 Bellinger DC, Wypij D, Rivkin MJ, et al. **Adolescents with d-transposition of the great arteries corrected with the arterial switch procedure: neuropsychological assessment and structural brain imaging.** Circulation. 2011;124(12):1361-9.

5 Egana-Ugrinovic G, Sanz-Cortes M, Figueras F, Couve-Perez C, Gratacos E. F**etal MRI insular cortical morphometry and its association with neurobehavior in late-onset small-for-gestational-age fetuses.** Ultrasound Obstet Gynecol. 2014;44(3):322-9.

6 Masoller N, Martinez JM, Gomez O, et al. **Evidence of second-trimester changes in head biometry and brain perfusion in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol. 2014;44(2):182-7.

7 Limperopoulos C, Tworetzky W, McElhinney DB, et al. **Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy.** Circulation. 2010;121(1):26-33.

8 Ortinau C, Alexopoulos D, Dierker D, Van Essen D, Beca J, Inder T. **Cortical folding is altered before surgery in infants with congenital heart disease.** The Journal of pediatrics. 2013;163(5):1507-10.

9 Licht DJ, Shera DM, Clancy RR, et al. **Brain maturation is delayed in infants with complex congenital heart defects.** J Thorac Cardiovasc Surg. 2009;137(3):529-36; discussion 36-7.

10 Masoller N, Sanz-Cortes M, Crispi F, et al. **Severity of Fetal Brain Abnormalities in Congenital Heart Disease in Relation to the Main Expected Pattern of in utero Brain Blood Supply**. Fetal Diagn Ther. 2016;39(4):269-78.

11 Matthiesen NB, Henriksen TB, Gaynor JW, et al. **Congenital Heart Defects and Indices of Fetal Cerebral Growth in a Nationwide Cohort of 924,422 Liveborn Infants.** Circulation. 2016.

12 **International Society of Ultrasound in O, Gynecology Education C. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram'.** Ultrasound Obstet Gynecol. 2007;29(1):109-16.

13 Clouchoux C, Kudelski D, Gholipour A, et al. **Quantitative in vivo MRI measurement of cortical development in the fetus.** Brain Struct Funct. 2012;217(1):127-39.

14 Mlczoch E, Brugger P, Ulm B, et al. **Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program.** Eur J Paediatr Neurol. 2012.

15 Pistorius LR, Stoutenbeek P, Groenendaal F, et al. **Grade and symmetry of normal fetal cortical development: a longitudinal two- and three-dimensional ultrasound study.** Ultrasound Obstet Gynecol. 2010;36(6):700-8.

16 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. **Prenatal diagnosis of congenital heart defects: accuracy and discrepancies in a multicenter cohort.** Ultrasound Obstet Gynecol. 2016;47(5):616-22.
17 Koning IV, van Graafeiland AW, Groenenberg IAL, et al. **Prenatal influence of congenital heart defects on trajectories of cortical folding of the fetal brain using three-dimensional ultrasound.** Prenat Diagn. 2017;37(10):1008-16.
18 Peng Q, Zhou Q, Zang M, et al. **Reduced fetal brain fissures depth in fetuses with congenital heart diseases.** Prenat Diagn. 2016;36(11):1047-53.
19 Masoller N, Sanz-Cortes M, Crispi F, et al. **Mid-gestation brain Doppler and head biometry in fetuses with congenital heart disease predict abnormal brain development at birth.** Ultrasound Obstet Gynecol. 2016;47(1):65-73.
20 Ortinau CM, Rollins CK, Gholipour A, et al. **Early-Emerging Sulcal Patterns Are Atypical in Fetuses with Congenital Heart Disease.** Cereb Cortex. 2018.
21 Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. **Delayed cortical development in fetuses with complex congenital heart disease.** Cereb Cortex. 2013;23(12):2932-43.
22 Jaimes C, Rofeberg V, Stopp C, et al. **Association of Isolated Congenital Heart Disease with Fetal Brain Maturation.** AJNR Am J Neuroradiol. 2020;41(8):1525-31.
23 Lauridsen MH, Uldbjerg N, Henriksen TB, et al. **Cerebral Oxygenation Measurements by Magnetic Resonance Imaging in Fetuses With and Without Heart Defects.** Circ Cardiovasc Imaging. 2017;10(11):e006459.
24 Rees S, Stringer M, Just Y, Hooper SB, Harding R. **The vulnerability of the fetal sheep brain to hypoxemia at mid-gestation.** Brain Res Dev Brain Res. 1997;103(2):103-18.
25 Sun L, Macgowan CK, Sled JG, et al. **Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.** Circulation. 2015;131(15):1313-23.
26 Kelly CJ, Makropoulos A, Cordero-Grande L, et al. **Impaired development of the cerebral cortex in infants with congenital heart disease is correlated to reduced cerebral oxygen delivery.** Sci Rep. 2017;7(1):15088.
27 Lim JM, Porayette P, Marini D, et al. **Associations Between Age at Arterial Switch Operation, Brain Growth, and Development in Infants With Transposition of the Great Arteries.** Circulation. 2019;139(24):2728-38.
28 Peschke C, Ziegler W, Eisenberger J, Baumgaertner A. **Phonological manipulation between speech perception and production activates a parieto-frontal circuit.** Neuroimage. 2012;59(1):788-99.
29 Gasquoine PG. **Contributions of the insula to cognition and emotion.** Neuropsychol Rev. 2014;24(2):77-87.
30 Morton PD, Ishibashi N, Jonas RA. **Neurodevelopmental Abnormalities and Congenital Heart Disease: Insights Into Altered Brain Maturation.** Circ Res. 2017;120(6):960-77.
31 Nakao T, Radua J, Rubia K, Mataix-Cols D. **Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication.** Am J Psychiatry. 2011;168(11):1154-63.

32 Zhu CZ, Zang YF, Cao QJ, et al. F**isher discriminative analysis of resting-state brain function for attention-deficit/hyperactivity disorder.** Neuroimage. 2008;40(1):110-20.

33 DeMaso DR, Calderon J, Taylor GA, et al. **Psychiatric Disorders in Adolescents With Single Ventricle Congenital Heart Disease.** Pediatrics. 2017;139(3).

34 Malinger G, Paladini D, Haratz KK, Monteagudo A, Pilu GL, Timor-Tritsch IE. **ISUOG Practice Guidelines (updated): sonographic examination of the fetal central nervous system. Part 1: performance of screening examination and indications for targeted neurosonography.** Ultrasound Obstet Gynecol. 2020;56(3):476-84.

Tables

Table 1 *Baseline characteristics for congenital heart defect cases and controls*

Characteristics	CHD cases	Controls	p-value
	97 subjects	**61 subjects**	
Maternal Age in years - Mean (SD)	29 (4.2)	32 (4.6)	0.01
BMI (kg/m2) - Mean (SD)	23.7 (4.3)	23.6 (3.9)	0.97
Maternal diabetes - n (%)	3 (3)	0	0.17
Primigravidae - n (%)	36 (37)	20 (33)	0.35
Male gender- n (%)	60 (62)	28 (46)	0.04
no. of CHD cases			**n.a.**
Aortic Arch Hypoplasia and/or Aortic Stenosis- n (%)	24 (25)		
Tetralogy of Fallot or Fallot-like defect n (%)	14 (14)		
Transposition of the Great Arteries - n (%)	14 (14)		
Tricuspid or Pulmonary Atresia n (%)	6 (6)		
HLHS - n (%)	5 (5)		
(un)balanced atrioventricular septal defect n (%)	5 (5)		
Ventricular Septal defect n (%)	3 (3)		
Other major CHD† n (%)	17 (18)		
Other minor CHD‡ n (%)	9 (9)		
Excluded cases n(%)			
Postnatal normal heart	4 (4)		
Non-isolated cases	8 (8)		
Pregnancy outcome n(%)			**n.a.**
Live birth	81 (95)	61 (100)	
Termination of Pregnancy	4 (5)	0 (0)	
Mean GA at scanning - Mean (SD)			
20 weeks	20.9 (0.8)	21.1 (0.8)	0.49
24 "	24.1 (0.8)	24.2 (1.0)	0.77
28 "	28.3 (0.8)	28.1 (0.8)	0.36
32 "	32.2 (0.8)	32.1 (0.6)	0.49
36 "	36.2 (0.7)	35.9 (0.5)	0.04

***P*=<0.05, Statistically significant.

† Other major CHD include: Truncus Arteriosus, Multiple level left obstruction syndrome (Shone's complex), Double Outlet Right Ventricle-TGA, Congenitally Corrected TGA without additional cardiac anomalies, AVSD with Pulmonary Atresia, Aortic-left ventricular tunnel with severe distention of the left ventricle.

‡ **Other minor CHD include:** Persistent left caval vein without obstruction of the left atrioventricular flow, Restrictive Foramen Ovale, mild pulmonary stenosis.

Table 2 *Results of mixed model analysis in fissure grading*

		Average difference in grade over all times			Difference in maturation speed
		difference	**95% CI**	***p***	***p***
Sylvian	*Unadjusted*	-0.11	-0.21 ; -0.01	0.03*	0.90
	Adjusted	-0.12	-0.23 ; -0.01	0.05*	0.97
Parieto-Occipital	*Unadjusted*	-0.08	-0.18 ; 0.02	0.11	0.01*
	Adjusted	-0.05	-0.16 ; 0.06	0.36	0.02*
Central	*Unadjusted*	-0.08	-0.19 ; -0.04	0.21	1.00
	Adjusted	-0.06	-0.21 ; 0.09	0.44	0.98
Cingulate	*Unadjusted*	-0.22	-0.34 ; -0.11	<0.01*	0.08
	Adjusted	-0.24	-0.37 ; 0.10	<0.01*	0.19
Calcarine	*Unadjusted*	-0.13	-0.24 ; 0.02	0.02*	0.04*
	Adjusted	-0.09	-0.21 ; 0.03	0.15	0.07

Overall difference in grading represents the mean difference between scores in CHD and control cases. Speed of maturation represents the test on differences in progression with GA between scores of CHD and control cases. *p <0.05 is considered statistically significant. Unadjusted: Outcome of mixed model analysis, unadjusted for confounders. Adjusted: Outcome of mixed model analysis adjusted for maternal age, maternal BMI, maternal diabetes, parity and fetal gender.

Table 3 *Results of mixed model analysis in cortical area grading*

		Average difference in grade over all times			Difference in maturation speed
		difference	95% CI	*p*	*p*
Frontal	*Unadjusted*	-0.01	-0.12 ; 0.13	0.97	0.10
	Adjusted	-0.11	-0.26 ; 0.04	0.14	0.38
Parietal	*Unadjusted*	-0.07	-0.19 ; 0.05	0.24	0.01*
	Adjusted	0.10	-0.25 ; 0.04	0.16	0.06
Temporal	*Unadjusted*	-0.09	-0.22 ; -0.03	0.15	0.01*
	Adjusted	-0.12	-0.26 ; 0.03	0.11	0.09
Occipital	*Unadjusted*	-0.07	-0.17 ; 0.03	0.23	0.08
	Adjusted	-0.08	-0.20 ; 0.04	0.17	0.47

Overall difference in grading represent the difference between scores in CHD and control cases. Speed of maturation represents the test on differences in progression with GA between scores of CHD and control cases. *p <.05 is considered statistically significant. Unadjusted: Outcome of mixed model analysis, unadjusted for confounders. Adjusted: Outcome of mixed model analysis, adjusted for maternal age, maternal BMI, maternal diabetes, parity and fetal gender.

Figure 1 *Sylvian fissure staging*

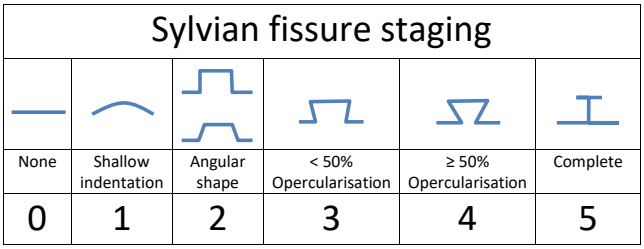
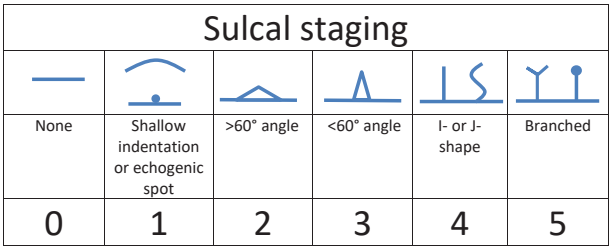
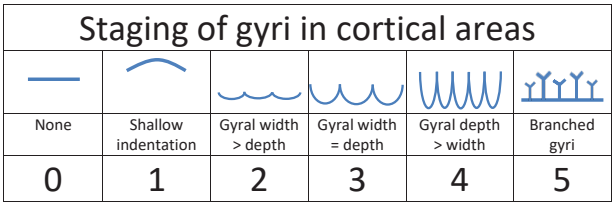


Figure 2 *Sulcal staging*



Sulcus: parieto-occipital, central, superior temporal, calcarine and cingulate progressing with gestational age from no visible echoscopic sign of sulcation, to end-stage sulcation.

Figure 3 *Cortical area staging*



Frontal, parietal, temporal and occipital cortical areas progression with gestational age from no visible echoscopic sign of sulcation, to end-stage sulcation.

Figure 4 *Progression of sulcal staging throughout pregnancy.*
X-axis: sulcal grades, y-axis: gestational age in weeks.
Continuous line (──): CHD-fetuses, dotted line (· · ·) control fetuses.

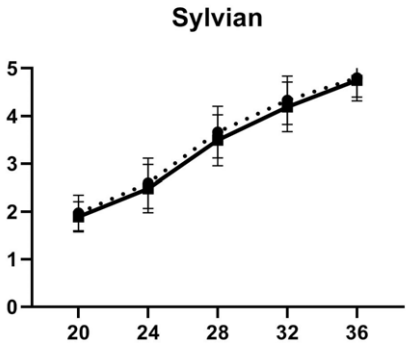


Figure 4a *Sylvian fissure development*

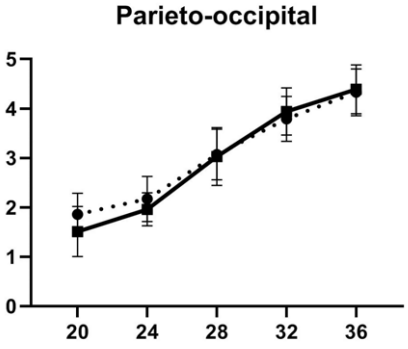


Figure 4b *Parieto-occipital sulcus development*

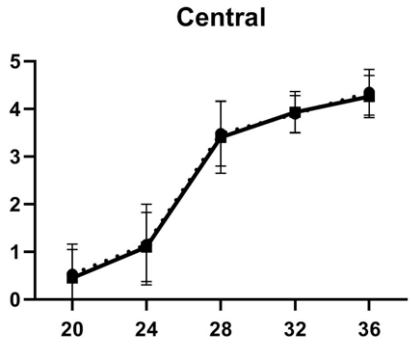


Figure 4c *Central sulcus development*

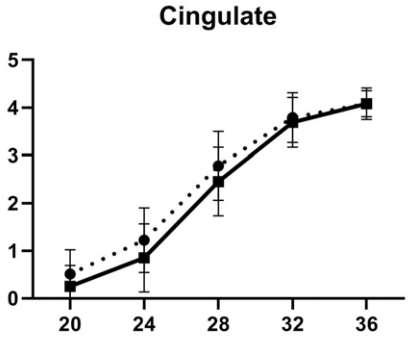


Figure 4d *Cingulate sulcus development*

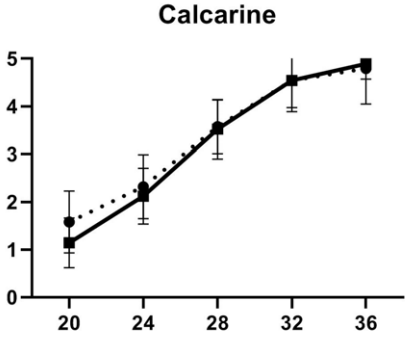


Figure 4e *Calcarine sulcus development*

Figure 5 *Progression of brain area staging throughout pregnancy.*
X-axis: sulcal grades, y-axis: gestational age in weeks.
Continuous line (──): CHD-fetuses, dotted line (· · ·) control fetuses

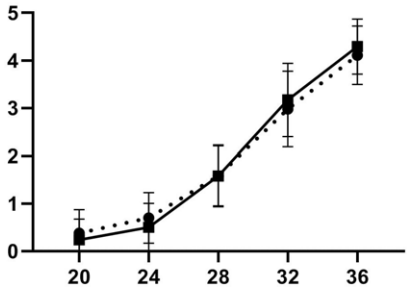


Figure 5a *Frontal area development*

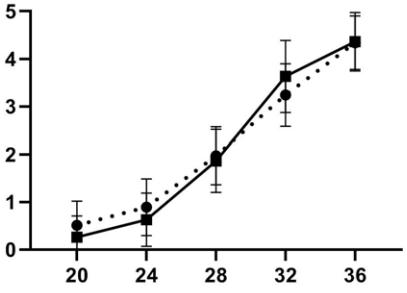


Figure 5b *Parietal area development*

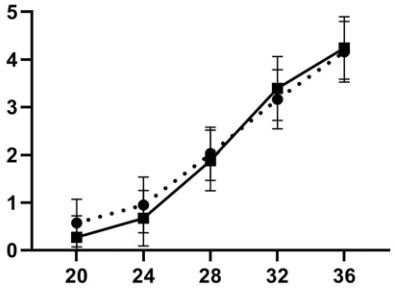


Figure 5c *Temporal area development*

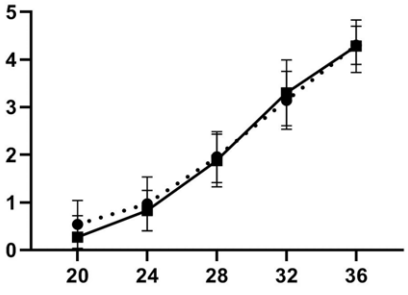


Figure 5d *Occipital area development*

CHAPTER

6

Cortical development in fetuses with congenital heart defects using an automated brain-age prediction algorithm

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ABSTRACT

INTRODUCTION: Congenital heart defects are associated with neurodevelopmental delay. It is hypothesized that fetuses affected by congenital heart defect (CHD) have altered cerebral oxygen perfusion and are therefore prone to delay in cortical maturation. The aim of this study was to determine the difference in fetal brain age between consecutive CHD cases and controls in the second and third trimester using ultrasound.

METHODS: Since 2014, we have included all isolated major and minor CHD cases in the Heart And Neurodevelopment(HAND)-study. Every four weeks, detailed neurosonography was performed in these fetuses, including the recording of a 3D volume of the fetal brain, from 20 weeks onward. 75 healthy fetuses underwent the same protocol to serve as a control group. The volumes were analyzed by automated age prediction software which determines gestational age by the assessment of cortical maturation.

RESULTS: In total 513 volumes were analyzed using the age prediction software (219 volumes of 104 CHD cases; 278 volumes of 75 controls). 16 (3.2%) volume recordings were excluded because of imaging quality. The age distribution was 19-33 weeks. Mixed model analysis showed that the age predicted by brain maturation was 2.9 days delayed compared to the control group (p = 0,064).

CONCLUSION: This study shows that fetuses with isolated cases of congenital heart defects show some delay in cortical maturation as compared to healthy control cases. The clinical relevance of this small difference is debatable. This finding was consistent throughout pregnancy and did not progress during the third trimester.

INTRODUCTION

Improvements over time in the quality of neonatal care and cardiothoracic surgery in children with congenital heart defects (CHD) have resulted in an increased survival of children with severe CHD. This has stimulated longer term follow up and a recognition that there is an association between CHD and impaired neurodevelopmental (ND) outcome.[1, 2] Developmental delay, decreased IQ and behavioural disorders have been reported, even in non-syndromic CHD children.[1] Previously, these sequelae were attributed to perioperative hypoxia or thromboembolic events during surgery. Recent studies suggest, however, that signs of abnormal neurological development may be present prior to surgery.[3-5] Imaging studies in pregnancy using Magnetic Resonance Imaging (MRI)[6, 7] and ultrasound [8, 9], have shown signs of delayed fetal brain development. It has been suggested that it is these abnormal findings that result in the altered neurological outcome later in life.[5, 6] The hypothesized mechanism is that the abnormal development of the brain is the result of altered brain oxygenation in fetal life.[10, 11]

However, there is no robust evidence for delayed fetal brain maturation, because the current studies are subject to potential bias due to the small number of included affected women and due to selection of participants with regard to the type of cardiac defect. [4]

Therefore, the aim of this study is to assess fetal brain development and maturational changes over time in a prospective, consecutive cohort of fetuses with isolated CHD, to avoid selection bias. In this study, ultrasound (US) imaging was used, this not only enables the inclusion of a larger number of fetuses (and thus reduces selection bias), but also facilitates multiple examinations in the same fetus, to evaluate brain development and changes over time. Furthermore, the used technique assesses brain maturation automatically and is therefore blinded, which, in combination with repeated measurements, are important differences with previous studies.

We hypothesize that the patterns of brain maturation of fetuses with CHD are delayed compared to control fetuses.

Methods

Data acquisition

All consecutive pregnant women, diagnosed with a fetal CHD before 32 weeks gestation at the Leiden University Medical Center between March 2014 and December 2016, were approached to participate in the Heart And Neurodevelopment (HAND)-study. To account for natural variation of cortical development in the healthy population, we constructed a control group by the recruitment of unselected pregnant women after a normal structural anomaly scan. Control cases were not offered addi-

tional genetic testing but had a postnatal visit in which dysmorphic featured were assessed. Gestational age (GA) in both the CHD cases and the control cases, was based on first-trimester ultrasound at approximately 10 weeks gestation, according to Dutch national guidelines. For both cases and controls we excluded: Maternal age <18 years, multiple gestation, genetic or syndromic defects (prenatally diagnosed or postnatally apparent up to the age of six months), cases with placental pathology (pre-eclampsia, severe growth restriction) and cases that showed normal cardiac anatomy after birth. In the CHD group, non-isolated cases were excluded. The reasons for only including isolated CHD was that altered neurodevelopment could otherwise be attributed to genetic or syndromic defects. Furthermore, cases with aortic valve stenosis that underwent fetal balloon valvuloplasty were excluded, since fetal brain oxygenation may have changed due to the intervention during pregnancy.[12] Also, strictly minor cases (persistent left caval vein, mild pulmonary stenosis and restrictive foramen ovale) were also excluded, since in these cases, blood flow towards the brain is expected to be uncompromised. The sample size calculation for the normal reference population was based on the available evidence from two MRI-studies[7, 13] that compared HLHS-fetuses to controls, to detect a difference in mean brain age of two weeks. The normal reference population was calculated to consist of 60 fetuses. The CHD-group contains all the women that met the inclusion criteria and were referred between March 2014 – December 2016.

A CHD in combination with minor associations – namely a single umbilical artery; enlarged first-trimester nuchal translucency with normal chromosomal analysis and small for gestational age with normal Doppler recordings, were considered as isolated CHD. These cases were not excluded unless genetic syndromes became apparent postnatally.

A detailed neurosonographic examination was performed in cases and controls every four weeks after the diagnosis or in the case of controls after normal standard anomaly scan. Examinations were undertaken by experienced sonographers (FJ/AT/SE) using a RAB 6-D three-dimensional transducer on a Voluson E8 or E10 (GE Healthcare ultrasound, Milwaukee, WI, USA). The examination was conducted transabdominally in four scanning planes: axial, coronal, sagittal and parasagittal. At these visits, we assessed the presence of structural brain anomalies and fetal biometry. Multiple 3D volume recordings were obtained in the axial plane, starting at head circumference level of the transthalamic plane. The 3D acquisition was performed in the maximum quality setting (6-12 seconds) or on high quality setting (2-8 seconds) to limit the amount of movement artefacts.

Brain-Age prediction algorithm

The evaluation of brain maturation by 2D ultrasound imaging is known to have an agreeable rate of inter-observer variation. Data from recent MRI studies show a strong correlation between the degree of gyrification and gestational age[14, 15], neuropathologists consider the appearance and stage of the sulci to be so precise[14] that cortical complexity can be used as an accurate proxy for intrauterine neurodevelopment. Therefore, we used a semi-automated age prediction algorithm as a proxy for cortical maturation. At each visit a mean of 2.7(0.9) 3D volumes for cases and 3.5(1.2) for controls were recorded. These volume recordings were examined to identify cases with poor acquisition quality due to fetal motion artifacts. The recording with the highest quality was selected to enter into the algorithm. All 3D volumes were processed with a study-code, which did not reveal the presence of a heart defect or not. Plane localization was annotated manually in each 3D volume using the ITK-SNAP tool.[16] The algorithmic details on the process of predicting brain maturation from a 3D ultrasound volume were previously described.[17] Briefly, a 3D surface-based coordinate system is spatially aligned to the cranial pixels in the image. This coordinate system allows for the sampling of brain regions based on surface locations. The US image and its corresponding surface are passed into a regression forest model, where they traverse the nodes of a set of pre-trained binary decision trees, within the forest. At each node, a binary test is applied to a sampled brain region to evaluate whether it is indicative of a more or less advanced stage of maturation. In this way, each brain region (eg callosal sulcus, thalamic region, cingulate sulcus, Parieto-Occipital Fissure (POF), Sylvian Fissure (SF), central sulcus and ventricular regions) votes for a particular brain age (figure 1). The final prediction of brain maturation is achieved by averaging the votes from the brain regions, across the full set of decision trees in the forest. Thus, the algorithm is able to estimate the brain-age according the pattern of gyrification of the fetal cortex, which varies during gestation (figure 2). Furthermore, since the true gestational age was known for each case, we were able to compare the brain-age with the true age to determine any delay in cortical maturation. A more extensive description of the algorithm is available as supplement material (Supporting information).

Data handling

The prenatal diagnosis was compared to the postnatal echocardiographic findings. In case of discrepancy, the postnatal diagnosis or the results of post mortem examination (PME) in case of pregnancy termination, were considered as the definitive cardiac diagnosis. In cases in which the parents did not give consent for PME, the prenatal diagnosis was used for this study. We have previously shown that the rate of discrepancies is low in our unit.[18]

Statistical analysis

We investigated evidence of the presence of systematic between-group differences in brain age, as calculated by the age prediction algorithm, between the CHD group and the control group. We have selected the data from measurements at 19 – 33 weeks since the age prediction algorithm had been validated in this gestational age.[17]

As multiple volume measurements were acquired from the same patient during pregnancy (longitudinal repeated-measures data) linear mixed modelling must be applied to account for systematic within-patient correlation. The mixed-effect regression model corrected for GA (assumed to relate linearly to the age prediction), group (CHD-cases vs controls) and the interaction between GA and group as fixed effects. Within-patient correlation was modelled by inclusion of a random-effect intercept per individual. The presence of a between-group difference was then assessed by removing both the interaction term and the main group effect from the full model and assess the associated likelihood ratio test with two degrees of freedom. As the likelihood ratio test confirmed the presence of group effect, two follow-up hypothesis tests were investigated. Firstly, the main group difference was assessed at the median GA by comparing the (marginal) mean brain-age in that set. Secondly, regression slopes were compared between CHD-cases and controls to assess whether groups differed in their maturation speed. In a sensitivity analysis we repeated the tests allowing for a quadratic effect of GA. All statistical analysis were performed using IBM SPSS statistics version 24.0.0.0 (IBM, Armonk, NY, USA). Statistical significance was determined when $p \leq 0.05$.

Ethical Approval

This study was approved by the local ethics committee on March 17, 2014 under ref. number P13.107.

Results

In the study period, 90 consecutive CHD cases and 75 controls were included (*see Table 1 for study characteristics).* The groups were not prospectively matched for baseline characteristics, however the groups did not differ significantly in maternal age, parity, BMI or maternal diabetes. We excluded 14 cases according the defined exclusion criteria, of which eight were postnatal diagnoses of genetic syndromes (three CHARGE syndrome, two Kabuki syndrome, three with postnatal multiple dysmorphic features, final genetic diagnosis pending). The genetic diagnosis of the CHD-cases was followed up until one year postnatally. 30 % of control cases opted for first-trimester screening. No genetic or structural abnormalities were found in the control-group up to six months postnatally. Thus, a total of 152 CHD cases and

controls were eligible for analysis. From these 152 women, in 493 scanning sessions, volume recordings were made. Of these volumes, 16 (3.2%) were excluded due to ultrasonographic factors (oblique insonation, fetal movement artifacts or very poor image quality), resulting in 477 volumes suitable for analysis by the age-prediction algorithm. In total, 199 volumes in 77 cases (mean of 2.4 recorded volumes per woman) and 278 volumes in 75 controls (mean of 3.7 recorded volumes per woman) were analyzed using the automated age prediction algorithm. The CHD cases were scanned at 1-5 different time points during pregnancy, with 63% of the women scanned more than once. For the control group, all cases were measured more than once.

The fetal brain-age of the healthy control cases was calculated by the age prediction algorithm. This cohort of normal fetuses showed a calculated brain-age by the algorithm which did not statistically differ from the true gestational age [17], based on first-trimester ultrasound suggesting the model is applicable to our cohort. The predicted brain-age increased perfectly linear in the second trimester and the algorithm tends to slightly underestimate the brain age during the third trimester (figure 3).

The overall test indicated that the time trend significantly differed between CHD-cases and controls (*p=0.005*) indicating that indeed there was a group effect. When comparing CHD cases with controls, the brain-age determined by the algorithm was lower compared to controls at the median true gestational age (26.20 vs 26.61 weeks; difference 3.0 days, 95% CI (1.07 – 4.63) *p = 0.001*. (figure 4) The speed of the development of the brain maturation (i.e. slopes of the curves), between both groups did not differ statistically significant. Cortical maturation was estimated to increase with 4.45 vs 4.52/days per week (*p = 0.78)* for CHD cases and controls, indicating similar speed of maturation between CHD cases and controls. This was also analyzed with a quadratic age trend analysis, which confirmed the similar increase in cortical maturation between cases and controls.

Discussion

In this study of a consecutive cohort of fetuses with isolated CHD fetuses, we found a delay in fetal brain-age of 3.0 days, compared to normal fetuses. The delay was continuous throughout our study period, which opposes the earlier findings that suggest further delay in cortical maturation with advancing gestation.[7, 19] This study is the first to implement a validated automated algorithm to assess fetal cortical development using ultrasound, to a clinically relevant group.

Neurodevelopmental delay in CHD children has been recognized for decades, even with optimized pre-operative and neonatal care.[20] prenatal brain damage is hypothesized to result from the altered hemodynamics caused by the cardiac defect,

which may result in decreased flow or oxygenation of the blood directed towards the brain [21, 22], resulting in delayed brain development. Increased NAA:Cho-ratio and increased lactate levels in MRI and spectroscopy studies support a decrease in brain oxygenation in the developing fetal brain of fetuses with CHD.[19, 23]

Cortical maturation by measuring fissure depth has been described before using both MRI [14] and US [24, 25]in non-CHD fetuses, application of these techniques show significant differences in the depth of the POF and Calcarine fissure in CHD cases as compared to controls. [6-9] These fissures were also reported to be shallower in CHD neonates when compared to controls with a comparable gestational age [3], which was explained as delayed maturation. The findings in these studies are, however, not in full agreement with each other. A significant decrease in depth of the SF, POF and Calcarine fissure was found by some authors [8] whereas others did not find a significant difference in the SF depth [6], but did find an overall decrease in brain maturation. [9] The differences in the results of these studies can be explained by the small sample sizes, different methodologies, and the differences in statistical analysis of the data.[9]

Our study is the first to convey the development of cortical maturation with ultrasound by using maturational age as an outcome measure. Thus, the used methodology in this study is capable to determine the extent of the delay, which was demonstrated to be small (3.0 days). Moreover, we do not see a difference in the slopes of the development between CHD and control cases, indicating no further delay in the cortical growth trajectory in the third trimester, as described by other authors.[7, 9] A possible explanation for this absence of third trimester difference, might be that the role of fetal brain oxygenation is being magnified in literature due to case selection. Decreased head growth as a proxy for brain development and developmental delay has however also been demonstrated in other types of CHDs which suggest a role for placental, genetic or epigenetic factors.

A common method of assessing fetal cortical development in the previously mentioned studies is a manual, sometimes unblinded, measurement of the depth of two-three fissures.[6-9] The applied algorithm in our study automatically selects the most age-discriminating regions of the entire fetal brain. As cortical maturation is an excellent proxy for brain age, this does not imply that the sulcation in itself is a linear phenomenon. [14, 26] The sampled locations (eg callosal, cingulate and central sulcus, thalamic region, POF and SF) are proven as the most distinct points to assess maturation speed, as the algorithm used automated deep learning in a large cohort of normal fetuses.[17] It is therefore arguable if the maturation patterns of the commonly chosen fissures in previous studies (SF, POF and calcarine fissure) are sensitive enough to detect brain maturation and representative of the global cortical

development, as our algorithm selected more sulci to be able to assess brain-age with a precision of 6 days.[17]

Another important difference with previous studies is the fact that we included cases with isolated CHD and have excluded neonates that were diagnosed with genetic syndromes (routinely tested with micro-array or whole exome sequencing) after birth. Although previous studies report the exclusion of aneuploid fetuses [6-8], only de Koning et al.[9] report the postnatal exclusion of syndromic cases. Since a significant amount of genetic syndromes present with mental retardation, abnormal brain development could be caused not solely by the CHD in itself.

Whether a delay of three days is clinically relevant, is debatable. On the other hand one could argue that even though differences are small, they could still have an impact on long term outcome, since the detected delay is visible in early life.[27] Two of the studies mentioned above[6, 8] found significant differences when correlating cortical maturation and neurodevelopmental outcome by performing Bayley Scales of Infant and Toddler Development (BSID). However, authors performed BSID in a minority of infants and, paradoxically, only in milder CHD cases. With this sparse evidence, it is undisputable that there is an urgent need to explore the relation between altered fetal brain maturation and neurodevelopmental outcome further. As this is a limitation of the current study, we are planning to correlate the findings in this cohort to postnatal neurodevelopment.

It is controversial which imaging modality is superior to detect abnormalities in fetal brain development. While we do acknowledge the fact that MRI is regarded as the gold standard for detecting structural brain abnormalities[28], both previously mentioned MRI-studies only comprise a single MRI acquisition during pregnancy, with slice thicknesses of 1,5-3 mm, which will influence the accuracy of the measurements as well. We believe that repeated measurements by US in the hands of experienced sonographers is sensitive enough to study brain maturation trajectories.

A limitation of this study is the assessment of all CHD-cases combined. We acknowledge that fetuses with lower oxygen delivery to the brain might be prone to delayed cortical development, reduced head circumference and brain lesions.[10, 19, 22, 29] However, reduced head circumference, as a proxy for brain development, has been reported in fetuses with only a single VSD.[30] We have chosen to not stratify according to CHD, as the current group is too small to make statements on cortical development. Stratification according lesion physiology will be possible in the future as we continue monitoring these cases.

A second limitation is the upper GA limit of included cases, because brain visibility is obscured due to acoustic shadowing and fetal position in the late third trimester.

Conclusion

In conclusion, this study shows that fetuses with isolated cases of congenital heart defects show some delay in cortical maturation as compared to healthy control cases. The clinical relevance of this small difference is debatable. This finding was consistent throughout pregnancy and did not progress during the third trimester.

References

1 Marino BS, Lipkin PH, Newburger JW, et al. **Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.** Circulation 2012;1269:1143-72.

2 Paladini D, Alfirevic Z, Carvalho JS, 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;492:287-8.

3 Licht DJ, Shera DM, Clancy RR, et al. **Brain maturation is delayed in infants with complex congenital heart defects.** J Thorac Cardiovasc Surg 2009;1373:529-36; discussion 36-7.

4 Jansen FA, Everwijn SM, Scheepjens R, et al. **Fetal brain imaging in isolated congenital heart defects - a systematic review and meta-analysis.** Prenat Diagn 2016;367:601-13.

5 Majnemer A, Limperopoulos C, Shevell MI, et al. **A new look at outcomes of infants with congenital heart disease.** Pediatric neurology 2009;403:197-204.

6 Masoller N, Sanz-Cortes M, Crispi F, et al. **Mid-gestation brain Doppler and head biometry in fetuses with congenital heart disease predict abnormal brain development at birth.** Ultrasound Obstet Gynecol 2016;471:65-73.

7 Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. **Delayed cortical development in fetuses with complex congenital heart disease.** Cereb Cortex 2013;2312:2932-43.

8 Peng Q, Zhou Q, Zang M, et al. **Reduced fetal brain fissures depth in fetuses with congenital heart diseases.** Prenat Diagn 2016;3611:1047-53.

9 Koning IV, van Graafeiland AW, Groenenberg IAL, et al. **Prenatal influence of congenital heart defects on trajectories of cortical folding of the fetal brain using three-dimensional ultrasound.** Prenat Diagn 2017;3710:1008-16.

10 Sun L, Macgowan CK, Sled JG, et al. **Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.** Circulation 2015;13115:1313-23.

11 Al NB, van Amerom JF, Forsey J, et al. **Fetal circulation in left-sided congenital heart disease measured by cardiovascular magnetic resonance: a case-control study.** J Cardiovasc Magn Reson 2013;15:65.

12 Prosnitz AR, Drogosz M, Marshall AC, et al. **Early hemodynamic changes after fetal aortic stenosis valvuloplasty predict biventricular circulation at birth.** Prenat Diagn 2018;384:286-92.

13 Mlczoch E, Brugger P, Ulm B, et al. **Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program.** Eur J Paediatr Neurol 2012.

14 Clouchoux C, Kudelski D, Gholipour A, et al. **Quantitative in vivo MRI measurement of cortical**

development in the fetus. Brain Struct Funct 2012;2171:127-39.

15 Studholme C, Rousseau F. **Quantifying and modelling tissue maturation in the living human fetal brain.** Int J Dev Neurosci 2014;32:3-10.

16 Yushkevich PA, Piven J, Hazlett HC, et al. **User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability.** Neuroimage 2006;313:1116-28.

17 Namburete AI, Stebbing RV, Kemp B, et al. **Learning-based prediction of gestational age from ultrasound images of the fetal brain.** Med Image Anal 2015;211:72-86.

18 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. **Prenatal diagnosis of congenital heart defects: accuracy and discrepancies in a multicenter cohort.** Ultrasound Obstet Gynecol 2016;475:616-22.

19 Limperopoulos C, Tworetzky W, McElhinney DB, et al. **Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy.** Circulation 2010;1211:26-33.

20 Dittrich H, Buhrer C, Grimmer I, et al. **Neurodevelopment at 1 year of age in infants with congenital heart disease.** Heart 2003;894:436-41.

21 Zeng S, Zhou J, Peng Q, et al. **Assessment by three-dimensional power Doppler ultrasound of cerebral blood flow perfusion in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2015;456:649-56.

22 Masoller N, Martinez JM, Gomez O, et al. **Evidence of second-trimester changes in head biometry and brain perfusion in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2014;442:182-7.

23 Azpurua H, Alvarado A, Mayobre F, et al. **Metabolic assessment of the brain using proton magnetic resonance spectroscopy in a growth-restricted human fetus: case report.** American journal of perinatology 2008;255:305-9.

24 Alonso I, Borenstein M, Grant G, et al. **Depth of brain fissures in normal fetuses by prenatal ultrasound between 19 and 30 weeks of gestation.** Ultrasound Obstet Gynecol 2010;366:693-9.

25 Alves CM, Araujo Junior E, Nardozza LM, et al. **Reference ranges for fetal brain fissure development on 3-dimensional sonography in the multiplanar mode.** J Ultrasound Med 2013;322:269-77.

26 Wright R, Kyriakopoulou V, Ledig C, et al. **Automatic quantification of normal cortical folding patterns from fetal brain MRI.** Neuroimage 2014;91:21-32.

27 Miller SL, Huppi PS, Mallard C. **The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome.** J Physiol 2016;5944:807-23.

28 Griffiths PD, Bradburn M, Campbell MJ, et al. **Use of MRI in the diagnosis of fetal brain abnormalities in utero (MERIDIAN): a multicentre, prospective cohort study.** Lancet 2017;38910068:538-46.

29 Kelly CJ, Makropoulos A, Cordero-Grande L, et al. **Impaired development of the cerebral cortex in infants with congenital heart disease is correlated to reduced cerebral oxygen delivery.** Sci Rep 2017;71:15088.

30 Matthiesen NB, Henriksen TB, Gaynor JW, et al. **Congenital Heart Defects and Indices of Fetal Cerebral Growth in a Nationwide Cohort of 924,422 Liveborn Infants.** Circulation 2016.

Tables

Table 1 *Baseline characteristics of included cases*

Characteristics	Value		
	CHD cases	Controls	Total
No. of women	**90**	**75**	
No. of analyzed volumes	**199 (42%)**	**278 (58%)**	**477 (100%)**
Characteristics			***P*-value**
Maternal Age in years (Mean(SD))	29.76 (4.2)	32.08 (4.39)	0.30
BMI (kg/m2) Mean(SD)	23.79 (4.2)	23.24 (3.8)	0.11
Primigravidae (%)	44 (42%)	25 (33%)	0.28
Diabetes n(%)	3(2.9%)	0(0%)	0.14
Total no. of CHD cases	**90**		**n.a.**
Major CHD			
HLHS	7		
Transposition of the Great Arteries	13		
Aortic Arch Hypoplasia and/or Aortic Stenosis	21		
Tricuspid or Pulmonary Atresia	11		
Tetralogy of Fallot or Fallot-like defect	15		
(un)balanced atrioventricular septal defect	7		
Other major CHDa	14		
Minor CHD			
Ventricular Septal defect	2		
Excluded Cases	**14**		**n.a.**
Fetal Intervention	3		
Postnatal non-isolated/syndromic	8		
Postnatal normal heart	3		
Pregnancy outcome			n.a.
Live birth	75 (83%)	75(100%)	
Termination of Pregnancy	15 (17%)	0(0%)	

*** Other major CHD include:**

Truncus Arteriosus, Multiple level left obstruction syndrome (Shone's complex), Double Outlet Right Ventricle-TGA, Congenitally Corrected TGA.

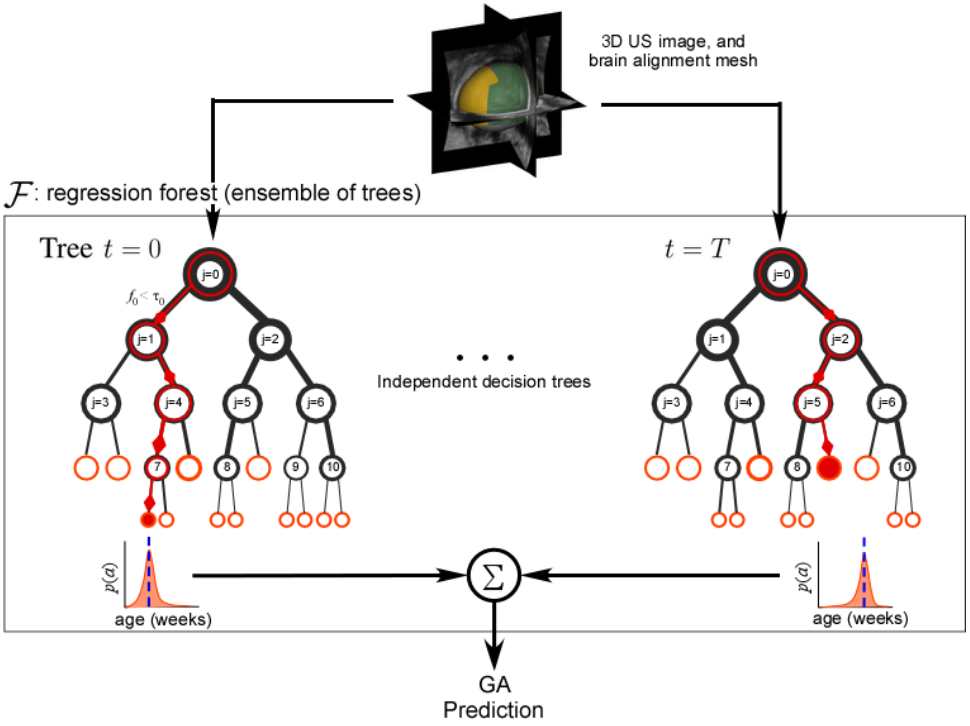

Figure 1 *Schematic representation of a regression forest. Different brain regions are sampled to calculate the brain-age in a 3D US volume*

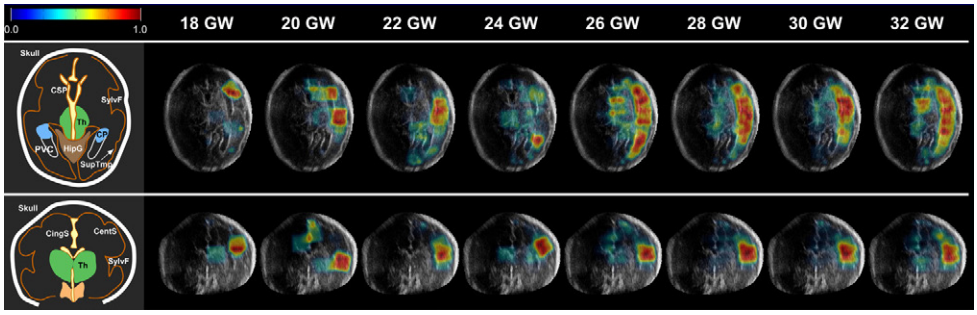

Figure 2 *A visual representation of gestational age discriminating brain regions between 18-32 weeks gestation. Colour scale is shown in the top left, top row: axial plane and bottom row: coronal plane. The colours closest to 1.0 represent brain regions that are selected most frequently by the algorithm.*

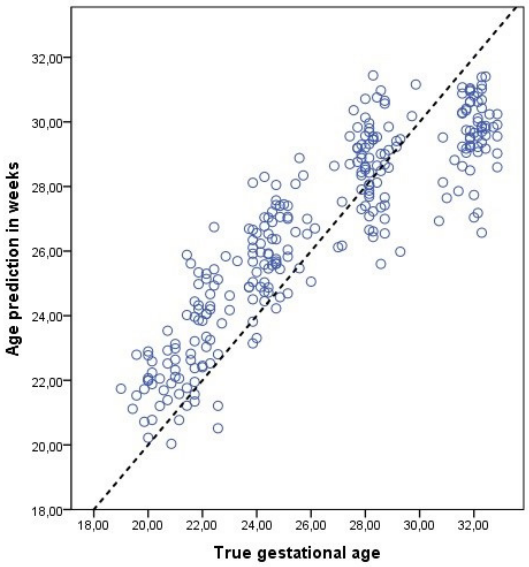

Figure 3 *Regression plot for 75 control cases: gestational age('true age') on the x-axis and age prediction on the y-axis.*

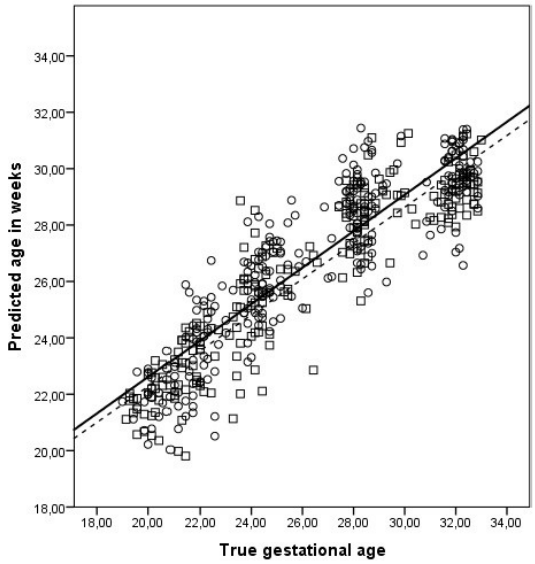

Figure 4 *The x-axis shows the gestational age at ultrasound('true age'), the y-axis shows age as predicted by the algorithm.*
Legend: *□ CHD cases, - - (interrupted line), ○ control cases. ── (continuous line).*

CHAPTER

6

The association between flow and oxygenation and cortical development in fetuses with congenital heart defects using a brain-age prediction algorithm

CHAPTER 7

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ABSTRACT

INTRODUCTION: Introduction: Presumably, changes in fetal circulation contribute to the delay in maturation of the cortex in fetuses with CHD. The aim of the current study is to analyze fetal brain development based on hemodynamic differences, using novel brain-age prediction software.

METHODS: We have performed detailed neurosonography, including acquiring 3D volumes, prospectively in cases with isolated CHD from 20 weeks onwards. An algorithm that assesses the degree of fetal brain-age automatically was used to compared CHD cases to controls. We stratified CHD cases according to flow and oxygenation profiles by lesion physiology and performed subgroup analyses.

RESULTS: 616 ultrasound volumes of 162 CHD cases and 75 controls were analyzed. Significant differences in maturation of the cortex were observed in cases with normal blood flow towards the brain (-3.8 days, 95%CI (-5.5 ; -2.0), p=<0.001) and low (-4.0 days, 95% CI (-6.7 ; -1.2) p=< 0.05)(hypoplastic left heart syndrome(HLHS)) and mixed (-4.4 days, 95%CI (-6.4 ; -2.5) p=<0.001) oxygen saturation in the ascending aorta (TGA) and in cardiac mixing (e.g. Fallot) cases.

CONCLUSION: The current study shows significant delay in brain-age in TGA- and Fallot-cases as compared to control cases. However, the small differences found in this study questions the clinical relevance.

INTRODUCTION

A significant proportion of children and adolescents with a congenital heart defect (CHD) show neurodevelopmental impairment [1, 2]. The lower IQ-scores, higher-order cognitive disorders and behavioural abnormalities that were found in previously mentioned studies, were attributed to the perioperative period, also in isolated cases[3-6]. More recent studies in both severe and mild CHD have shown altered neurological development on fetal and pre-operative neonatal examinations using MRI and ultrasound (US) [7-15]. These studies formed the basis for the hypothesis that altered circulation in CHD-fetuses causes an abnormal development of the fetal brain[16]. Specifically, brain maturation (e.g. the forming and developing of the gyri and sulci) has been shown to be delayed in fetuses with CHD[12, 17, 18]. The studies that address abnormal brain maturation in fetuses with CHD are, however, hampered by a limited number of cases and a selection bias towards fetuses with severe CHD. To overcome these difficulties, we decided to prospectively study fetal brain development in all consecutive isolated CHD cases that were referred to our unit. We have used repetitive US examinations to record brain maturation trajectories over the course of pregnancy. Also, to overcome possible errors that can arise with manual segmentation techniques, we have analyzed cases with a validated brain-age estimation algorithm[19]. The aim of this study was to compare fetal brain maturation with the use of automated brain-age estimation software in fetuses with CHD to control fetuses and to establish whether fetal brain maturation is affected by hypothesized cerebral oxygenation.

Methods

All consecutive cases of isolated CHD referred to Leiden University Medical Center between September 2013 and August 2018 were included in the Heart and Neurodevelopment (HAND)-study. Exclusion criteria were: non-isolated CHD cases, meaning cases with abnormal results of genetic testing, cases with apparent genetic abnormalities postnatally up to at least 6 months of age and cases with additional anomalies in ultrasound. We furthermore excluded: cases that were referred after 32 weeks' gestation, maternal age <18 years, multiple gestation, cases with a normal cardiac anatomy postnatally (mainly left-right asymmetry without coarctation) and fetal growth restriction, defined as an estimated fetal weight <10th percentile. Furthermore, cases with aortic valve stenosis that underwent fetal balloon valvuloplasty were excluded, since fetal brain oxygenation may have changed due to the intervention[20]. Minor additional findings, such as a single umbilical artery or increased first-trimester nuchal translucency with normal results of genetic testing were still considered as isolated CHD and thus included. However, The sample size

calculation (at least 75 cases and 75 controls) was based on the available evidence from two MRI-studies[21, 22] that compared hypoplastic left heart syndrome(HLHS)-fetuses to controls, to detect a difference in mean brain age of 2 weeks.
A control group was constructed, that consisted of healthy women with uneventful pregnancies, enrolled after a normal structural anomaly scan with a low risk of pregnancy-related complications. All participants provided informed consent. Aneuploidy screening (combined test or NIPT) was offered to all control participant according to national guidelines, which was chosen by 30%, comparable to the Dutch background population[23]. The neonates of the control group did not display any evidence of abnormalities after birth.
Gestational age (GA) in both the CHD cases and the control cases, was based on first-trimester US at approximately 10 weeks' gestation, according to the Dutch national guidelines.
Detailed neurosonography was performed every four weeks by an experienced operator (SE/FJ/AT). CHD-cases were enrolled after the fetal echo in our center and controls after a normal standard anomaly scan at mid-gestation. All scans were performed on a Voluson E8/10 (GE Healthcare ultrasound, Milwaukee, WI, USA) using a RAB 6-D three-dimensional abdominal transducer, with a frequency range of 2-7 MHz. Neurosonography was performed in axial, coronal, sagittal and parasagittal planes to assess the presence of structural anomalies (e.g. cysts, white-matter injury or intracranial fluid excess). Fetal biometry and biophysical profile were assessed. At each visit, several 3D volumes were obtained starting in the axial plane. Depending on fetal movement, we have used high to maximum quality settings for the 3D volume acquisition. An acquisition was considered successful if the following criteria were met: the cranium occupies ≥50% of the volume, the distal cerebral hemisphere is clearly visible, the interhemispheric fissure is clearly visible and the intracranial structures (Sylvian fissure, thalami, ventricles and cavum septum pellucidum) are clearly visible.

Brain-age algorithm
Intrauterine development of the fetal brain follows a distinct pattern of emerging and progressing sulcation. The use of an automated brain-age algorithm is based on the assumption that the aging of the fetal cortex is so precise that it can be used as a proxy for brain maturation[24]. We have used the brain-age algorithm to analyze fetal brain age in both CHD and control fetuses, to assess differences in maturation patterns in affected and unaffected fetuses. The exact formulation of the brain-age algorithm and the implementation details are published elsewhere [19]. The recorded volumes underwent post-examination processing: from each scanning-session, the best quality volumes were stored under a study-code, which did not reveal patient

identification nor the allocated group. In short, a system of coordinates is aligned to the outer surface of each 3D-volume. Sampling of different brain regions is then allowed on the volume surface. The 3D volume and its custom coordinate system are passed into a regression forest machine learning model[25]. Within the forest, each volume passes through a set of binary nodes, in several decision trees, which were pre-trained with a control group of more than 400 volumes from the INTERGROWTH-21 database[26]. At every node, a sample of the volume is assessed to be either older or younger than the previous node depending on the intensity of gray tones formed by the gyri and sulci in the 3D-volume. To conclude the brain-age prediction, the votes of all the decision trees in the forest are averaged. For each GA, varying brain regions are found to be the most discriminative to assess brain-age, however, the callosal sulcus, thalamic region, cingulate sulcus, Parieto-Occipital Fissure (POF), Sylvian Fissure (SF), central sulcus and ventricular regions are the most commonly encountered regions. Since the true GA was assessed accurately in the first trimester according to the ISUOG-guideline[27], we were able to compare the brain-age as assessed by the algorithm to the true GA to detect any delay in maturation. Although we have *perform*ed US scans in included fetuses up till 36 weeks of gestation, US scans were included in the analysis until 33+6 weeks gestation. As was explained in the original article, this cutoff was chosen because the algorithm tends to underestimate fetal brain age after 34 weeks gestational age[19].

Clustering of CHDs

As previously described [28], we allocated the cases to different groups according to hypothesized prenatal oxygen saturation in the ascending aorta and the aortic arch flow, as an indication of oxygen delivery to the brain. The cases were clustered in different groups having either: low, mixed or normal oxygen delivery to the brain; or reversed, obstructed or normal aortic arch flow. The combination of these characteristics resulted in six subgroups of CHD, the exact details of which are discussed in Supplement 1. The classification into different groups was made using the postnatal diagnosis and using the prenatal diagnosis in cases in which termination of pregnancy was chosen by the parents. In the latter group there was consensus concerning the diagnosis by a team of highly experienced echocardiographic consultants. This approach has shown to lead to a very low rate of discrepancies in our unit[29].

Statistical analysis

Linear mixed models were used to account for multiple volume measurements acquired at different gestational ages from the same fetus during pregnancy. Covariates in the first mixed-effect regression model were GA (a linear increase of brain maturation with GA was supposed), group ('CHD-cases versus controls') and

the interaction between GA and group. To account for between-patient variation, a random intercept was added per patient. A likelihood ratio test with 2 degrees of freedom (main and interaction effect of group) was performed to ascertain difference in brain-age. This test confirmed differences between the whole case group and the control group. Three additional linear mixed models were performed zooming in on subgroups of the cases: one with the cases divided according to blood flow towards the brain and one with division according to oxygen saturation, and one assessing cross-combinations. In each model, the differences were quantified at the median GA by comparing the mean brain age of the case groups against the control group and slopes were compared to assess differences in developmental velocity between the case groups and the control group.
The results were verified with quadratic time trends, which confirmed the results assuming linear time trends.
All statistical analyses were performed using IBM SPSS statistics version 24.0.0.0 (IBM, Armonk, NY, USA). Statistical significance was set at $p \leq 0.05$.

Results

Between March 2014 and August 2018, we have included 162 consecutive CHD cases and 75 controls. The groups did not differ significantly in parity, maternal age, or BMI. Baseline characteristics are shown in Table 1. After the manual selection of the coded volumes, 3 CHD-cases did not yield any 3D-volumes of sufficient quality. Twenty cases were excluded for the following reasons: 3 due to fetal aortic balloon valvuloplasty, 13 due to genetic abnormalities (3 CHARGE-syndrome, 2 Kabuki-syndrome, 1 PAX-2 mutation, 1 DAAM-1 mutation, 1 SCNA8-mutation with severe epilepsy, 1 14q11.2 deletion, 1 case with heterozygous MYBPC 3 cardiomyopathy and pylorus hypertrophy, 1 case with a copy number variant on chromosome 14, 2 cases with clear dysmorphic features after birth; final diagnosis unknown (both patients died), and 4 cases because of a structural normal heart after birth (all cases with suspected aortic coarctation). No cases were excluded in the control group, as no genetic of structural abnormalities were found in follow-up until at least 1 year postnatally.

Thus, 142 CHD-cases and 75 controls were considered for analysis. Table 2 shows the allocation of the CHD cases in the different flow and saturation groups. A total of 660 US examinations were performed in these subjects. The US examinations were performed every 4 weeks starting at 20 weeks onwards, the median GA for the examinations was 20.9 (18.7-21.9), 24.1 (22-25.9), 28.4 (26-29.9) and 32.1 (30-33.3) in the CHD-group and 21.0 (19-21.9), 24.3 (22-25.9), 28.1 (26-29.9) and 32 (30-32.9) in the control group.

Due to technical factors (tilted insonation, inferior quality, motion artefacts), 39 volumes (5.9%) were excluded. A median of 2.0 (1-5) volumes for the CHD group and 4.0 (1-5) for the controls were recorded per case.
Brain-age as determined by the algorithm was compared between cases and controls at the median gestational age (26.16 weeks). Cases were found to have significantly less mature brains than controls: -3.2 days, 95%CI (1.6 ; 4.8) *p=<0.001*[30](fig. 1). When comparing the slopes, no significant difference was found in maturation velocity between cases and controls (maturation difference = 0.2 days/week, 95%CI (-0.2 ; 0.5) *p=0.31),* meaning that the difference between the cases and controls was consistent throughout pregnancy.

Sub group analysis

Blood flow
In the analyses categorizing cases according to the hypothesized flow (either reversed, obstructed or normal) , an overall test showed significant differences when comparing the three case groups and the control group (*p=0.001,* df=3), with all three case groups showing lower mean brain-age at the median GA than the controls. The largest differences were found between the cases with normal flow to the fetal brain (n=228) compared to controls: -3.8 days, 95%CI (-5.5 ; -2.0) , *p=<0.001* and the cases with reversed flow (n=17) towards the brain compared to the controls: reversed flow: -3.4 days, 95% CI (-8.8 ; 2.0) *p= 0.21.* For cases with obstructed flow towards the brain (n=94) the difference was -1.5 days, 95% CI (-3.9 ; 0.8) *p=0.20* (fig. 2) The speed of maturation did not differ significantly between the groups (overall test for difference in slopes *p=0.545,* df=3).

Oxygen Saturation
In the analyses categorizing cases according to the hypothesized oxygen saturation in the fetal ascending aorta (either low, mixed or normal) , an overall test showed significant differences comparing the three case groups and the control group (*p= <0.001,* df=3), with all three case groups showing lower mean brain age at the median GA than the controls. The largest differences were found between the cases with low oxygen saturation in the ascending aorta (n=61) compared to controls: -4.0 days, 95% CI (-6.7 ; -1.2) *p=< 0.05* and the cases with mixed oxygen saturation (n=152) in the ascending aorta compared to the controls: -4.4 days, 95%CI (-6.4 ; -2.5) *p=<0.001.* The cases with normal oxygen saturation to the fetal brain (n=126) did not significantly differ from controls: -1.1 days, 95%CI (-3.2 ; 1.0) *p=0.28* (fig. 3). The speed of maturation did not differ significantly between the groups (overall test for the difference in slopes *p= 0.303,* df=3).

Flow/saturation subgroups
In the analyses categorizing cases according to the combination in aforementioned flow and saturation in six CHD-diagnosis groups, an overall test showed significant differences comparing the six case group and the control group (*p=0.002,* df=6), with all six case groups showing lower mean brain age at the median GA than the controls (fig. 4). For two groups the difference compared to controls was statistically significant: the group with normal flow, but decreased oxygen flow towards the brain, mainly TGA (n=61) : Difference -4.0 days, 95% CI (-6.7 ; -1.2), *p=0.006* and the group with normal flow, but intracardiac mixing, mainly AVSDs and Tetralogy of Fallot cases (n=110) : -4.5 days, 95% CI (-6.8 ; -2.3) *p=<0.001* (fig. 5). The speed of maturation did not differ significantly between the groups (overall test for the difference in slopes p= 0.393, df=6).

Discussion

In this study of a relatively large cohort of consecutive CHD fetuses we found a brain maturation delay of four days in the subgroups of fetuses with TGA and the group with intracardiac mixing (e.g. Fallot and AVSD).

Delayed neurodevelopment in children born with congenital heart defects is hypothesized to be caused by the altered cerebral perfusion in fetal life, either caused by decreased flow or decreased oxygenation of the cerebral blood, due to the abnormal cardiac anatomy. MRI studies such as phase-contrast magnetic resonance[31] and T2*[32] showed decreased intracerebral oxygen levels in fetuses with CHD cases, which confirmed the hypothesized decreased cerebral oxygenation.
The rapid fetal brain maturation during gestation has been previously studied by US[10, 11] and MRI[9, 21, 33] in fetuses with CHD. In these studies the depth of several fissures (e.g. POF, SF, central sulcus and calcarine sulcus) were measured manually to represent cortical development. A significant difference in fissure depth was found in CHD cases, compared to controls, starting in the second trimester and progressing towards the end of pregnancy. Our study confirms these findings of altered brain development, but beyond that, is able to assess the brain at multiple regions at once. Furthermore, the machine learning algorithm allowed for an automated selection of the most age-discriminating brain regions, which varied during gestation. And finally, this method is able to convey the extent of the delay, whereas others can only report on differences in depth of fissures.

Some of the previously mentioned authors analyzed their data regarding cortical development stratified by CHD-subgroup as well[9, 10, 21]. Our findings did not corroborate two studies that reported on delayed brain maturation in left obstructive lesions

in which the delay increased in the third trimester and found significant differences in delay in cortical folding and a more shallow SF depth in the second trimester [10, 21]. Furthermore the delay increased in the third trimester. Our study did not show significantly less mature brains in HLHS fetuses (hypothesized mixed oxygenation with reversed flow), and the magnitude of the delay did not increase during gestation. Masoller et al. divided CHD cases into two groups: a low and a normal cerebral oxygenation group, they observed global delay in several fissures, but did not find any differences in subgroup analyses[9].

As mentioned above, the magnitude of the delay in this study was at maximum only four days, which is marginal, compared to other severe delays in cortical maturation found by other authors[9, 12, 15]. Possible explanations for this difference are the inclusion of a relatively large cohort of consecutive cases, preventing selection bias and the exclusion of syndromic cases[7-15]. The reason for the differences, observed in these studies, compared to our results, could be the bias towards more severe cases. One of the largest observed findings in our study was a delay with a magnitude of almost four days in fetuses with TGA. This confirms the earlier reported decreased fissure depth in TGA cases [10]. The delay in cortical maturation in TGA in imaging studies during fetal and neonatal stages raises, however, questions, since TGA is known as a defect with a generally good prognosis in adolescent life[34]. It is therefore arguable whether the detected delay in prenatal brain maturation is a reflection of neurodevelopment later in life, or a true finding after all. The fact that 4 days is below the accuracy range of 6.1 days of the used algorithm may also mean that this is a random finding and confirms our hypothesis that this finding has little clinical effect.

Our results did not confirm earlier studies that suggested decreased flow towards the brain as an important etiological factor for the delay in maturation. It seems that our current understanding of fetal neurodevelopment in CHD and the observed deviations from normal in these fetuses should at least be considered multifactorial. Current studies show a larger prevalence of *de novo* non-syndromic gene mutations in CHD cases with affected children having lower scores on neurodevelopmental tests at 14 months of age[35, 36]. This could imply a common (non-syndromic) genetic contribution that affects both the maldevelopment of the heart *and* altered development of the fetal brain. Moreover, besides genetic causes, mechanisms for altered cortical development could originate from insufficient nutrient delivery from the maternal circulation. Placental maldevelopment in CHD fetuses has been described by Llurba et al.[37] and although we excluded cases of pregnancy-related hypertension and pre-eclampsia from our cohort and therefore cannot conclude on prevalence, others have described a high prevalence of such complications[38]. Thus, in our

opinion the effect of the placenta on neurodevelopmental outcome in pregnancies complicated by CHD needs to be studied further.

One of the key strengths of the current study is the fact that we have thoroughly reviewed each case and included only isolated CHD cases, since it is known that altered neurodevelopment could be attributed to genetic or syndromic defects in non-isolated cases. Also, we have reviewed all included cases up to one year postnatally and have excluded cases with genetic syndromes. The minority of authors[7, 11] mention the follow-up period for excluding genetic or syndromic affected cases, other studies seem not to perform any reviewing of postnatal outcome after the study MRI or US examination. Another strength of this study is the prospective inclusion of consecutive CHD cases. To our knowledge, this is largest cohort of consecutive CHD-cases to have been analyzed using 3D US recordings.

Limitations of the current study is the uneven distribution of cases throughout the presumed flow and oxygenation groups. Notably, in the aortic arch flow analysis, significant differences were found in the group with presumed normal flow towards the fetal brain (also containing TGA and Fallot cases). However, the aforementioned group consisted of 228 measurements versus only 17 in the reversed flow group. The findings in this subgroup could therefore be the result of a lack of power and have to be considered with caution. Secondly, a limitation that was previously mentioned is the more erroneous brain-age predictions in the third trimester that were described in the algorithm. The prediction error is low in the second trimester and increases with advancing gestational age, however, since we have analyzed both controls and cases with the same protocol, they can be accurately compared. The increase in prediction error in the late third trimester is the reason why we have not included cases beyond 34 weeks gestation, which we considered the most important limitation of our study.

Another limitation is the lack of long-term neurodevelopmental follow-up. Although we have found very small differences in brain maturation opposed to the controls in this study, but as these findings are early in fetal life, this might have a large effect on long term follow-up.

This study describes the magnitude of delay in brain maturation in fetuses with CHD, stratified to the different types of flow and saturation of the blood towards the brain in strictly isolated CHD-cases. We conclude that cases with TGA or cases with intracardiac mixing show the largest difference in brain maturation as compared to controls. Whether these findings are solely explained by reduced oxygenation of the

blood towards the fetal brain remains uncertain. In our opinion the image-based results in brains of CHD fetuses and neonates is the result of a complicated multi-factorial process and the actual effect of intellectual performance at school age and adolescence has not been investigated so far.

References

1 Majnemer A, Limperopoulos C, Shevell MI, et al. **A new look at outcomes of infants with congenital heart disease.** Pediatric neurology 2009;403:197-204.

2 Mahle WT, Tavani F, Zimmerman RA, et al. **An MRI study of neurological injury before and after congenital heart surgery.** Circulation 2002;10612 Suppl 1:I109-I14.

3 Marino BS, Lipkin PH, Newburger JW, et al. N**eurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association.** Circulation 2012;1269:1143-72.

4 Sarrechia I, Miatton M, De WD, et al. **Neurocognitive development and behaviour in school-aged children after surgery for univentricular or biventricular congenital heart disease.** Eur J Cardiothorac Surg 2016;491:167-74.

5 Paladini D, Alfirevic Z, Carvalho JS, 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;492:287-8.

6 Calderon J, Jambaque I, Bonnet D, et al. **Executive functions development in 5- to 7-year-old children with transposition of the great arteries: a longitudinal study.** Dev Neuropsychol 2014;395:365-84.

7 Andescavage N, Yarish A, Donofrio M, et al. **3-D volumetric MRI evaluation of the placenta in fetuses with complex congenital heart disease.** Placenta 2015.

8 Brossard-Racine M, du Plessis AJ, Vezina G, et al. Prevalence and spectrum of in utero structural brain abnormalities in fetuses with complex congenital heart disease. AJNR Am J Neuroradiol 2014;358:1593-9.

9 Masoller N, Sanz-Cortes M, Crispi F, et al. **Mid-gestation brain Doppler and head biometry in fetuses with congenital heart disease predict abnormal brain development at birth.** Ultrasound Obstet Gynecol 2016;471:65-73.

10 Peng Q, Zhou Q, Zang M, et al. **Reduced fetal brain fissures depth in fetuses with congenital heart diseases.** Prenat Diagn 2016;3611:1047-53.

11 Koning IV, van Graafeiland AW, Groenenberg IAL, et al. Prenatal influence of congenital heart defects on trajectories of cortical folding of the fetal brain using three-dimensional ultrasound. Prenat Diagn 2017;3710:1008-16.

12 Licht DJ, Shera DM, Clancy RR, et al. **Brain maturation is delayed in infants with complex congenital heart defects.** J Thorac Cardiovasc Surg 2009;1373:529-36; discussion 36-7.

13 Licht DJ, Wang J, Silvestre DW, et al. **Preoperative cerebral blood flow is diminished in neonates with severe congenital heart defects.** J Thorac Cardiovasc Surg 2004;1286:841-9.
14 Peyvandi S, De Santiago V, Chakkarapani E, et al. **Association of Prenatal Diagnosis of Critical Congenital Heart Disease With Postnatal Brain Development and the Risk of Brain Injury.** JAMA Pediatr 2016;1704:e154450.
15 Kelly CJ, Makropoulos A, Cordero-Grande L, et al. **Impaired development of the cerebral cortex in infants with congenital heart disease is correlated to reduced cerebral oxygen delivery.** Sci Rep 2017;71:15088.
16 Williams IA, Fifer C, Jaeggi E, et al. **The association of fetal cerebrovascular resistance with early neurodevelopment in single ventricle congenital heart disease.** Am Heart J 2013;1654:544-50.
17 Andropoulos DB, Hunter JV, Nelson DP, et al. **Brain immaturity is associated with brain injury before and after neonatal cardiac surgery with high-flow bypass and cerebral oxygenation monitoring.** J Thorac Cardiovasc Surg 2010;1393:543-56.
18 Dimitropoulos A, McQuillen PS, Sethi V, et al. **Brain injury and development in newborns with critical congenital heart disease.** Neurology 2013;813:241-8.
19 Namburete AI, Stebbing RV, Kemp B, et al. **Learning-based prediction of gestational age from ultrasound images of the fetal brain.** Med Image Anal 2015;211:72-86.
20 Prosnitz AR, Drogosz M, Marshall AC, et al. **Early hemodynamic changes after fetal aortic stenosis valvuloplasty predict biventricular circulation at birth.** Prenat Diagn 2018;384:286-92.
21 Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. **Delayed cortical development in fetuses with complex congenital heart disease.** Cereb Cortex 2013;2312:2932-43.
22 Mlczoch E, Brugger P, Ulm B, et al. **Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program.** Eur J Paediatr Neurol 2012.
23 van Schendel RV, van El CG, Pajkrt E, et al. **Implementing non-invasive prenatal testing for aneuploidy in a national healthcare system: global challenges and national solutions.** BMC Health Serv Res 2017;171:670.
24 Clouchoux C, Kudelski D, Gholipour A, et al. **Quantitative in vivo MRI measurement of cortical development in the fetus.** Brain Struct Funct 2012;2171:127-39.
25 Breiman L. **Random forests.** Mach Learn 2001;451:5-32.
26 Papageorghiou AT, Ohuma EO, Altman DG, et al. **International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project.** Lancet 2014;3849946:869-79.
27 Salomon LJ, Alfirevic Z, Bilardo CM, et al. **ISUOG practice guidelines: performance of first-trimester fetal ultrasound scan.** Ultrasound Obstet Gynecol 2013;411:102-13.
28 Jansen FA, van Zwet EW, Rijlaarsdam ME, et al. **Head growth in fetuses with isolated congenital heart defects: lack of influence of aortic arch flow and ascending aorta oxygen saturation.** Ultrasound Obstet Gynecol 2016;483:357-64.
29 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. **Prenatal diagnosis of congenital heart defects: accuracy and discrepancies in a multicenter cohort.** Ultrasound Obstet Gynecol 2016;475:616-22.

30 Everwijn SMP, Namburete AIL, van Geloven N, et al. **Cortical development in fetuses with congenital heart defects using an automated brain-age prediction algorithm.** Acta Obstet Gynecol Scand 2019.

31 Sun L, Macgowan CK, Sled JG, et al. **Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.** Circulation 2015;13115:1313-23.

32 Lauridsen MH, Uldbjerg N, Henriksen TB, et al. **Cerebral Oxygenation Measurements by Magnetic Resonance Imaging in Fetuses With and Without Heart Defects.** Circ Cardiovasc Imaging 2017;1011:e006459.

33 Ortinau CM, Rollins CK, Gholipour A, et al. **Early-Emerging Sulcal Patterns Are Atypical in Fetuses with Congenital Heart Disease.** Cereb Cortex 2018.

34 Escobar-Diaz MC, Freud LR, Bueno A, et al. **Prenatal diagnosis of transposition of the great arteries over a 20-year period: improved but imperfect.** Ultrasound Obstet Gynecol 2015;456:678-82.

35 Gaynor JW, Stopp C, Wypij D, et al. **Neurodevelopmental outcomes after cardiac surgery in infancy.** Pediatrics 2015;1355:816-25.

36 Newburger JW, Sleeper LA, Bellinger DC, et al. **Early developmental outcome in children with hypoplastic left heart syndrome and related anomalies: the single ventricle reconstruction trial.** Circulation 2012;12517:2081-91.

37 Llurba E, Syngelaki A, Sanchez O, et al. **Maternal serum placental growth factor at 11-13 weeks' gestation and fetal cardiac defects.** Ultrasound Obstet Gynecol 2013;422:169-74.

38 Ruiz A, Cruz-Lemini M, Masoller N, et al. **Longitudinal changes in fetal biometry and cerebroplacental hemodynamics in fetuses with congenital heart disease.** Ultrasound Obstet Gynecol 2017;493:379-86.

Tables

Table 1 *Baseline characteristics of included cases*

Characteristics	CHD cases	Controls	*p-value*
	162 subjects	**75 subjects**	
	343 volumes	**278 volumes**	
Maternal Age in years (Mean(SD))	29.9 (±4.5)	32.1 (±4.4)	0.17
BMI (kg/m2) Mean(SD)	24.0 (±4.4)	23.2 (±3.8)	***0.11***
Primigravidae (%)	67 (41)	25 (33)	0.24
Male gender	99 (61)	36 (48)	0.02*
Total no. of CHD cases n (%)	**162(100)**		**n.a.**
HLHS	10 (6.2)		
Transposition of the Great Arteries	24 (14.8)		
Aortic Arch Hypoplasia and/or Aortic Stenosis	28 (17.2)		
Tricuspid or Pulmonary Atresia	17 (10.5)		
Tetralogy of Fallot or Fallot-like defect	21 (13)		
Balanced/unbalanced atrioventricular septal defect	9 (5.6)		
Ventricular Septal defect	8 (4.9)		
Other major CHD†	24 (14.8)		
Other minor CHD‡	21 (13)		
Excluded Cases n (%)	**20 (100)**		**n.a.**
Fetal Intervention	3 (15)		
Postnatal non-isolated/syndromic	13 (65)		
Postnatal normal heart	4 (20)		
Pregnancy outcome n(%)	**162**		**n.a.**
Live birth	132 (81%)	75(100%)	
Termination of Pregnancy	30 (19%)	0(0%)	

***P*=<0.05,** Statistically significant.

† Other major CHD include:

Truncus Arteriosus, Multiple level left obstruction syndrome (Shone's complex), Double Outlet Right Ventricle-TGA, Congenitally Corrected TGA without additional cardiac anomalies, AVSD with Pulmonary Atresia, Aortic-left ventricular tunnel with severe distention of the left ventricle.

‡ Other minor CHD include:

Persistent left caval vein without obstruction of the left atrioventricular flow, Restrictive Foramen Ovale, mild pulmonary stenosis.

Table 2 *Clustering of types of congenital heart defects (CHD) according to aortic arch flow and oxygen saturation for 142 fetuses in our cohort.*

	Ascending aorta oxygen saturation			
	Low	**Mixed**	**Normal**	**Total**
Aortic arch flow				
Reversed	0	**11 (17)** -3.4 (-8.8 ; 1.9) *p=0.21*	0	**11 (17)** -3.4 (-8.8 ; 2.0) *p=0.21*
Obstructed	0	**15 (27)** -3.4 (-7.4 ; 0.6) *p=0.09*	28 (67) -0.8 (-3.5 ; 1.9) *p=0.56*	**43 (94)** -1.5 (-3.9 ; 0.8) *p=0.20*
Normal	**21 (61)** -4.0 (-6.7 ; -1.2) *p=0.01**	**43 (110)** -4.5 (-6.8 ; -2.3) *p=<0.001**	**24 (57)** -1.6 (-4.5 ; 1.2) *p=0.26*	**88 (228)** -3.8(-5.5 ; -2.0) *p=<0.001**
Total	**21 (61)** -4.0 (-6.7 ; -1.2) *p=0.01**	**69 (152)** -4.4 (-6.4 ; -2.5) *p=<0.001**	**52 (126)** -1.1 (-3.2 ; 1.0) *p=0.28*	**142 (339)** -3.2 (-4.9 ; -1.6) *p=<0.001**

Legend:

n (amount of volumes) Difference compared to controls shown as:

days, 95% CI(lower bound-upper bound) p.

Flow: aortic arch blood flow.

O2: Oxygen saturation in the ascending aorta.

Normal flow + Low O2: Transposition of the great arteries,

Obstructed flow + Normal O2: Aortic Obstruction and Small left heart syndrome,

Reversed flow + mixed O2: Severe CHD with reversed aortic arch flow,

Obstructed flow + Mixed O2: Severe cardiac mixing cases with aortic obstruction,

Normal flow + Mixed O2: Severe cardiac mixing cases without aortic obstruction,

Normal flow + Normal O2: No mixing, no obstructed flow.

For detailed description of included cases in each group, see supplement 1.

******p* = **<0.05,** Statistically significant.

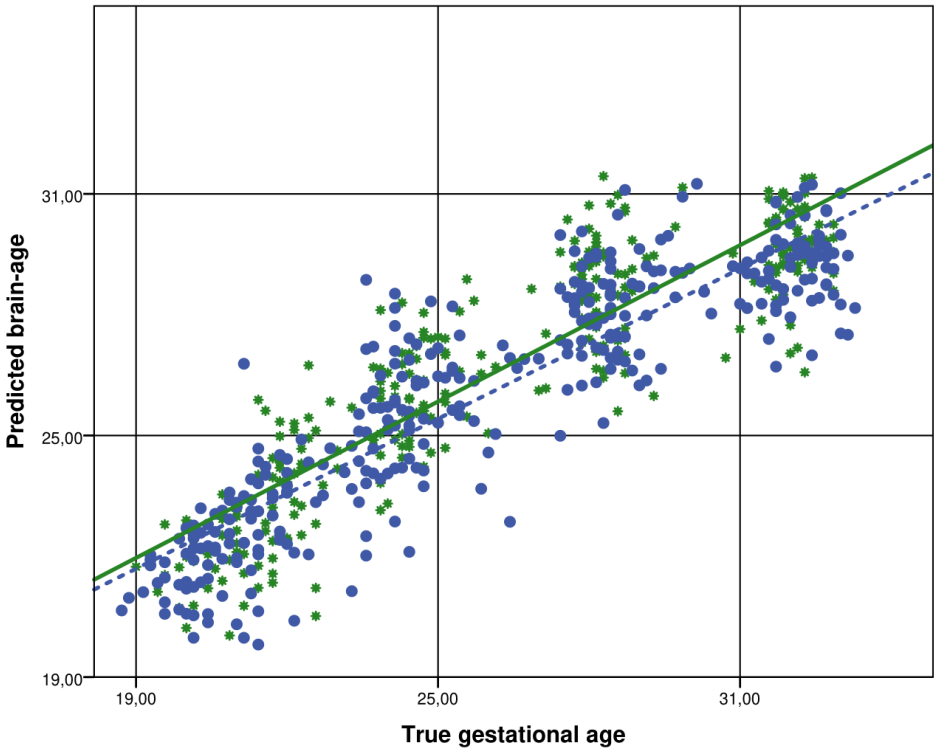


Figure 1 *Predicted brain age (y-axis) is plotted against the true gestational age (x-axis) in weeks. Linear lines show results of linear mixed model regression. Green star (✱) and continuous line (—) all controls. Blue dot (●) and - - - (dashed line) all cases.*

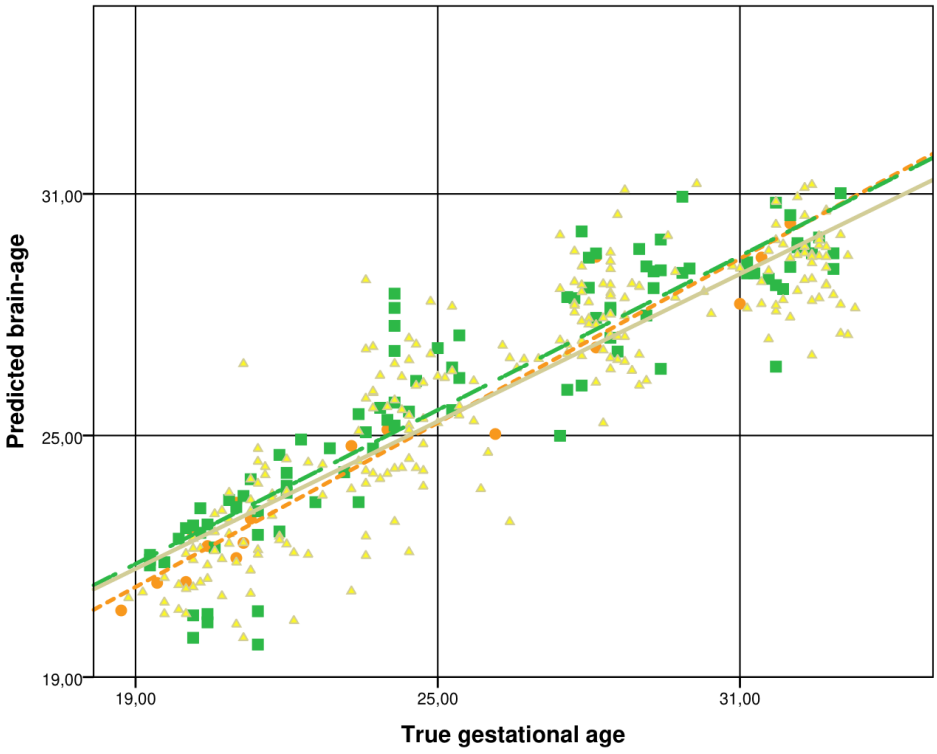


Figure 2 *Influence of aortic arch flow towards the fetal brain on brain-age in fetuses with isolated congenital heart defects. Linear lines show results of sub-analysis with linear mixed model regression. Green square (■) and interrupted line (– –) obstructed flow, orange dot (●) and dashed line (- - -) reversed flow, yellow triangle (▲) and continuous line (—) normal flow towards the fetal brain.*

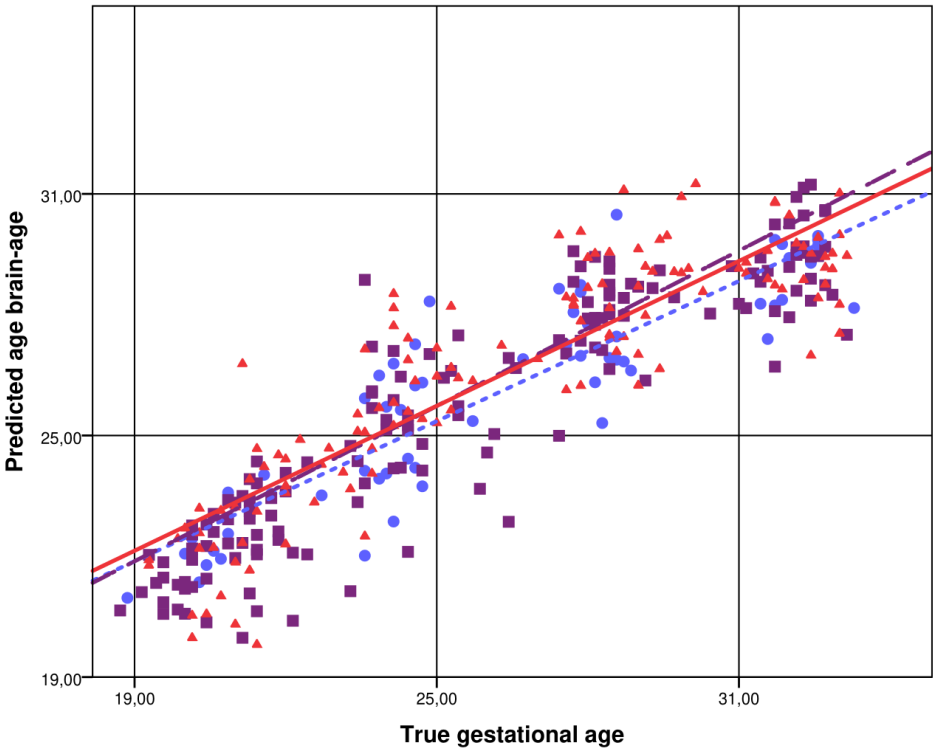


Figure 3 *Influence of ascending aorta oxygen saturation on brain-age in fetuses with isolated congenital heart defects. Linear lines show results of subanalysis with linear mixed model regression. Blue dot (●) and dashed line (- - -) low oxygen saturation, purple square (■) and interrupted line (— —) mixed oxygen saturation, red triangle (▲) and continuous line(——) normal oxygen saturation.*

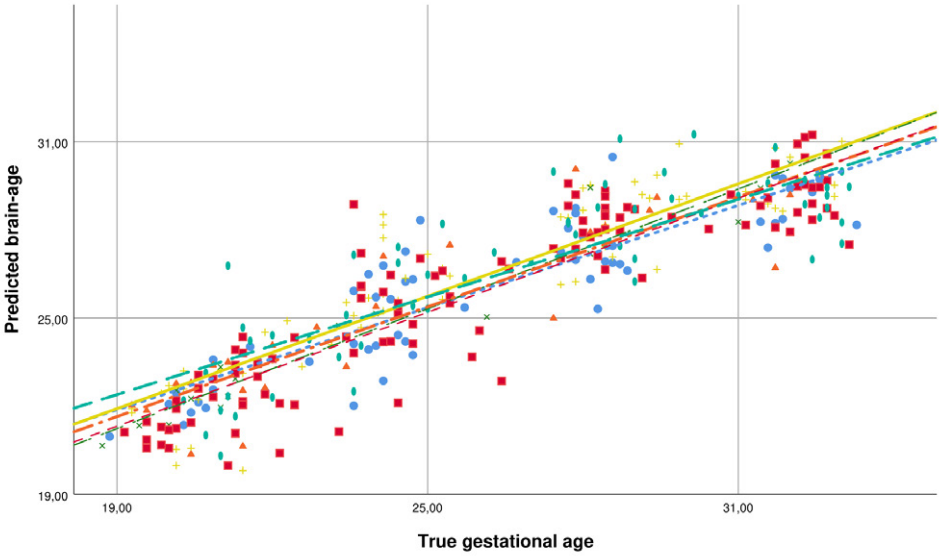


Figure 2 *Influence of CHD diagnoses on estimated brain-age. Linear lines show results of subanalysis with linear mixed model regression.*
Blue dot (●) and dashed line (- - -) normal flow + low O2: Transposition of the great arteries.
Yellow plus (+) and continuous line (——) obstructed flow + normal O2: Aortic Obstruction and Small left heart syndrome.
Green cross (✖) and thin dashed line (- - -) reversed flow + mixed O2: Severe CHD with reversed aortic arch flow.
Orange triangle (▲) and thick dashed line (- - -) obstructed flow + mixed O2: Severe cardiac mixing cases with aortic obstruction.
Red square (■) and thin interrupted line (— —) normal flow + mixed O2: Severe cardiac mixing cases without aortic obstruction (e.g. Fallot/Fallot-like).
Turquoise elipse (⬮) and thick interrupted line (— —) normal flow + normal O2: No mixing, no obstructed flow.

A detailed description of included cases in each group can be accessed online at https://obgyn.onlinelibrary.wiley.com/journal/10970223

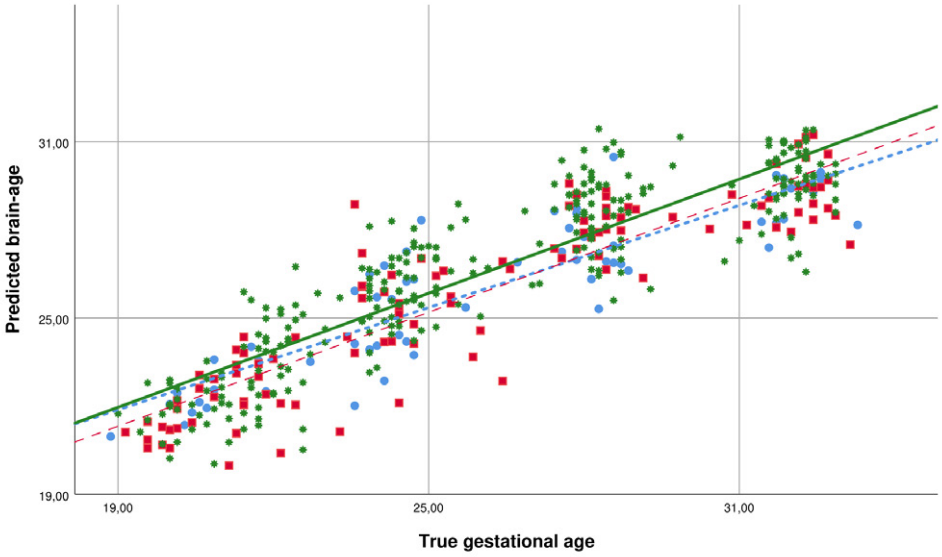


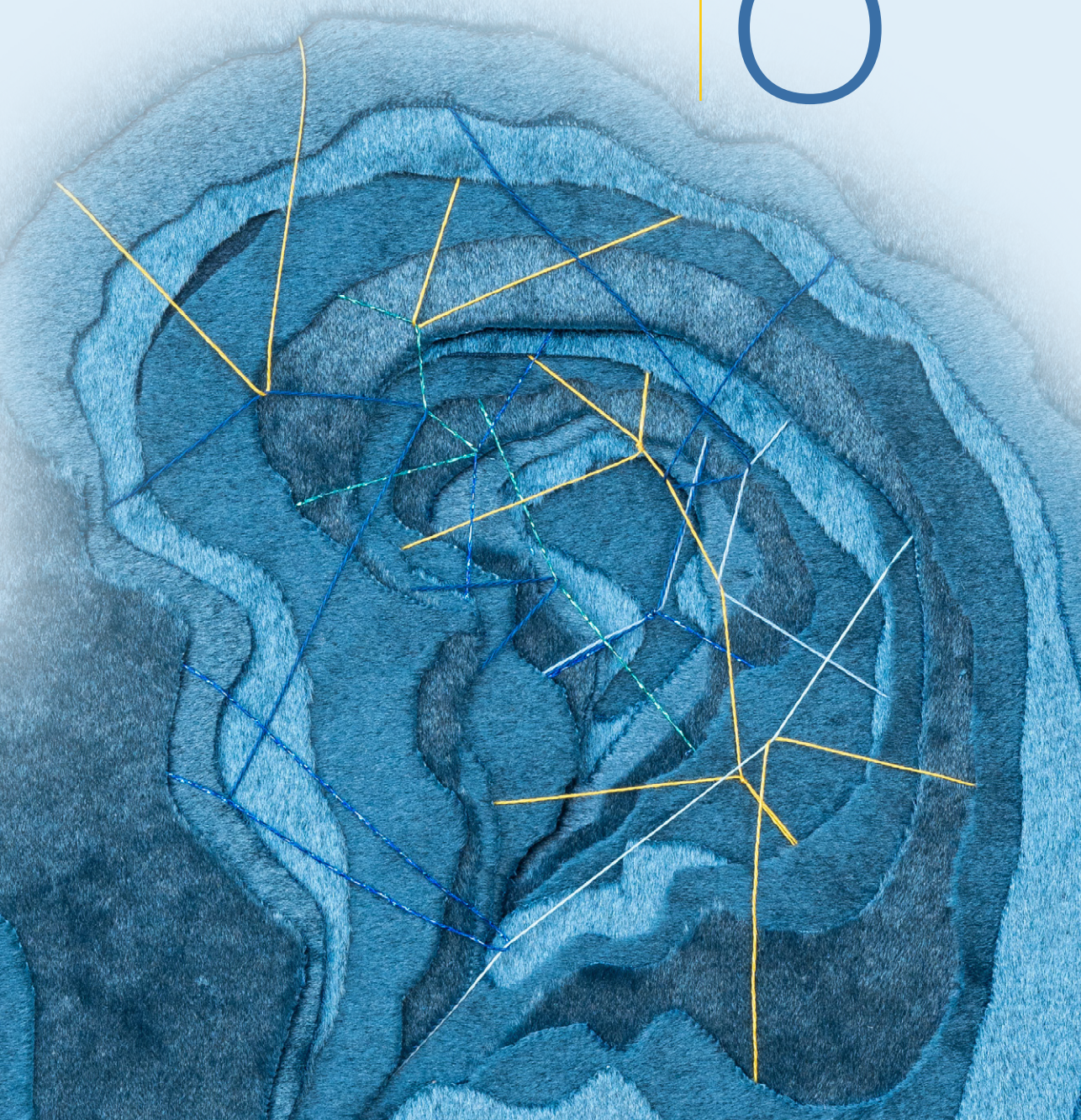
Figure 5 *Significant influence of transposition of the great arteries and Fallot/ Fallot-like congenital heart defects on prenatal brain-age as compared to controls. Linear lines show results of subanalysis with linear mixed model regression. Green star (✱) and continuous line (—) : all controls. Blue dot (●) and dashed line (- - -) normal flow + low O2: Transposition of the great arteries. Red square (■) and thin interrupted line (– –) normal flow + mixed O2: severe cardiac mixing cases without aortic obstruction (e.g. Fallot/Fallot-like).*

CHAPTER 7

General Discussion

CHAPTER

8



Congenital heart defects (CHD) are the most prevalent congenital defect with 6-8/1000 live births.

A prenatal diagnosis (PD) of a CHD results in a decrease of mortality and morbidity, underlining the importance of screening for CHD. The vast majority of children with a CHD survive infancy nowadays due to optimized neonatal and peri-operative care, but these children still face an increased risk of morbidity. Deficits in neurocognitive development are increasingly recognized, even in purely isolated heart defects. These deficits are described in multiple domains such as cognition, behavior, planning, execution as well as academic achievements and are linked to white matter injury. White matter injury has been attributed as a complication of (open) heart and percutaneous surgeries in the first year of life, leading to periods of low cardiac output and hypoxia, which is associated with abnormal cerebral development and delayed neurological development. Most recently, smaller cerebral volumes and decreased head circumferences were also seen in imaging studies in infants with less severe cardiac defects which only required percutaneous intervention or no intervention at all. This shows that neurocognitive impairment in children with CHD might not only arise from peripartum or perioperative circumstances. Adjacent to this, studies that showed brain injury on MRI in CHD-children prior to surgery have led to the hypothesis that these alterations might originate earlier than previously thought. Furthermore, fetal data has even shown signs of altered cortical development in CHD fetuses, thus prior to birth. This poses the hypothesis: is it possible that neurodevelopmental delay in congenital heart defects originates in fetal life? Can fetal brain injury worsen by postnatal circumstances, for example cardiopulmonary bypass or low cardiac output (second-hit theory)? If the altered fetal circulation plays a role, does this mean that certain cerebral areas or certain cardiac defects are more prone to delay than others?
This thesis focusses on in-utero neurological development, as well as the prenatal detection of two common congenital heart defects, to prevent postnatal brain injury.

Prenatal detection of congenital heart defects

The prenatal detection of congenital heart defects has been a topic of great interest over the last decades. Ultrasound as an medical imaging modality was introduced in the sixties and became more widespread and was introduced in obstetrical care early after its development. At first, ultrasound was only applied to determine fetal position. Secondly, it became apparent that it was possible to diagnose severe malformations, with anencephaly as the first diagnosed abnormality. In the decades thereafter, it gradually became clear that the diagnosis of a congenital defect could lead to optimized care and thus better outcome in the majority of the defects. This

was the start of the introduction of routine screening for congenital abnormalities using ultrasound in pregnancy.

In the Netherlands, prenatal screening for fetal anomalies was introduced relatively late compared to the surrounding countries. This was mainly caused by the concerns of Christian-Democratic politicians, who feared an increase in pregnancy termination after the introduction of prenatal screening and considered abortion not as 'treatment' for a disease, which was the most important prerequisite for the introduction of a test in the population-health program according the Dutch law. Eventually, all parties agreed that expecting parents had the right of equal access to prenatal care and freedom in their reproductive choices [1]. Thus, the routine screening program was implemented in 2007 in the Netherlands and is monitored by the government. Health-care professionals performing the standard anomaly scan (SAS) are bound to an annual number of scans and regular quality monitoring. The officially registered uptake of the 20 weeks' anomaly scan reports around 95% in the Netherlands, while the majority of the remaining 5% is scanned in a department for PD because of increased risk. The majority of the ultrasonographers in the screening setting are midwives that have received a standardized training in ultrasound to detect fetal anomalies.

Evaluation of the fetal heart is considered one of the more difficult parts of screening for anomalies. Possibly, the movement of the organ, the small size and the necessity to evaluate the organ in different planes, might play a role in the historically low rates of prenatal detection of congenital heart defects. Furthermore, the large variation in morphologic variants that can present with a completely normal four-chamber view and the relative low exposure to abnormalities, makes it difficult to recognize CHD. Furthermore, an interview study among sonographers showed that they experience a high threshold in referring a patient to a tertiary center, because they want to be absolutely sure that there is an anomaly, to not produce unnecessary parental anxiety [2]. Anomalies visible in the four-chamber view are detected in > 93% in our geographic region (Amsterdam – Leiden) after introduction of the SAS[3]. This means that recognition of very abnormal planes is excellent when prenatal screening follows a uniform protocol. Cardiac abnormalities that present with a normal four-chamber view, which encompass mainly outflow tract anomalies, for example tetralogy of Fallot and transposition of the great arteries (TGA), are detected less often. Since these abnormalities can be more subtle on prenatal ultrasound, they are perceived as more difficult. In chapter 2, the detection rates of these two common outflow-tract anomalies is described, before and after the introduction of the three-vessel view in our screening program. The reason why we chose to study the detection rate of Fallot and TGA lies in the fact that in TGA, prenatal detection can

prevent postnatal hypoxia and ultimately cardiac arrest, and in Fallot it creates the opportunity for genetic testing in pregnancy.
In TGA, the foramen ovale closes immediately after birth and the ductus arteriosus constricts within the first days of life, transitioning the heart to the circumstances outside the womb. If a fetus with TGA is undetected before birth, they have an increased risk of mortality and morbidity due to the severe hypoxia which is the result of the fact that the oxygenated blood cannot reach the systemic circulation.
It is known that Tetralogy of Fallot cases have an increased risk of genetic abnormalities. These cases benefit from increased detection prior to 24 weeks gestation (the legal limit for pregnancy termination in the Netherlands), providing the opportunity to offer additional genetic testing. When a genetic anomaly is present, the long term outcome for children with Fallot is worse than children with an isolated defect, thus a prenatal diagnosis allows parents to reach a decision on whether or not to continue with the pregnancy.

The current mandatory planes in the Dutch screening program consist of four cardiac planes; the four-chamber view, left- and right outflow tracts and the three-vessel view. This is quite sparse considering the fact that in the diagnostic setting, more than 60 minutes and over a hundred planes and sequences is no exception. Our study showed that the simple addition of the three-vessel view as a mandatory plane to the national screening program leads to the increased detection of TGA and Fallot. Following this example, the national screening guideline could be expanded with the 'V-sign/three vessel trachea view', the aortic arch in the sagittal plane and measurements of the semilunar valves to detect anomalies like aortic coarctation, pulmonary artery or aortic valve stenosis and total pulmonary venous return, which all present with very subtle changes in the planes. Preliminary research shows that the detection rate of aortic coarctation has been consistently low at a rate of 25% and has not risen in recent years, despite efforts in quality control and regular schooling of ultrasonographers. Children suffering from aortic coarctation benefit from an early detection, as a postnatal detection can lead to cyanosis and morbidity and even cardiac arrest, therefore we emphasize the need for a prenatal detection in these cases. Adding additional planes could increase the detection further. Moreover, to eliminate human error to some extent, we expect innovation and increased detection if automated image detection is implemented (with artificial intelligence). Studies regarding this topic are already being undertaken and show promising results.

Cortical development in fetal life

Prenatal brain development in CHD has gained increased interest as it became apparent that fetuses showed delayed Head Circumference (HC)-growth compared with normal controls. Secondly, pre-operative delays in cortical development in CHD children has furthered the interest in fetal cortical development for the etiology of altered neurodevelopment in CHD.

The human fetus follows a predictable pattern of emerging and specific temporal evolution of gyri and sulci. It is known from studies in brain malformations that cortical development is complex and is orchestrated by neuronal migration, which can be influenced by abnormal circumstances. Several studies showed that sustained or intermittent periods of cerebral hypoxia can lead to abnormal cerebral development[4]. This has been shown in lamb fetuses that were exposed to cerebral hypoxemia by transient bilateral carotid clamping, in which signs of decreased oligodendrocyte maturation were seen[5]. Oligodendrocytes synthesize myeline, which, if disturbed, results in white matter injury, since pre-oligodendrocytes are specifically prone to ischemic injury. The pre-oligodendrocytes in lamb fetuses with CHD are more easily damaged compared to those in healthy controls. Next to disturbances in oligodendrocyte maturation, studies with hypoxic animals also describe abnormal cortical development, such as reduced cortical volume and microstructural maturation. With the use of pre-operative MRI and neurological examinations in CHD neonates, more abnormalities were found compared to control neonates[6, 7], which supports the hypothesis that neurodevelopmental delays may be present before surgery and not all damage occurs in the peri-operative period.

In chapter 5 the ultrasonographic assessment of cortical development in fetal life showed delays in cortical maturation. This was, however, only statistically significant in the Sylvian, calcarine and the cingulate fissures. Alterations in the cingulate fissure area, are associated with attention deficit disorder, which is significantly more prevalent in CHD children. Therefore, it is possible the delay in maturation found in the Sylvian and cingulate fissures are associated with altered cognitive and behavioral problems in later life. The differences in cortical maturation found in the prenatal ultrasound studies described in this thesis (chapter 5,6 and 7) are, however, small. In chapter 6 we describe a brain maturation delay of four days using an brain-age estimation algorithm in all CHD's combined. After stratification, these delays were mainly seen in subgroups with TGA and cases with intra-cardiac mixing.

The results of our studies show an important difference with other studies exploring this subject, describing a more severe delay of up to 3 weeks in ultrasound[8, 9] and MRI studies[10-12], however, in our studies, we only see a delay of 4 days.

This could have two reasons: Firstly, the generalizability of some MRI studies are questionable, as these studies included severe cases (for example HLHS) more often than

moderate or minor cases, which might have introduced selection bias[11]. Secondly, some of the studies concerned one or two measurements in a broad range of gestational ages, producing results with broad confidence intervals, this could have over-exaggerated their findings[10, 13, 14]. Furthermore, it is frequently mentioned that ultrasound has a lower accuracy than MRI to pick up brain abnormalities. We do not agree with this statement, as it has been proven that neurosonography performed by experienced sonographers in a tertiary setting is not inferior to MRI[15, 16].
On the other hand, we need to keep in mind that even small differences found prenatally could have a big impact on development in later life. Fetuses are known for an accelerated growth trajectory during pregnancy. If a disturbance takes place, it could mean this hampers physiological brain development. This could lead to a cumulative effect, since the foundation of the fetal brain has been flawed. In other words: the small deviations we have found prenatally with ultrasound studies might still have a clinical relevance in CHD-children.

Etiology of prenatal brain damage

The incidence and severity of neurologic deficits are still very difficult to predict, even if the cardiac defect is known prenatally, and birth is uncomplicated and timely, takes place in an center equipped for cardiac care. The multitude of published data on prenatal, neonatal and adolescent neurodevelopment is testament to the diversity of proposed etiologies. In this thesis we explore the theory on altered cerebral hemodynamics in CHD in several chapters. Also, altered placental function and different genetic make-up in CHD are possible etiologies that might play a role.

Children born with CHD are prone to brain damage in the first weeks of life and during the pre-operative period. Prolonged ventilation, cardiopulmonary-bypass and ICU admission are all, on itself, associated with white matter injury. *Pre*-operative brain abnormalities, such as white matter injury and arterial ischemic stroke, are seen in 19-52% of cases with different imaging modalities. Furthermore, in *fetuses* with CHD, head growth and intracerebral blood flow are found to be abnormal, even in-utero[17]. This poses the theory that altered neurodevelopment in CHD children might have its origin in prenatal life. Theoretically, fetuses with left obstructive lesions (Aortic arch anomalies, HLHS) have diminished flow, and fetuses with intra-cardiac mixing lesions (TGA, DORV, Fallot) have diminished oxygenation of the brain. In these lesions it was shown that impaired development of the fetal cortex in a small group of severe CHD cases (mainly TGA and left-sided obstructive lesions) with MRI[18]. The largest finding in this thesis (chapter 7) is a delay in cortical maturation of almost 4 days is found in fetuses with TGA. Yet, these findings in fetuses with TGA are in large contradiction to the neurologic(al) follow-up studies in children with TGA;

they are known to have normal cognitive development with a normal IQ. Yet they show a somewhat higher prevalence of behavioral disorders (for example attention deficit disorder)[19]. Furthermore, in our review of the literature on prenatal neurodevelopment in CHD (chapter 3), we have included studies in which MCA measurements (middle cerebral artery) were available. A total of 1412 fetuses with different CHD raging from moderate to severe were included. In one of the largest prospective studies, 72 fetuses with single ventricle morphology, increased cerebral blood flow was correlated with better ND-outcome in later life [20]. Theoretically, decreased resistance in the middle cerebral artery due to vasodilatation should be present in the case of a CHD, to enhance cerebral oxygen and nutrient delivery. In growth restricted fetuses, this is called the brainsparing effect, but in growth restriction this correlated with a worse long-term outcome. Since brainsparing in CHD-cases was correlated with a more favorable neurological outcome, a different etiology than growth restricted fetuses might be playing a role. The contradictory effect of the same intracerebral flow pattern (i.e. brain perfusion) in different pathologies, makes it questionable if the proposed theory that altered neurodevelopment is the effect of altered brain perfusion is correct.
Finally, as mentioned previously, the methodology of several imaging studies in CHD-fetuses, show selection bias. Fetuses with severe CHD (univentricular heart defects, HLHS) are included in these studies more often than moderate/minor CHD. These children are prone to more complex interventions and lengthy ICU stays as compared to children in the minor end of the CHD spectrum, with subsequent worse neurological outcome. Furthermore, genetic abnormalities are more often present in severe CHD-cases. This makes it difficult to draw conclusions on brain development of the full CHD -spectrum. Also, severe delays in-utero are not always correlated to postnatal findings, meaning that the clinical relevance of these findings still remains uncertain.

Lower birth weight and delayed HC-growth have been reported in CHD-fetuses, and is in itself associated with poor neurodevelopmental outcome as well. This has led to the thought that placental function in CHD cases might be abnormal and might also be influenced by the cardiac lesion. An association between non-genetic severe CHD cases (for example HLHS) and decreased HC-growth was not found, however, placental related complications, such as: growth restriction and pre-eclampsia, are seen more often in CHD-cases. Therefore, placental pathology might play a role in the etiology of altered neurodevelopment in CHD. Underlining this hypothesis, it has been shown that growth-factors that promote growth and angiogenesis, like VEGF (Vascular Endothelial Growth Factor), PIGF (Placental Endothelial Growth Factor) and soluble fms-like tyrosine kinase-1 (sFlt-1) are abnormal in mothers carrying

CHD fetuses. Also, the development of the placenta and the heart share the same regulatory pathways, such as Wnt (Wingless and Int-1). Wnt signaling pathways are involved in embryonic development and are necessary for normal cardiogenesis, they also play an important role in implantation, decidualization and placental differentation[21]. The strongest indication that placenta formation and heart development are intertwined, was seen in studies with knock-out mice. They were given specific genetic defects that are associated with congenital heart defects and showed abnormal placentation. After post-mortem examination, researchers found that these mice died because of severe growth restriction due to abnormal placentation[22].

Furthermore, a combination of aforementioned etiologies are involved in the presence of altered neurodevelopment in CHD, and therefore, we must consider it to be a multifactorial problem.

Parental counseling on neurodevelopment in CHD

The above described knowledge on the appearance and variation of neurodevelopmental delay in CHD cases, makes the prediction of outcome in later life extremely imprecise and difficult.

If parents are faced with the fact that the heart defect of their fetus may also influence brain development, but the extent of this delay cannot be predicted, this uncertainty may be very hard to cope with.

The American heart association (AHA) published a statement in 2012 stating that 'children with CHD are at increased risk of developmental disorder or disabilities' after evaluation of literature published on the matter between 1966 – 2011, advising physicians to strive for early detection of problems, in order to support them accordingly. For fetal medicine, the ISUOG released a statement in 2016 that is much more conservative than the statement of the AHA. They state that deficiencies in neurodevelopment are more prevalent in CHD children. The prenatal findings must, however, be correlated to postnatal neurologic testing, in order to validate the hypothesis that these findings really cause delay in neurodevelopment in later life. Since the release of this statement, a quick search reveals more than 200 peer-reviewed published articles on the subject. The association has been thoroughly confirmed, and in our opinion a new recommendation should amend the previous ISUOG statement. Numerous imaging studies have shown alterations in cortical development, decreased brain volume and white matter injury. Although the extent and timing of occurrence has not been sufficiently clarified, the link between neurodevelopment and congenital heart defects has been well-established. We would suggest to inform the parents on the multifactorial theory on neurodevelopmental delay in CHD children. Moreover, decreased cerebral oxygenation in-utero might be the

most obvious originator, but there must be more factors, seeing as the expression of altered cerebral development in CHD presents in a broad spectrum of defects. Although the timing might not be fully understood, exacerbating factors like preterm birth, perioperative complications, CPB and prolonged ventilation (e.a.) must be avoided if possible. Physicians should be aware of the increased vulnerability, and provide expecting parents with tailored information on altered neurodevelopmental outcome in CHD. This message should of course be delivered with some caution, because prenatal alterations have not sufficiently been correlated to postnatal outcome yet. Fortunately, the most frequent findings in neurodevelopmental testing concern learning difficulties, behavioral problems and executive functions, and not severe neurodevelopmental impairment (NDI) leading to complete dependency of medical care.

Future perspectives and conclusion

This thesis presents a number of prospective, well-executed studies in a cohort of consecutive cases with isolated CHD ranging from minor to severe defects of disease. These studies were specifically conducted to thoroughly explore brain development with multiple measurements in pregnancy, because previous studies had various methodological pitfalls (e.g. single measurements, selection bias, non-isolated caseload). Our studies showed significant but slight variations in development of the brain compared to normal fetuses. Repeated examinations allowed for the analysis of development during pregnancy and these analyses did not see a worsening during the third trimester as suggested by other publications.

We propose the following implementation in all tertiary centers in the Netherlands to gather a large set of CHD-types for research purposes.

The questions that still need answering are the following: Do children that display worse neurodevelopmental outcome later in life, have abnormal brain development in their fetal life? Can the severity of impaired neurodevelopment be predicted in pregnancy?

Is there an association between placental pathology and CHD, and also: does placenta pathology lead to worse neurodevelopmental outcome in CHD?

The methodology for research projects that will provide the answers to these knowledge gaps is a multicenter prospective cohort study according to the following guidelines:

Thorough neurosonography needs to be performed in the second and third trimester including: fetal growth, hemodynamics and cortical development. Specific attention should be aimed towards deviations from the normal sulcation patterns and structural anomalies. Furthermore, an MRI needs to be performed in moder-

ate/severe CHD cases using a uniform protocol (including the assessment of cortical development, volumetry and white matter injury) at least prenatally (around 34 weeks) and pre-and postoperatively. These MRI's need to be assessed by an experienced team, and use a standardized method for analysis of cortical development [23]. Whole exome sequencing (WES), has to be added as a standard genetic testing offer (now consisting of QF-PCR and SNP-array). Furthermore, placental tissue needs to be analyzed by a specialized pathologist to investigate signs of hypoxic-ischemic damage, and tissue needs to be stored in biobanks for further research. The infrastructure for such a collaboration has already been established in CAHAL (Center for congenital heart defects Amsterdam – Leiden), and can easily be rolled out in the rest of the Netherlands.

The focus of further study possibilities in cerebral development in CHD cases should address etiology and if prevention is deemed impossible, therapies should be developed to prevent further damage to the brain.
To further the understanding of the etiology of cerebral dysgenesis in CHD fetuses, we propose to look into placental pathology in combination with CHD. Fetal growth restriction and placental diseases like pre-eclampsia are more common in pregnancies complicated by CHD. A correlation between delayed neurodevelopment in fetuses with CHD with abnormal placentation might be explanatory in this issue. Therefore, biobanking of placental tissue is being conducted, which might clarify the contribution of the placenta further. In such a prospective cohort, all prenatal and perioperative data should be correlated to neuro-cognitive examinations and intellectual performance in school-aged children, to interpret the minor delays we have found with the presented studies. This requires the collaboration of maternal-fetal medicine specialists with pediatric cardiologists, and although this is not common practice everywhere, within CAHAL the collaboration is well-established. Ongoing research is being performed in our group, to gather an even more robust cohort of CHD, which allows for stronger subgroup analyses.

Although the etiology of prenatal brain damage in fetuses with congenital heart disease might not be completely understood, researchers are looking in to protective measures in peri-operative and neonatal ICU-care. Preventive measures such as the prenatal administration of Allopurinol[24, 25] or intranasal bone marrow cell therapy[26], might possibly be available in the future, to diminish the aforementioned 'second hit' caused by peripartum or perioperative circumstances.

Neurocognitive deficits in children with congenital heart defects are common and the mechanism behind it remains unclarified. A combination of genetics, hemo-

dynamics, placental factors and peri-operative complications are considered risk factors for worse neurodevelopmental outcome. One of the largest protecting factors is prenatal detection of a congenital heart defects. Uniform screening protocols with standard planes are known to increase detection of CHD, and we have demonstrated that adding new planes to this uniform program adds to the recognition of CHD. From a global perspective, other countries could model after our well organized national screenings program, since it has led to one of the highest detection rates of CHD. Although we are currently still dependent on human identification of imaging, automated image analysis has shown promising results and will definitely play a role in the future. This would benefit both prenatal detection of CHD and identify children who are prone to worse neurodevelopmental outcome.
Attention for the brain development is crucial in children with CHD as the increased vulnerability for altered neurodevelopment has been well established. With the right support, families, teachers and other caretakers of children with CHD could help them to thrive by meeting their specific needs.

References

1 Slagboom. Echo: Augustus; 2011.

2 Oosterhuis JJ, Gillissen A, Snijder CA, et al. **Decision-making in the referral process of sonographers in primary care screening centers.** Prenat Diagn 2016;366:555-60.

3 van Velzen CL, Clur SA, Rijlaarsdam ME, et al. **Prenatal detection of congenital heart disease--results of a national screening programme.** BJOG 2016;1233:400-7.

4 Sun L, Macgowan CK, Sled JG, et al. **Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease.** Circulation 2015;13115:1313-23.

5 Morton PD, Ishibashi N, Jonas RA. **Neurodevelopmental Abnormalities and Congenital Heart Disease: Insights Into Altered Brain Maturation.** Circ Res 2017;1206:960-77.

6 Miller SP, McQuillen PS, Vigneron DB, et al. **Preoperative brain injury in newborns with transposition of the great arteries.** Ann Thorac Surg 2004;775:1698-706.

7 Te Pas AB, van Wezel-Meijler G, Bokenkamp-Gramann R, et al. **Preoperative cranial ultrasound findings in infants with major congenital heart disease.** Acta paediatrica 2005;9411:1597-603.

8 Koning IV, van Graafeiland AW, Groenenberg IAL, et al. **Prenatal influence of congenital heart defects on trajectories of cortical folding of the fetal brain using three-dimensional ultrasound.** Prenat Diagn 2017;3710:1008-16.

9 Peng Q, Zhou Q, Zang M, et al. **Reduced fetal brain fissures depth in fetuses with congenital heart diseases.** Prenat Diagn 2016;3611:1047-53.

10 Masoller N, Sanz-Cortes M, Crispi F, et al. **Mid-gestation brain Doppler and head biometry in fetuses with congenital heart disease predict abnormal brain development at birth.** Ultrasound Obstet Gynecol 2016;471:65-73.

11 Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. **Delayed cortical development in fetuses with complex congenital heart disease.** Cereb Cortex 2013;2312:2932-43.

12 Ortinau CM, Rollins CK, Gholipour A, et al. **Early-Emerging Sulcal Patterns Are Atypical in Fetuses with Congenital Heart Disease.** Cereb Cortex 2018.

13 Jaimes C, Rofeberg V, Stopp C, et al. **Association of Isolated Congenital Heart Disease with Fetal Brain Maturation.** AJNR Am J Neuroradiol 2020;418:1525-31.

14 Mlczoch E, Brugger P, Ulm B, et al. **Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program.** Eur J Paediatr Neurol 2012.

15 Griffiths PD, Bradburn M, Campbell MJ, et al. **Use of MRI in the diagnosis of fetal brain abnormalities in utero (MERIDIAN): a multicentre, prospective cohort study.** Lancet 2017;38910068:538-46.

16 Malinger G, Ben-Sira L, Lev D, et al. **Fetal brain imaging: a comparison between magnetic resonance imaging and dedicated neurosonography.** Ultrasound Obstet Gynecol 2004;234:333-40.

17 Peng Q, Zeng S, Zhou Q, et al. **Different vasodilatation characteristics among the main cerebral arteries in fetuses with congenital heart defects.** Sci Rep 2018;81:4544.

18 Kelly CJ, Makropoulos A, Cordero-Grande L, et al. I**mpaired development of the cerebral cortex in infants with congenital heart disease is correlated to reduced cerebral oxygen delivery.** Sci Rep 2017;71:15088.

19 Bellinger DC, Wypij D, Rivkin MJ, et al. A**dolescents with d-transposition of the great arteries corrected with the arterial switch procedure: neuropsychological assessment and structural brain imaging.** Circulation 2011;12412:1361-9.

20 Williams IA, Fifer C, Jaeggi E, et al. **The association of fetal cerebrovascular resistance with early neurodevelopment in single ventricle congenital heart disease.** Am Heart J 2013;1654:544-50.

21 Sonderegger S, Pollheimer J, Knofler M. **Wnt signalling in implantation, decidualisation and placental differentiation--review.** Placenta 2010;3110:839-47.

22 Park SG, Kim EK, Nam KH, et al. **Heart defects and embryonic lethality in Asb2 knock out mice correlate with placental defects.** Cells Dev 2021;165:203663.

23 Garel C, Chantrel E, Elmaleh M, et al. **Fetal MRI: normal gestational landmarks for cerebral biometry, gyration and myelination.** Childs Nerv Syst 2003;197-8:422-5.

24 Stegeman R, Lamur KD, van den Hoogen A, et al. **Neuroprotective Drugs in Infants With Severe Congenital Heart Disease: A Systematic Review.** Front Neurol 2018;9:521.

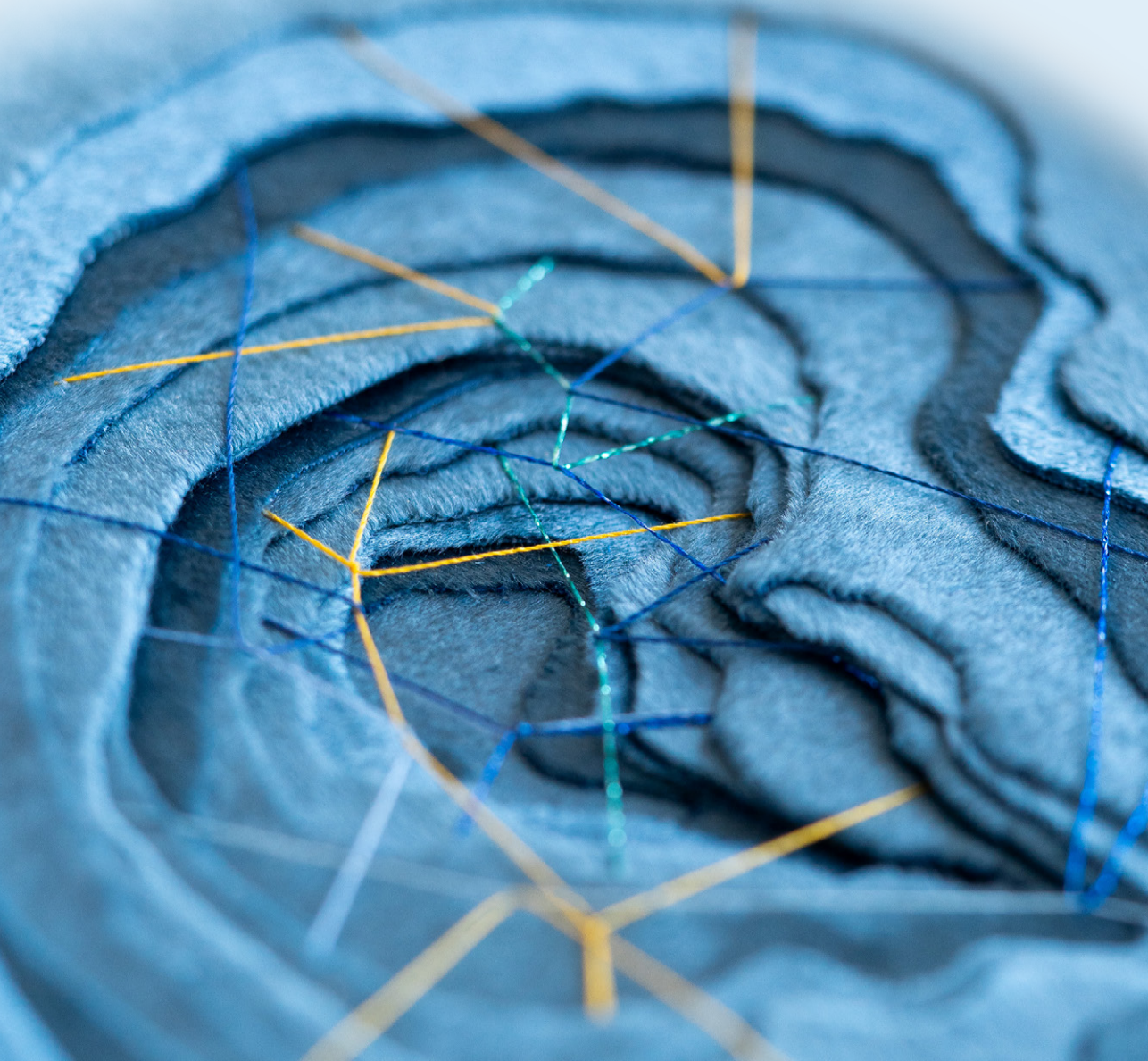
25 Kaandorp JJ, Benders MJ, Schuit E, et al. **Maternal allopurinol administration during suspected fetal hypoxia: a novel neuroprotective intervention? A multicentre randomised placebo controlled trial.** Arch Dis Child Fetal Neonatal Ed 2015;1003:F216-23.

26 Baak LM, Wagenaar N, van der Aa NE, et al. **Feasibility and safety of intranasally administered mesenchymal stromal cells after perinatal arterial ischaemic stroke in the Netherlands (PASSIoN): a first-in-human, open-label intervention study.** Lancet Neurol 2022;216:528-36.

CHAPTER

9

Summary



SUMMARY

Children affected by congenital heart defects (CHD), are nowadays known to live longer and with less morbidity than children with CHD that were born some decades ago, due to the advances in perinatal, perioperative and intensive care. Long-term follow-up studies of children born with (isolated) CHD however show a high prevalence of morbidity, especially neurodevelopmental impairment. These impairments include neurologic, cognitive and behavioral deficits. In **chapter 1,** the introduction of this thesis, this topic is discussed in greater detail.

Detecting CHD antenatally has a positive effect on survival and long-term neurodevelopmental outcome in isolated CHD. Efforts that are made to increase prenatal detection of CHD are therefore of the utmost importance. Historically, CHDs that present with an abnormal four chamber-view on ultrasound, have better prenatal detection rates than CHDs that have a normal four-chamber view. The latter CHDs may present with abnormal views of the outflow tracts, which a far more difficult to recognize. **Part one, chapter 2** of this thesis shows the comparison of the prenatal detection of transposition of the great arteries (TGA) and Tetralogy of Fallot (ToF) which usually have a normal four chamber view. The detection of these two CHD-types was compared between two time periods: before and after the introduction of the three vessel view. The addition of this plane as a mandatory item in the Dutch screening protocol for the 20 weeks anomaly scan resulted in an increase of the detection of TGA from 44% to 82%, and the detection of ToF from 43% to 67%.

To explore the impact of a prenatal diagnosis on mortality rates between detected and undetected cases, we studied mortality rates of neonates with TGA within the Amsterdam-Leiden geographic region, between 2007 and 2015. Assuming that these neonates would have received immediate postnatal care, at least 4 of 9 deaths might have been prevented if they were detected prenatally. This also highlights the importance of a prenatal diagnosis to decrease mortality in TGA cases.

In **part two** of this thesis, prenatal neurodevelopment in CHD cases is studied. Chapter 3 presents a systematic review and meta-analysis of studies on this subject published before November 2015. This review showed that fetuses with CHD consistently show significantly smaller head circumferences as compared to controls across all studies; either measured by MRI, ultrasound or postnatally. The maturation of the brain was mainly studied by MRI, in which signs of delay were encountered in several studies. The studies that were included in this systematic review however, showed several methodological weaknesses such as selection bias (only the most severe end

of the CHD spectrum was studied), the absence of comparing the results with the actual outcome after birth and the inclusion of fetuses without information about the presence of a genetic syndrome. Furthermore, structural brain malformations were attributed to acquired injury, due to hemodynamic changes during pregnancy, although that actual correlation was not studied.

To explore prenatal neurodevelopment in CHD cases without the encountered weaknesses of the described studies, the HAND (Heart And Neurodevelopment) study was started in 2014. It explores cerebral development in cases with isolated congenital heart defects. All consecutive cases that are referred to our tertiary center, Leiden University Medical Center, are studied by neurosonography every 4 weeks until delivery. Since the HAND-study includes only isolated CHD cases, fetuses with known and suspected genetic malformations are not included, or excluded if a genetic syndrome became apparent after birth. The infants were followed up until the age of one year. In the neurosonography examinations, the cortical development was assessed in 2D and 3D imaging. These data resulted in the following chapters.

In **chapter 4,** we assessed the feasibility of neurosonography in a clinical setting, which means that the examinations were performed in a limited time frame. Both fetuses with a CHD as well as normal fetuses were examined. The visibility of different brain structures was scored by researchers, blinded for the presence or absence of a CHD. Intra-observer and interobserver variation turned out to be excellent. A neurosonographic examination was considered complete when at least 7/9 analysed brain structures were visible in the recorded images and clips. In the CHD group this was encountered in 79% of examinations, and in the control group in 90% of the examinations. In both groups, the examinations that were conducted between 22-34 weeks yielded the highest results, this time frame is thus considered as the optimal time for neurosonography. Also, the structures that are visible in the axial plane (lateral ventricle, cerebellum and cavum septum pellucidum), and the coronal plane (frontal horns) were visible in almost all examinations. The influence of confounding factors on visibility of cerebral structures was also considered. We found no differences in common factors that could have influenced visibility, such as mothers with BMI higher that 30, non-cephalic presenting fetuses, and non-anterior placenta's. Thus, this study showed that neurosonography for the purpose of surveillance in a clinical setting is possible, but when there is an indication for extended neurosonography and the need to see every structure of the brain, larger time-slots than then the 30 minutes that were used should be planned. Extended neurosonography with a (seemingly) unlimited time-frame, in which there is time to await a favorable fetal position, is not a realistic representation of day-to-day practice.

The cortical development in CHD-cases and controls assessed by 2D ultrasound during pregnancy is studied in **chapter 5.** Nine different brain sulci (Sylvian, parieto-occipital, central, calcarine and cingulate fissures) and brain areas (frontal, parietal, occipital and temporal areas) were scored using a chart with a range from 0 (no cortical maturation) until 5 (end-stage maturation). Of the analysed cortical structures, only the cingulate fissure and the Sylvian fissure were significantly delayed in CHD fetuses compared to controls. Alterations in these fissures are also encountered in children presenting with behavioral problems, for example in attention deficit disorder. Possibly the delays in cortical development that were found in this study could be a first expression of altered neurodevelopment in children with CHD. The differences that were found in this study were however very subtle (-0.25 grade point per fissure), which raised uncertainty about the clinical significance of this finding. On the other hand, small deviations from normal development in very early life may lead to large differences in later life, as certain developmental milestones might be missed and lead to larger developmental delays.

For the analyses in **chapter 6** and **7** a deep learning software algorithm was applied to the 3D brain volumes acquired by ultrasound, to assess the cortical age. Cortical age was plotted against true gestational age in CHD-cases and controls, to represent the speed of maturation. And thus, cortical or brain age was used as a proxy for brain development. The use of a deep-learning algorithm to analyse cortical development is superior to manual analysis, since the algorithm is trained with a large dataset of normally developed brains. The algorithm displays exactly which areas represent cortical development at every stage of prenatal development for which it was trained. Theoretically, the algorithm determines the cortical development more accurate, compared to manually selected and scored cortical fissures and areas.

In **chapter 6,** a group of 90 isolated CHD-fetuses and 75 controls were sequentially scanned and the brain-age was calculated using the aforementioned algorithm. This article represents the first clinical application of the presented algorithm. The main finding was a significant but small delay of 3 days in CHD-fetuses compared to control fetuses. This delay was consistent throughout pregnancy, defying previous reports on further deteriorating delay in cortical maturation with advancing gestational age. Again, the small delay in brain-age that was found in this study may represent a clinical significant delay in cortical development that might have a profound effect in later life. It is, however, also plausible that other factors that occur later in life play a role in neurodevelopmental delay in CHD children.

To address the influence of flow and oxygenation toward the brain in different types of CHD, a subgroup analysis is presented in **chapter 7.** A total of 660 US examinations that were performed in 142 CHD-cases and 75 controls were analysed using the brain-age algorithm. In the two subgroups, that contain TGA-cases and intracardiac mixing cases (e.g. Tetralogy of Fallot and AVSD), a significant delay of maximum 4 days compared to controls was found. Whether these findings could only be attributed to the altered cerebral oxygenation because of the cardiac lesion, remains uncertain. A great number of factors play a role in the etiology of neurodevelopmental delay in CHD-children, some of which might not be known yet.

Lastly, in **chapter 8,** all the findings in this thesis are discussed, and recommendations to improve prenatal screening are suggested. Furthermore, future research areas, such as correlation with placenta pathology, are presented.

SAMENVATTING

Kinderen geboren met aangeboren hartafwijkingen (aangeboren hartafwijking, AHA) leven tegenwoordig langer en gezonder dan kinderen met AHA die enkele decennia geleden geboren werden, dankzij de vooruitgang in perinatale, perioperatieve - en neonatale zorg. Lange termijn studies naar kinderen geboren met (geïsoleerde) AHA tonen echter vaker problemen die buiten de cardiologische problematiek liggen, vooral neurologische ontwikkelingsstoornissen. Daarbij zijn verschillende domeinen van de ontwikkeling aangedaan en laten kinderen onder andere neurologische, cognitieve en gedragsstoornissen zien. In **hoofdstuk 1,** de introductie van dit proefschrift, wordt dit in meer detail besproken.

Wanneer AHA prenataal gedetecteerd worden, laat dat een positief effect op de overleving en de neurologische ontwikkeling op lange termijn bij geïsoleerde hartafwijkingen zien. Verhogen van deze detectie is daarom van groot belang om de langetermijn-uitkomsten te verbeteren. Historisch gezien worden AHA met een echografisch abnormaal vier-kamerbeeld beter gedetecteerd dan hartafwijkingen met een normaal vier-kamerbeeld. De laatstgenoemde hartafwijkingen hebben immers afwijkende uitstroombanen, die aanzienlijk moeilijker te herkennen zijn. **Deel één, hoofdstuk 2** van dit proefschrift toont de vergelijking van de detectie van transpositie van de grote vaten (TGA) en tetralogie van Fallot (ToF) tussen twee tijdsperioden: vóór en na de introductie van het three-vesselview. De toevoeging van dit vlak als verplicht item in het Nederlandse screeningsprotocol voor de twintig weken echo resulteerde in een toename van de detectie van TGA van 44% naar 82%, en de detectie van ToF van 43% naar 67%.

Om de invloed van een prenatale diagnose op sterftecijfers tussen gedetecteerde en niet-gedetecteerde gevallen te onderzoeken, bestudeerden we sterftecijfers van pasgeborenen met TGA binnen de geografische regio Amsterdam-Leiden tussen 2007 en 2015. Ervan uitgaande dat deze pasgeborenen onmiddellijk postnatale zorg zouden krijgen, hadden 4 van de 9 sterfgevallen mogelijk voorkomen kunnen worden met een prenatale diagnose. Dit hoofdstuk onderschrijft derhalve het belang van een prenatale diagnose om de mortaliteit in TGA-gevallen te verlagen.

In **deel twee** van dit proefschrift wordt de prenatale neurologische ontwikkeling bij CHD onderzocht. **Hoofdstuk 3** is een systematisch literatuur-onderzoek en meta-analyse van studies over dit onderwerp die voor november 2015 zijn gepubliceerd. Deze review toonde aan dat foetus met hartafwijkingen een significant kleinere hoofdomtrek laten zien in vergelijking met controles; dit was gemeten met

MRI, echoscopie of postnataal. De rijping van de hersenen werd voornamelijk bestudeerd met MRI, waarbij in verschillende studies tekenen van vertraagde rijping werden gevonden. De studies die werden opgenomen in deze systematic review vertoonden echter verschillende methodologische tekortkomingen, zoals selectiebias (in sommige studies werden alleen ernstige hartafwijkingen met slechte lange termijn-uitkomsten bestudeerd), bij sommige studies ontbrak de daadwerkelijke correlatie met de uitkomst na de geboorte en werden foetussen met genetische syndromen niet geëxcludeerd. Bovendien werden structurele hersenafwijkingen toegeschreven aan verworven letsel als gevolg van hemodynamische veranderingen tijdens de zwangerschap, hoewel die feitelijke correlatie niet werd bestudeerd.

Om de prenatale neurologische ontwikkeling te onderzoeken zonder de zwakke punten van de beschreven studies, werd in 2014 de HAND (Heart And Neurodevelopment) studie gestart, die de cerebrale ontwikkeling onderzoekt bij foetussen met geïsoleerde aangeboren hartafwijkingen. Alle opeenvolgende gevallen die naar ons tertiaire centrum, het Leids Universitair Medisch Centrum, zijn verwezen, werden tot aan de bevalling elke 4 weken onderzocht middels neurosonografie. Omdat de HAND-studie alleen geïsoleerde AHA omvat, werden foetussen met bekende en vermoede genetische afwijkingen niet geïncludeerd, of na de geboorte geëxcludeerd wanneer dit het geval was. Tijdens de neurosonografische onderzoeken gedurende de zwangerschap, werden zowel 2D als 3D beelden gemaakt. De gegevens van deze onderzoeken resulteerden in de volgende hoofdstukken.

In **hoofdstuk 4** onderzochten we de haalbaarheid van neurosonografie in een klinische setting, wat betekent dat de onderzoeken in een beperkt tijdsbestek werden uitgevoerd. Zowel foetus met een congenitale hartafwijking als normale foetus werden onderzocht. De zichtbaarheid van verschillende hersenstructuren werd gescoord door onderzoekers, die geblindeerd waren voor de aan- of afwezigheid van een hartafwijking. De intra-observer en interobserver variatie bleek uitstekend te zijn. Een neurosonografisch onderzoek werd als compleet beschouwd als ten minste 7/9 geanalyseerde hersenstructuren zichtbaar waren in de opgenomen beelden en clips. In de CHD-groep werd dit in 79% van de onderzoeken aangetroffen en in de controlegroep in 90% van de onderzoeken. In beide groepen leverden de onderzoeken die werden uitgevoerd tussen 22-34 weken zwangerschap de beste resultaten op, en deze termijn wordt dus beschouwd als het optimale tijdstip voor neurosonografie. Ook waren de structuren die zichtbaar zijn in het transversale of axiale vlak (laterale ventrikel, cerebellum en cavum septum pellucidum) en het coronale vlak (voorhoornen) in vrijwel alle onderzoeken zichtbaar. Er werd ook gekeken naar veel voorkomende factoren die mogelijk van invloed kunnen zijn op de zichtbaar-

heid van hersenstructuren. De volgende factoren bleken niet statistisch significant; moeders met een BMI hoger dan 30, foetus die zich in stuit of dwarsligging presenteerden en placenta's die niet aan de voorkant lagen. Deze studie toonde aan dat neurosonografie om de hersenontwikkeling te monitoren mogelijk is, maar als er een indicatie is voor uitgebreide neurosonografie en de noodzaak om elke structuur van de hersenen te zien, moeten er langere tijdslots worden gepland dan de 30 minuten die beschikbaar waren. Uitgebreide neurosonografie met een onbeperkt tijdslot, waarin er ook tijd is om te wachten tot de foetus zich uit een ongunstige positie beweegt, geeft geen realistische weergave van de dagelijkse praktijk.

De prenatale corticale ontwikkeling die werd gemonitord middels 2D echoscopie en vergeleken in AHA en controles, wordt beschreven in **hoofdstuk 5.** Negen verschillende sulci (Sylvius, parieto-occipitale, centrale, calcarine en cingulate fissuren) en hersengebieden (frontale, pariëtale, occipitale en temporale gebieden) werden gescoord met behulp van een classificering tussen 0 (geen corticale maturatie) tot 5 (volledige maturatie). Van de geanalyseerde corticale structuren waren alleen de cingulate fissuur en de fissuur van Sylvius significant vertraagd in vergelijking met controles. Veranderingen in deze fissuren komen ook voor bij kinderen met gedragsproblemen, zoals ADHD. Mogelijk zijn de vertragingen in de corticale ontwikkeling die in dit onderzoek werden gevonden, een eerste uiting van een veranderde neurologische ontwikkeling bij kinderen met AHA. De verschillen die in deze studie werden gevonden, waren echter zeer subtiel (-0,25 punt per fissuur), waardoor het onduidelijk is hoe klinisch relevant deze bevindingen zijn. In theorie zouden deze kleine verschillen van de normale ontwikkeling op zeer jonge leeftijd kunnen leiden tot grote verschillen op latere leeftijd, omdat het missen van bepaalde ontwikkelingsmijlpalen zou kunnen leiden tot grote ontwikkelingsachterstanden.

Voor de analyses in **hoofdstuk 6** en **7** werd een deep-learning software algoritme toegepast op de 3D-hersenvolumes verkregen met de HAND-studie, om de corticale leeftijd te bepalen. De corticale leeftijd werd vergeleken met de werkelijke zwangerschapsduur bij foetus met AHA en controles, om zo de snelheid van corticale rijping weer te geven. Het gebruik van een deep-learning algoritme om de corticale ontwikkeling te analyseren bleek beter dan handmatige analyse. Dit is het gevolg van het feit dat het algoritme getraind is met een grote dataset van normaal ontwikkelde hersenen en precies kan weergeven welke gebieden de corticale ontwikkeling vertegenwoordigen in elke fase van de prenatale ontwikkeling. Theoretisch bepaalt het algoritme de corticale ontwikkeling nauwkeuriger in vergelijking met handmatig geselecteerde en gescoorde corticale sulci en hersengebieden.

In **hoofdstuk 6** werd het bovengenoemde algoritme voor het eerst in een klinische setting toegepast. Een groep van 90 geïsoleerde foetussen met AHA en 75 controles onderzocht middels echografie waarvan de corticale rijping werd berekend werd met behulp van het bovengenoemde algoritme. De belangrijkste bevinding was een significante maar kleine vertraging van drie dagen bij foetussen met AHA vergeleken met controle foetus. Deze vertraging bleef gelijk gedurende de hele zwangerschap, wat eerdere rapporten over toenemende vertraging gedurende de zwangerschap tegenspreekt. Zoals eerder genoemd, zou de kleine vertraging in corticale rijping, die werd gevonden in deze studie, mogelijk een klinisch significante vertraging in de corticale ontwikkeling kunnen vertegenwoordigen die een effect zou kunnen hebben in het latere leven. Het is echter ook aannemelijk dat andere factoren die later in het leven optreden, een rol kunnen spelen in de neurologische ontwikkelingsachterstand van kinderen met AHA.

Om de rol van bloeddoorstroming en zuurstoftoevoer naar de hersenen van foetussen met verschillende typen AHA te onderzoeken, wordt in **hoofdstuk 7** een subgroepanalyse gepresenteerd. In totaal werden 660 echoscopische onderzoeken uitgevoerd bij 142 AHA-foetussen en 75 controles geanalyseerd met behulp van het algoritme voor de corticale ontwikkeling. In de twee subgroepen met TGA en AHA waarbij het bloed intracardiaal gemengd wordt (bijv. ToF en AVSD) werd een significante vertraging van maximaal vier dagen gevonden vergeleken met controles. Of deze bevindingen alleen kunnen worden toegeschreven aan de veranderde zuurstoftoevoer naar de hersenen als gevolg van de AHA, blijft onzeker. Een groot aantal factoren zou een rol kunnen spelen in de ontstaanswijze van neurologische ontwikkelingsachterstand bij kinderen met AHA, waarvan sommige misschien nog niet bekend zijn.

Tot slot worden in **hoofdstuk 8** alle bevindingen in dit proefschrift besproken en worden aanbevelingen gedaan om prenatale screening te verbeteren. Verder worden toekomstige onderzoeksgebieden, zoals de correlatie met placentapathologie, gepresenteerd.

CHAPTER

9

CHAPTER

10

Appendices

LIST OF ABBREVIATIONS

3v	Third Ventricle
3vv	Three-vessel view
4v	Fourth Ventricle
AC	Abdominal Circumference
ADHD	Attention Deficit Hyperactivity Disorder
AI	Artificial Intelligence
AU	Uterine Artery
AS	Aortic Stenosis
ASO	Arterial Switch Operation
AVSD	Atrio-ventricular Septal Defect
BPD	Biparietal Diameter
BMI	Body Mass Index
BSID	Bayley's Scale of Infant Development
CB	Cerebellum
CC	Corpus Callosum
CHARGE-syndrome	Coloboma, Heart defects, choanal Atresia, growth
	Retardation, Genital abnormalities and Ear abnormalities
CHD	Congenital Heart Defect
CM	Cisterna Magna
CMR	Cardiac Magnetic Resonance
CNS	Central Nervous System
CPR	Cerebro-Placental Ratio
CoA	Coarctation of the Aorta
CS	Caesarean Section
CSF	Cerebro-spinal Fluid
CSP	Cavum Septum Pellucidum
DORV	Double Outlet Right Ventricle
DMT	Discontinued Medical treatment
DR	Detection Rate
Ebstein	Ebstein's anomaly
EEG	Electro encephalogram
(E)RP	(Emergency) Rashkind Procedure
FH	Frontal Horns
FL	Femur Length
FO	Oval Foramen
GA	Gestational Age
HAND-study	Heart and NeuroDevelopment-study

HC	Head Circumference
HLHS	Hypoplastic Left Heart Syndrome
ICU	Intensive care Unit
IQ	Intelligence Quotient
IUFD	Intra-uterine Fetal Death
LV	Left Ventricle
MCA	Middle Cerebral Artery
MOF	Multi-Organ Failure
MRI	Magnetic Resonance Imaging
ND	Neurodevelopment
NIPT	Non Invasive Prenatal Test
PA	Pulmonary Atresia
PD	Prenatal Diagnosis
PI	Pulsatility Index
POF	Parieto-Occipital Fissure
PS	Pulmonary Stenosis
RI	Resistance Index
SAS	Standard Anomaly Scan
SD	Standard Deviation
SF	Sylvian Fissure
TA	Tricuspid Atresia
TI	Tricuspid Insufficiency
TGA	Transposition of the Great Arteries
TOD	Thalamo-Occipital Depth
ToF	Tetralogy of Fallot
TOP	Termination of Pregnancy
Truncus	Truncus Arteriosus
RSOL	Right Sided Obstructive Lesions
UA	Umbilical Artery
US	Ultrasound
VOCAL	Virtual Organ Computer-aided Analysis
VSD	Ventricle Septal Defect

LIST OF PUBLICATIONS

Everwijn SMP, *van Bohemen J., Jansen FAR, Teunissen AKK, Haak MC,*
Feasibility of neurosonography in CHD-fetuses and controls in a clinical tertiary setting. Submitted for publication.

Everwijn SMP, *van Bohemen JF, van Geloven N, Jansen FAR, Teunissen AKK, Rozendaal L, Blom N, van Lith JMM, Haak MC, Sylke J. Steggerda.*
Serial neurosonography in fetuses with congenital heart defects shows mild delays in cortical development. Prenatal Diagnosis. 2021 Dec;41(13):1649-1657

Everwijn SMP, *Namburete AIL, van Geloven N, Jansen FAR, Papageorghiou AT, Teunissen AKK, Rozendaal L, Blom N, van Lith JMM, Haak MC.*
The association between flow and oxygenation and cortical development in fetuses with congenital heart defects using a brain-age prediction algorithm
Prenatal Diagnosis. 2021 Jan;41(1):43-51

Everwijn SMP, *Namburete AI, van Geloven N, Jansen FAR, Papageorghiou AT, A. J. Noble, Teunissen AKK, Rozendaal L, Blom NA, van Lith JMM, Haak MC.*
Cortical development in fetuses with congenital heart defects using an automated brain-age prediction algorithm.
ACTA Obstetricia et Gynecologica Scandinavica 2019 Dec;98(12):1595-1602

Jansen FAR, van Zwet EW, ***Everwijn SMP,*** *Teunissen AKK, Rozendaal L, van Lith JMM, Blom NA, Haak MC.*
Fetuses with Isolated Congenital Heart Defects Show Normal Cerebral and Extracerebral Fluid Volume Growth: A 3D Sonographic Study in the Second and Third Trimester.
Fetal Diagnosis Therapy. 2019 Jan 17:1-9

Everwijn SMP, *van Nisselrooij AEL, Rozendaal L, Clur SB, Pajkrt E, Hruda J, Linskens IH, van Lith JM, Blom NA, Haak MC.*
The effect of the introduction of the three-vessel view on the detection rate of transposition of the great arteries and tetralogy of Fallot*. Prenatal Diagnosis. 2018 Nov;38(12):951-957. *cover story.

Peeters-Scholte CMPCD, Adama van Scheltema PN, Klumper FJCM, ***Everwijn SMP,*** *Koopmans M, Hoffer MJV, Koopmann TT, Ruivenkamp CAL, Steggerda SJ, van der Knaap MS, Santen GWE.*
Genotype-phenotype correlation in ATAD3A deletions: not just of scientific relevance. Brain. 2017 Nov 1;140(11):e66

Everwijn SMP, *Jansen FAR, Scheepjens R, Stijnen T, Peeters-Scholte CM, van Lith JM, Haak MC.*
Fetal brain imaging in isolated congenital heart defects - a systemic review and meta-analysis.
Prenatal Diagnosis. 2016 Jul;36(7):601-13

van de Mheen L, ***Everwijn SMP,*** *Haak MC, Manten GT, Zondervan HA, Knapen MF, Engels MA, Erwich JJ, Coumans AB, van Vugt JM, Bilardo CM, van Pampus MG, de Groot CJ, Mol BW, Pajkrt E.*
Outcome of Multifetal Pregnancy Reduction in Women with a Dichorionic Triamniotic Triplet Pregnancy to a Singleton Pregnancy: A retrospective Nationwide Cohort Study. Fetal Diagnosis Therapy. 2016;40(2):94-9

van de Mheen L, ***Everwijn SMP,*** *Knapen MF, Haak MC, Engels MA, Manten GT, Zondervan HA, Wirjosoekarto SA, van Vugt JM, Erwich JJ, Bilardo CM, van Pampus MG, de Groot CJ, Mol BW, Pajkrt E.*
Pregnancy outcome after fetal reduction in women with dichorionic twin pregnancy. Human Reproduction. 2015 Aug;30(8):1807-12

van de Mheen L, ***Everwijn SMP,*** *Knapen MF, Oepkes D, Engels M, Manten GT, Zondervan H, Wirjosoekarto SA, van Vugt JM, Erwich JJ, Nij Bijvank SW, Ravelli A, Heemelaar S, van Pampus MG, de Groot CJ, Mol BW, Pajkrt E.*
The effectiveness of multifetal pregnancy reduction in trichorionic triplet gestation. American Journal of Obstetrics Gynecology. 2014 Nov;211(5):536

CURRICULUM VITAE

Sheila Maria Pieternella Everwijn werd op 9 februari 1986 thuis geboren te Udenhout terwijl de ijsbloemen op de ramen stonden. Al vroeg in haar leven wist zij dat ze een passie voor geneeskunde bezat. Zelfs na twee uitlotingen liet ze deze passie niet uit het oog verliezen, niet toen ze kortstondig Farmaceutische wetenschappen studeerde, en ook niet toen zij volledig genoot van het niet-academische leven.

Zo begon ze in 2007 met veel energie aan de Universiteit van Amsterdam met de opleiding Geneeskunde. Tussendoor werd ze verrast door het overlijden van haar vader, wat een diepe indruk naliet. Niet veel later leerde ze haar huidige partner kennen en begon het leven een steeds verantwoordelijkere wending te krijgen. Niet onverdienstelijk begon zij aan haar wetenschappelijke stage naar de effecten van selectieve reductie bij drielingenzwangerschappen onder leiding van Prof. Dr. E. Pajkrt. Zij presenteerde de resultaten van dit onderzoek op het internationale congres van de Society for Maternal Fetal Medicine (SMFM) in Dallas, Texas, en ontving hiervoor de Van Walree beurs van het KNAW. Aan het einde van haar coschappen bracht ze vier maanden door in het Haydom Lutheran Hospital, aldaar zagen meerdere Sheila's het leven en ook één Wim, de naam van haar vader. Hoewel de toekomst in de Gynaecologie al steeds meer wortels kreeg, vond ze deze nadruk alsmaar meer tijdens haar oudste coschap in het Zaans Medisch Centrum.

De basis van dit proefschrift werd gelegd in het LUMC van 2015 - 2019, op de afdeling prenatale diagnostiek en foetale behandeling waar Sheila in 2015 begon als arts-echoscopist. Onder de vleugels van Prof. Dr. M. Haak verzamelde zij de gegevens voor de HAND-studie. Haar eerste dochter Ella werd tijdens deze periode geboren in Amsterdam. Na een bijzonder plezierige periode in het LUMC en na het maken van vrienden voor het leven, ging Sheila verder als ANIOS op de afdeling gynaecologie/verloskunde in het Haaglanden Medisch Centrum te 's Den Haag.

In 2020 is zij begonnen met de specialistenopleiding tot gynaecoloog in het Haaglanden Medisch centrum te Den Haag (opleider dr. M. Kagie en later dr. W. Hermes), en is sinds 2022 weer werkzaam in het LUMC voor het academische gedeelte van de opleiding (opleider dr. M. Sueters). Nadat zij niet alleen een stap maakte in haar carrière, verhuisde ze samen met haar gezin naar Den Haag. Waar in 2021 haar tweede dochter, Philo het leven zag.

Sheila woont samen met haar geliefde Rik en hun dochters Ella (2017) en Philo (2021) en poes Lou (2014).


Monique
Ana
Nico
Belangrijke zaken: Brecht,
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La Familia Madrigal!
Vrienden en vriendinnen
Alle lieve (oude) buren
(Schoon) familie
mama
Ella & Philo
Rik ♥

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