Congenital heart defects: from a prenatal perspective

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Congenital heart defects from a fetal perspective

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Congenital heart defects
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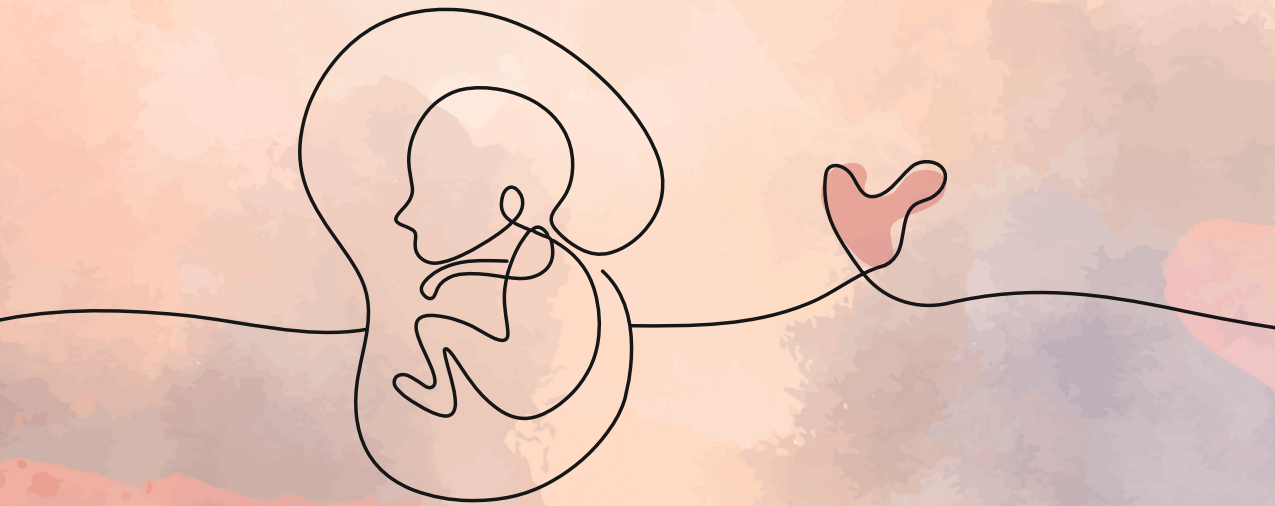

CHAPTER 1

General introduction

Congenital heart defects (CHD) are the most common congenital defect with a prevalence of 6-10 per 1 000 newborns.[1] These concern a severe heart defect in 2-3 per 1 000 newborns, which is defined as the need for surgery or an intervention within the first year of life.[2] The clinical presentation of (severe) congenital heart defects can vary significantly between diagnoses. Timely recognition of CHD is therefore warranted to prevent potentially life-threatening situations or permanent damage, as CHD remain one of the leading causes of congenital defect-related infant morbidity and mortality.[3] The majority of infant deaths attributable to CHD occur within the first 28 days after birth (also known as the neonatal period).[4]

One of the major changes in the transition from fetal to neonatal circulation is the closure of the ductus arteriosus, which generally closes within 72 hours after birth.[5] Neonates with a ductal-dependent CHD, however, rely upon a patent ductus arteriosus (PDA) to enable sufficient blood supply to the pulmonary or systemic circulation or allow adequate mixing between parallel circulations. Prostaglandin administration directly after birth can prevent acute cardiac decompensation in these patients and bridge the time until surgery is feasible.[6]

The majority of critical CHD neonates are symptomatic and therefore identified soon after birth. However, up to 30% of critical CHD neonates appear normal on routine examination and may be discharged home without diagnosis.[7, 8] The mortality risk in critical CHD neonates that remain unidentified during birth hospitalization, is reported to be as high as 30%.[9] If the time to diagnosis and referral to a tertiary care center with expertise and optimal neonatal care is longer, the morbidity and mortality risk in these cases has proven to increase as well.[9, 10] Timing of clinical manifestation may vary significantly between critical cardiac defects. Especially cardiac defects that do not depend upon a patent ductus arteriosus are unlikely to become symptomatic within the first 72 hours, yet a delayed diagnosis can result in similarly poor outcomes. Besides that, cyanosis is often not clinically recognized in mild desaturation (>80% saturation), anemia or darkly pigmented infants. Most developed countries therefore implemented the use of pulse oximetry as a routine screening tool in asymptomatic newborns to improve recognition of (cyanotic) cardiac defects after birth.[11, 12]

Antenatal screening for CHD

Following the first paper on the use of sonography in obstetrics in 1958[13], ultrasound has become an essential tool to visualize fetal (cardiac) anatomy with great detail and diagnose structural anomalies as soon as mid-gestation. A prenatal diagnosis enables a planned delivery in a neonatal care facility with prompt and optimal treatment, and therewith reduce mortality and improve long-term outcome as described above.[14-19]

Furthermore, prenatal diagnosis allows for further monitoring and the opportunity to perform genetic analyses to optimize prenatal counseling and prepare parents for having a child with CHD. In severe cases, this may even lead to the parent's decision to opt for termination of pregnancy.

Only 10% of all fetuses with a cardiac defect occur in pregnancies with known risk factors to develop CHD. On the other hand, 90% of fetuses with CHD are encountered unexpectedly in otherwise healthy families.[20, 21] Therefore, most developed countries have set up population-based screening programs to optimize fetal detection rates for congenital heart defects. Although these programs vary between and even within countries, the majority offer a routine anomaly scan for all pregnant women around 20 weeks of gestation, regularly preceded by a first trimester anomaly scan to detect major congenital anomalies. Practice guidelines for sonographic screening at mid-gestation recommend diagnostic imaging of the 'four-chamber view' as well as the different outflow tract views. This systematic approach aids to identify fetuses at risk for structural anomalies in a low-risk setting, to maximize the detection of cardiac anomalies during a second-trimester scan.[22-24]

The structural anomaly scan (SAS) was introduced as part of the national screening program for congenital anomalies in the Netherlands in 2007. The Dutch screening program has a unique character, due to the fact that its training program and quality monitoring is regulated on a national level. All sonographers operate following a standardized scanning protocol and are certified after uniform training requisites. Eight regional centers therewith monitor the quality of the ultrasound images for all sonographers, as well as the minimum scans that should be performed by each sonographer on a yearly basis, in order for them to stay qualified.[25, 26]

Despite standardized guidelines and protocols, structural cardiac anomalies remain the most frequently missed congenital defect on fetal ultrasound.[27] Screening programs have reported detection rates between 30–75% in most developed countries.[28-34] Single-center studies, on the other hand, achieve detection rates up to 80-90%.[35-37] Heart defects that present with a normal four-chamber view appear the most likely to be missed, especially in the absence of known risk factors or additional structural anomalies.[26, 38, 39] Although never studied in current literature, it is commonly thought that these discrepancies in detection rates rely on differences in (modifiable) factors, such as examiner experience, maternal obesity, gestational age and fetal position. If modifiable factors that impede prenatal detection can be identified, the sensitivity of national or regional screening programs may potentially increase to the 80–90%, as reported in single-center studies.

Prenatal counseling

One of the advantages of a prenatal diagnosis is the ability to inform and counsel parents before birth. It is essential for counseling that the prenatal diagnosis is accurate, ideally with an evidence-based estimate of the prognosis. This information is crucial for optimal postnatal care management, as well as informed decision making in case parents consider pregnancy termination.

Currently available literature, however, often focuses on the prognosis of neonates with a certain CHD following (successful) surgical correction or intervention.[3,40] Fetuses diagnosed with a CHD comprise a significantly different population, as those that demise before birth or do not make it to surgery are not included. To improve prenatal counseling at mid-gestation, fetal studies that report on the prognosis of these cardiac diagnoses from a fetal perspective are needed to facilitate reliable and patient-centered estimates of pre- and postnatal outcome.

Congenital heart defects are associated with additional extracardiac anomalies, neurodevelopmental delay and chromosome or genetic alterations.[41-44]. These co-morbidities may have a significant impact on the prognosis as well. After initial recognition that the heart is abnormal on fetal ultrasound, an advanced ultrasound examination by a fetal echocardiography expert is performed, to ascertain the diagnosis and assess the presence of other structural anomalies. A prenatal diagnosis also enables further genetic testing to timely identify genetic alterations in the fetal DNA, as CHD have been described as part of many genetic syndromes. Thus far, only a few cohort studies have reported the estimated incidence of genetic diagnoses in fetuses diagnosed with a CHD. Many of these studies are somewhat outdated, as they mainly focus on numerical chromosome abnormalities, such as trisomies or triploidy, or 22q11.2 deletion syndrome specifically.[45-47] Recently, innovative techniques including microarray analysis and exome sequencing allow to diagnose other copy number variations or genetic mutations as well. As chromosomal and genetic alterations can have a major impact on (neuro-)development, quality of life and life expectancy of children with a CHD, most developed countries already offer microarray analysis as the standard of care in the fetal setting to identify copy number variations.[48] The use of targeted or whole-exome sequencing to detect subtle genetic variations, commonly referred to as 'genetic mutations', is often preserved for a minority of CHD cases. Population-based studies that evaluate the direct relation between specific cardiac defects and the prevalence of genetic syndromes to this extend, have hardly been published in current literature. Evidence on the prevalence of these genetic variants can improve prenatal counseling in cases with severe CHD and aid professionals to decide whether the use of currently available additional tests, such as exome sequencing (ES) or genetic testing for specific genetic syndromes is indicated.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to study factors that influence the potential to detect congenital heart defects before birth and gather knowledge on the prognosis of antenatally detected cases with a congenital heart defect in order to improve prenatal counseling and postnatal care management.

Prenatal detection

This part of this theses aims to evaluate the influence of risk factors and identify causes for a missed prenatal diagnosis at mid-gestation to improve prenatal detection rates for congenital heart defects. The results of our case-control study are presented in **chapter 2**, where we describe differences in baseline characteristics, sonographer's experience and quality of ultrasound exams between prenatally undetected and detected cases with a congenital heart defect.

Outcome

The second part of this thesis focuses on the prognosis of congenital heart defects following a prenatal diagnosis at mid-gestation. In **chapter 3** we explored the prevalence of genetic diagnoses in the majority of congenital heart defects, either isolated or non-isolated, and evaluated the impact of these diagnoses on mortality and morbidity. As suggested in a Letter to the Editor, we additionally report the proportion of genetic diagnoses that is detectable with either chromosome microarray analysis (CMA) and exome sequencing for each congenital heart defect separately in **chapter 4,** to aid clinicians determine the genetic tests that should be performed.

Neurodevelopmental delay is frequently encountered in children with congenital heart defects. In **chapter 5**, we report on a cohort study evaluating the impact of extracardiac pathology on fetal head circumference - as a marker for brain development – to identify whether delayed neurodevelopment can be purely attributed to hemodynamic changes in utero caused by these heart defects.

The final chapters of this thesis focus on prenatal diagnoses that are challenging to counsel at mid-gestation, due to limited evidence on the outcome of these particular findings from a prenatal perspective. Ventricular size disproportion - for example - is a marker for aortic coarctation (CoA) in fetal life. Approximately 50% of these fetuses do, however, not develop CoA after birth. In **chapter 6**, we evaluate the postnatal outcome of cases with ventricular disproportion in the absence of CoA in a cohort of prenatally detected cases. **Chapter 7** describes a cohort study and systematic literature review on the outcome of fetal common arterial trunk beyond the neonatal period and

with regard to the presence of genetic diagnoses, extracardiac malformations and neurodevelopment. In **chapter 8**, we report on an international retrospective case series and systematic literature review to provide information on the outcome of fetuses with aorto-left ventricular tunnel, a diagnosis that is rarely made before birth.

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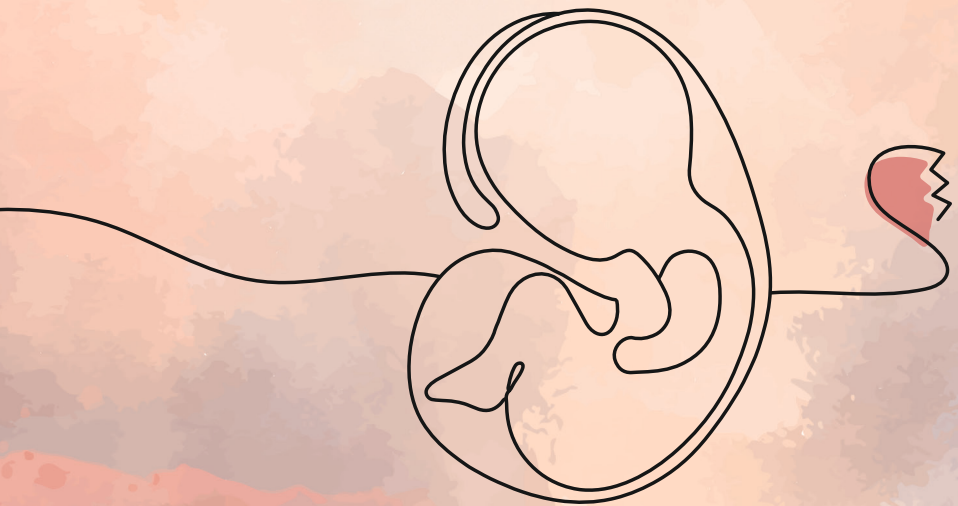

CHAPTER 2

Why are congenital heart defects being missed?

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ABSTRACT

Objectives

Congenital heart defects (CHD) are still frequently missed in prenatal screening programs, which can result in severe morbidity or even death. The aim of this study was to evaluate the quality of fetal heart images, obtained during the second-trimester standard anomaly scan (SAS) in cases of CHD, to explore factors associated with a missed prenatal diagnosis.

Methods

All cases born with an isolated severe CHD from 2015 to 2016 were extracted from the PRECOR registry. Severe CHD was defined as the need for surgical repair in the first year of life. Each cardiac view (four-chamber view (4CV), three-vessel (3V) view and left and right ventricular outflow tract (LVOT, RVOT) views) obtained during the SAS was scored for technical correctness on a scale of 0 to 5 by two fetal echocardiography experts, blinded to the diagnosis of CHD and whether it was detected prenatally.

Results

A total of 114 isolated CHD cases were analyzed, of which 58 (50.9%) were missed and 56 (49.1%) were detected on the SAS. The defects comprised transposition of the great arteries (17%), aortic coarctation (16%), tetralogy of Fallot (10%), atrioventricular septal defect (6%), aortic valve stenosis (5%), ventricular septal defect (18%) and other defects (28%). No differences were found in fetal position, obstetric history, maternal age or body mass index (BMI) or gestational age at examination between missed and detected cases. Compared with the detected group, the missed group had significantly lower cardiac examination quality scores (adequate score (≥ 12) in 36% *vs* 68%; $P = 0.002$), rate of proper use of magnification (58% *vs* 84%; $P = 0.01$) and quality scores for each individual cardiac plane (4CV (2.7 *vs* 3.9; $P < 0.001$), 3V view (3.0 *vs* 3.8; $P = 0.02$), LVOT view (1.9 *vs* 3.3; $P < 0.001$) and RVOT view (1.9 *vs* 3.3; $P < 0.001$)). In 49% of missed cases, the lack of detection was due to poor adaptational skills resulting in inadequate images; in 31%, the images showed an abnormality (mainly septal defects and aortic arch anomalies), which had not been recognized at time of the scan; whereas in 20%, the cardiac planes had been properly obtained, but showed normal anatomy.

Conclusions

A lack of adaptational skills, as opposed to circumstantial differences, appears to play an important role in prenatally undetected CHDs. Despite adequate quality of the images, the CHD was not recognized in 31% of cases. A high volume of SAS performed by each sonographer, in particular when performed in a large screening center, contributes to prenatal detection. In 20% of the undetected cases, the CHD was not visible, even though the quality of the images was good.

INTRODUCTION

Congenital heart defects (CHD) are the most common birth defect, with a prevalence of approximately 5-8 per thousand live births. Ultrasound in pregnancy enables prenatal diagnosis of CHD, which allows for delivery in a facility with appropriate postnatal care. Prenatal identification of CHD has been shown to decrease mortality and perioperative morbidity and may improve neurodevelopmental outcome.[1-5]

Prenatal detection of CHD, however, still fails in approximately half of the cases.[6] Screening programs in most developed countries have reported a detection rate (DR) of only 30-60%, which varies according to type of cardiac defect.[7-19] Although prenatal DRs have increased gradually over the past few years, the identification of modifiable factors, if targeted appropriately, could potentially increase the sensitivity of current screening programs to achieve a DR of 80-90%, as reported in single centers-studies.[20-22]

The most commonly missed severe CHDs are conotruncal lesions, such as transposition of the great arteries, tetralogy of Fallot, double outlet right ventricle and truncus arteriosus, as the four-chamber view may be falsely reassuring in the majority of these cases. The outflow tract views, which are necessary to detect these lesions, are more challenging to capture in a routine screening setting.[13] Cases with an isolated heart defect that present with a normal four-chamber view therefore appear to be the most likely to be missed, especially in the absence of known risk factors or additional structural anomalies.[23-28] It has also been speculated that human factors, such as experience, might be associated with failure to detect fetal CHD, as large differences in DRs can be found between healthcare facilities and geographical areas within the same country.[24, 25]

Therefore, this study aimed to identify factors that contribute to the failure to detect CHD, by auditing *original* images obtained during the second-trimester standard anomaly scans (SAS) of fetuses with undetected and detected CHD, in order to potentially improve antenatal DRs.

METHODS

Selection of cases

Screening for congenital anomalies is performed in The Netherlands based on a strict national SAS protocol, similar to the ISUOG protocol.[29] Every sonographer who performs SAS examinations is required to pass a national standardized examination and is monitored every two years, by evaluation of three randomly selected SAS, in order to assess their competence. If a sonographers does not pass this assessment, their qualification is withdrawn, and they are no longer able to perform SAS. This national screening program has resulted in one of the highest DRs for CHD worldwide[6, 30], which is reflected in the 82% detection rate for transposition of the great arteries.[31]

The Amsterdam University Medical Centers, location AMC and VUMC, and Leiden University Medical Center collaborate in the care for children with CHD in the Amsterdam-Leiden regions. All subjects with either a prenatal diagnosis of CHD or a postnatal diagnosis of *severe* CHD in these regions have been registered in the PRECOR database since 2002. Severe CHD is defined as the need for surgery or therapeutic cardiac catheterization within the first year of life. Data collection for this registry has been described previously.[23] This registry was used to identify all cases of *severe* isolated CHD, delivered in the period 2015-2016. We decided to include only recent cases, as the three-vessel view was introduced as a mandatory plane in 2012 and to ensure retrieval of the original ultrasound images in the majority of cases. Cases that did not undergo SAS in the second trimester, were excluded.

The mothers of CHD subjects were sent a letter with information regarding the study and an informed consent form to return if they were willing to participate. Mothers of CHD subjects that were not alive at the time of recruitment were excluded from the study, as requested by the ethical review board of our institution, but we ascertained the type of lesion in these cases from the PRECOR database. Following receipt of informed consent, mothers were contacted once to retrieve the location at which the SAS had been performed. Included subjects were allocated to either the group with or without a prenatal diagnosis of CHD.

Data collection

We collected the original ultrasound images from the SAS and pregnancy data. If data were missing, midwives were contacted for additional information. From 2007 onwards, the national prenatal screening database PERIDOS has registered all pregnant women who undergo SAS in The Netherlands. This database was used to retrieve information regarding the volume of SAS performed per year at each prenatal screening center,

and by each sonographer, as well as the sonographer's years of experience at the time of the SAS.

We developed a standard form to assess the quality of the ultrasound examination, as an indicator of the sonographer's technical skills, and additional parameters of interest. In order to assess objectively the quality of the cardiac examination, each of the four standard cardiac planes received a score between 0 and 5, resulting in a maximum total score of 20 for the entire cardiac examination. The score was based on the number of quality criteria met for that specific plane (Table 1). For example, if 3/5 criteria were met, the plane received a score of 3 (adequate quality). If the sonographer obtained multiple images of the same cardiac plane, these were assessed together. In case clips were recorded, they were assessed in the same manner. A cardiac examination with a total score of ≥ 12 (average score of ≥ 3 for each plane) was considered adequate, whereas a total score < 12 was considered inadequate. Examples of cardiac images with their respective scores, are depicted in Figure 1. A fetal medicine consultant [M.C.H.], specialized in fetal cardiology, and a senior cardiac sonographer [A.K.K.T.] scored the images together and were blinded to patient characteristics, diagnosis and whether the CHD had been detected prenatally. To quantify the reliability of this scoring system, 27 cases were scored twice to calculate the intraclass correlation coefficient (ICC)[32]. The time interval between the first and repeat assessments was more than 6 months in order to avoid recall bias.

Table 1. Criteria for quality assessment of cardiac planes obtained during second-trimester standard anomaly scan.

Four-chamber view (4CV)	**Left ventricular outflow tract (LVOT)**
• Complete depiction of both atrial chambers • Complete depiction of both ventricles • Cardiac crux visible • Clear visualisation of both AV valves • Clear visualisation of the ventricular septum	• Plane approximately at level of LVOT • Chosen plane at the maximum size of the vessel • Visibility of the aortic valve • Perimembranous septum visible in the plane • Complete long-axis from LV apex to ascending aorta visible
Right ventricular outflow tract (RVOT)	**Three-vessel view (3VV)**
• Plane approximately at level of RVOT • Depicted at maximum size of the vessel • Visibility of the pulmonary valve • Upper part of RV visible • Pulmonary artery visible from RV to arterial duct	• True transverse plane through the chest • Pulmonary artery (PA) visible from right ventricle to arterial duct • Valve (PA) visible • Clear depiction of the aorta • Clear depiction of right superior caval vein

AV, atrioventricular; LV, left ventricle; LVOT, left ventricular outflow tract; RV, right ventricle; RVOT, right ventricular outflow tract

The collected baseline characteristics comprised gestational age at screening, maternal age, body mass index, obstetric and medical history, multiple pregnancy, fetal gender, CHD diagnosis and the sonographer's and screenings center's experience and volume. We evaluated fetal position, resolution of the ultrasound images (amount of detail in the image that could be obtained), use of magnification, visibility of the heart defect and quality of each of the four cardiac planes: four-chamber view (4CV), three-vessel view (3VV) and the left and right outflow tracts (LVOT, RVOT).

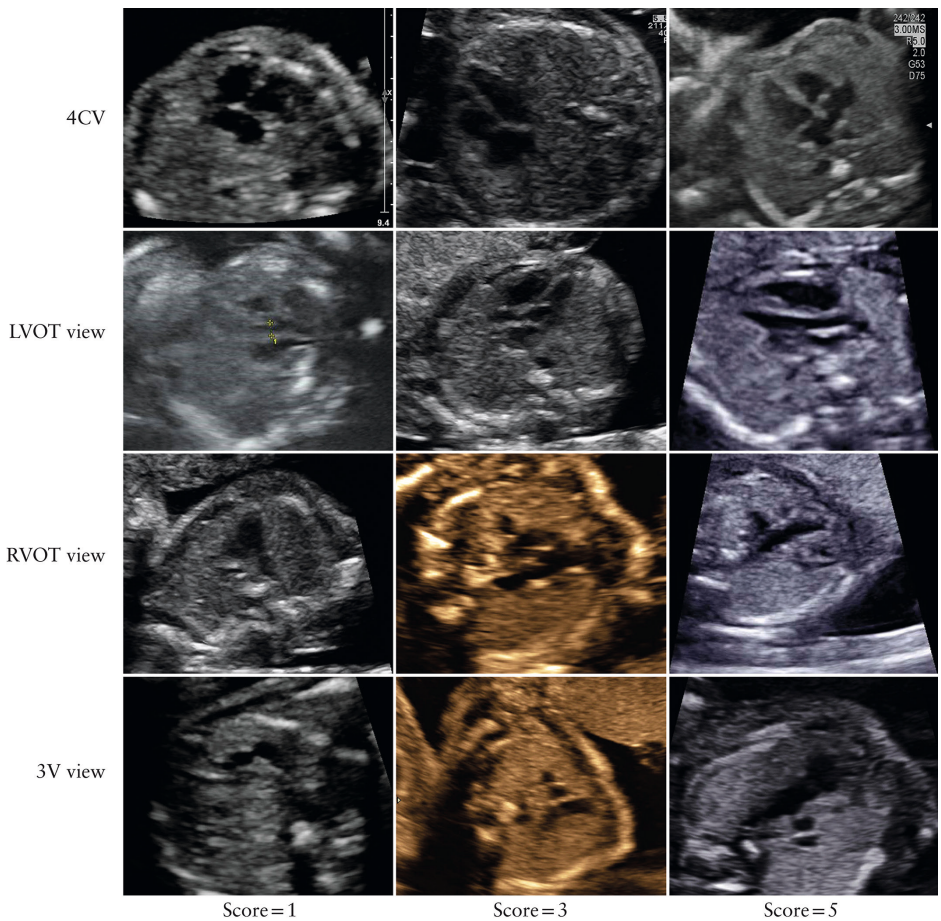

Figure 1. Visual scoring system. Examples of ultrasound images of the fetal heart in four-chamber (4CV), left ventricular outflow tract (LVOT), right ventricular outflow tract (RVOT) and three-vessel (3V) views, that obtained quality score of 1, 3 or 5, in cases with severe congenital heart disease at birth.

Fetal position was classified based on the position of the spine on an analog clock. A position of the spine from 10 to 2 o'clock (clockwise) was considered to be unfavorable, whereas the rest was scored as favorable. Sonographer and screening center volume, as well as the experience of the sonographer in years, was assessed for the year in which the SAS had been performed. Image resolution was scored on a five-point Likert scale, in which 1 represented poor resolution (lots of noise, multiple speckles, grey amniotic fluid) and 5 represented good resolution (clear black amniotic fluid, lots of detail visible in the image).

In order to gain insight into the completeness and quality of the SAS in normal cases, we retrieved the results from routine quality monitoring assessments in the Leiden region (in 2015). In these assessments, the four standard cardiac planes were scored as either 0 (inadequate), 1 (adequate) or a (absent). Normal cases could not be recruited in the same way in which CHD cases were as, in The Netherlands, only the physician who treated the patient is allowed to approach them. First, we collected the results from three normal scans obtained by sonographers in the Leiden region who missed a heart defect in the current cohort. Second, results from three normal scans performed by a random sample of 40 sonographers in the Leiden region were evaluated. We then assessed whether cardiac examination quality differed significantly between these two groups. As the evaluation in the national monitoring system had been performed in less detail, direct comparison of scores between the normal and CHD cases was not possible.

Statistical analysis

All variables of interest, that may possibly influence the ability to detect CHD prenatally, were compared between the undetected and detected group. Univariate and multivariate regression analyses were performed to assess whether the quality of the ultrasound examination was influenced by the sonographer's or screening center's experience.

To identify potential causes for a missed prenatal diagnosis, we considered the adequacy of the cardiac examination (total score < or ≥ 12) alongside the visibility of the heart defect, as assessed by the expert examiners [M.C.H. and A.K.K.T.], in all undetected cases. These were used to define three types of causes for a missed prenatal diagnosis. The first involved the sonographer being unable to obtain technically correct cardiac planes in cases with abnormal anatomy. These cases were missed due to *a lack of adaptational skills* and comprised all undetected cases in which the total quality score was < 12 and the heart defect was not clearly visible, according to our

experts examiners, because of suboptimal planes. The second cause was when the heart defect was *not recognized*, despite being clearly visible on the retrieved images, irrespective of the quality of the planes. Undetected heart defects that were not visible despite good quality of the images (total score ≥ 12) were classified as *inevitable (3)*. These three causes of a missed diagnosis in undetected cases were assessed according to the type of CHD.

Categorical variables were compared using a χ^2-test and continuous variables were compared using the independent t-test. ICC estimates and their 95% CI were calculated based on a mean-rating (k = 2), consistency-agreement, two-way mixed-effects model. IBM SPSS Statistics 23.0 for Windows (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. A P-value < 0.05 was considered statistically significant.

RESULTS

A total of 198 cases of severe CHD without an additional anomaly, born in 2015-2016, were extracted from the PRECOR registry. All mothers were approached to participate in the study, except for 12 cases (6.1%), in which the infant was not alive at time of recruitment. These 12 cases comprised univentricular heart defects (67%; all of which were detected prenatally) and other defects (33%; of which 80% were detected and 20% were undetected). We did not receive a response from 51 subjects (25.8%) and 10 (5.1%) chose not to participate in the study. Eleven subjects (5.6%) did not undergo SAS in the second trimester, because they had indications, mainly increased nuchal translucency, for an advanced diagnostic scan, including fetal echocardiography. This resulted in a total of 114 cases eligible for inclusion, of which 58 (50.9%) were undetected and 56 (49.1%) were detected prenatally (Figure 2).

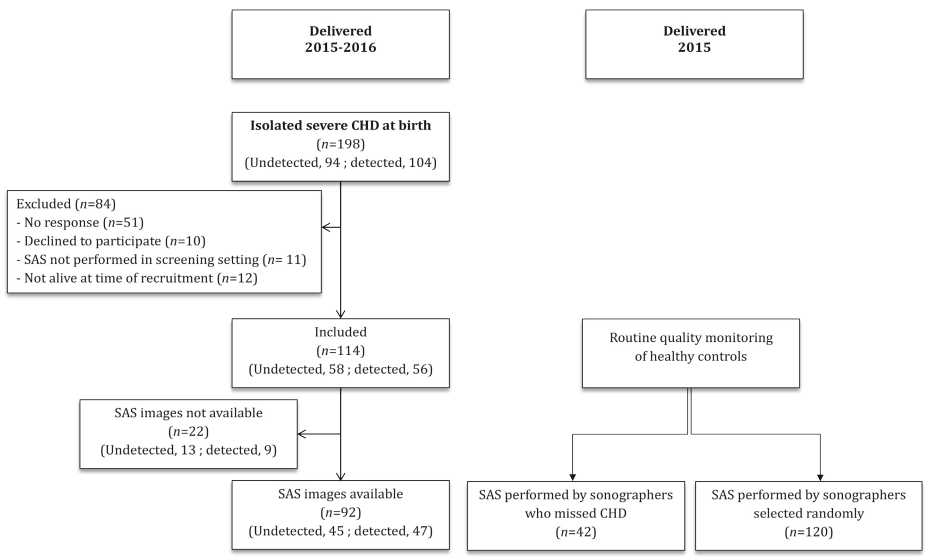

Figure 2. Flowchart summarizing inclusion of fetuses with severe congenital heart disease (CHD) at birth and normal controls. SAS, standard anomaly scan.

At baseline, significantly more women had a history of abdominal surgery in the undetected group (33.3%) compared to the detected group (9.5%) (p=0.01). This difference, however, could not be accounted for in subsequent analysis, as this information was missing in 39% of cases. The two groups did not differ significantly in any of the other parameters assessed at baseline (Table 2).

Table 2. Baseline characteristics

	Undetected (n=58)		Detected (n=56)		P	(95% CI)
Characteristic						
Gestational age at screening (weeks)	20.0	(±0.72)	20.0	(±0.95)	0.96	(-0.31 ; 0.32)
Ultrasound scan repeated	5	12.5%	6	15.0%	0.75	
Maternal age (years)#	31.6	(±4.33)	31.6	(±4.62)	0.98	(-1.68 ; 1.64)
Maternal obesity †	22	(53.7%)	17	(34.0%)	0.06	
Multigravid	31	(62.0%)	38	(69.1%)	0.45	
History of abdominal surgeries	9	33.3%	4	9.5%	**0.01***	
Pregnancy complication	12	40.0%	6	21.4%	0.13	
Multiple pregnancy	3	6.5%	6	10.7%	0.46	
Fetal sex male	38	65.5%	27	48.2%	0.06	
Experience						
Sonographer (yr) §	5.6	(2.67)	5.6	(2.98)	0.92	(-1.09 ; 1.21)
Sonographer (SAS/yr) §	343.4	(247.00)	410.0	(289.50)	0.22	(-173.12 ; 39.88)
Screening center (SAS/yr) §	1289.3	(1041.80)	1157.5	(1076.21)	0.54	(-290.92 ; 554.53)

Data is given as n (%) or mean (± SD).
* A p-value < 0.05 was considered statistically significant.

mean is given as this did not differ significantly from median (interval not skewed).
† Obesity is defined as a body mass index (BMI) > 25 kg/m2.
§ Calculated using data from end of year in which case underwent prenatal screening.

Ultrasound images could be retrieved from the initial screening center in 92/114 (80.7%) CHD cases. Sonographer use of magnification was significantly poorer amongst sonographers in the undetected group (p=0.01). The proportion of cases with unfavorable fetal position did not differ significantly between the groups, which demonstrates that the majority of sonographers waited until the fetus was in a favorable position to assess the heart. The quality of the cardiac examination, overall and for each of the cardiac planes separately, was significantly lower in undetected CHD cases. In the undetected group, the cardiac examination was more frequently incomplete i.e. ≥1 cardiac planes not obtained or saved (46.7% vs 22.2%; p=0.02). The expert assessors classified the defect as being clearly visible in 83.7% of detected cases, compared to only 31.1% of undetected cases (p<0.001), due mainly to technically incorrect cardiac planes (Table 3). The cardiac examination received an inadequate score in a higher proportion of missed CHD cases (64.4%) than in normal controls evaluated by the same

sonographers (14.7%), although different scoring systems were used. With regards to the completeness and quality of the cardiac examinations in normal cases, those performed by sonographers who missed a defect did not differ significantly from those performed by randomly selected sonographers (Table 4). The ICC for quality scoring of the overall cardiac examination (0.88, 95% CI 0.75 – 0.95) and for each of the cardiac planes separately (varying from 0.77 to 0.89) demonstrated good to excellent intrarater agreement.

Table 3. Analysis of the standard anomaly scan

	Undetected CHD (n=45)		Detected CHD (n=47)		p	95% CI of difference
Characteristic						
Unfavorable fetal position[a]	4	8.9%	3	6.7%	1.00	
Amniotic fluid volume†	3.0	(±0.16)	3.0	(±0.00)	0.32	(-0.08 ; 0.03)
Use of magnification						
Poor	2	5.0%	3	6.8%		
Average	15	37.5%	4	9.1%	**0.01***	
Good	23	57.5%	37	84.1%		
Image resolution						
Poor	4	9.1%	0	0.0%		
Below average	8	18.2%	9	21.4%		
Average	21	47.7%	17	40.5%	0.18	
Above average	9	20.5%	10	23.8%		
Good	2	4.5%	6	14.3%		
Quality assessment						
Quality score [0-20] ‡	9.4	(±5.24)	14.2	(±5.51)	**<0.001***	(-7.05 ; -2.52)
Four-chamber view [0-5]	2.7	(±1.47)	3.9	(±1.26)	**<0.001***	(-1.78 ; -0.62)
Three vessel view [0-5]	3.0	(±1.58)	3.8	(±1.57)	**0.02***	(-1.46 ; -0.14)
Left ventricular outflow tract [0-5]	1.9	(±1.57)	3.3	(±1.75)	**<0.001***	(-2.02 ; -0.62)
Right ventricular outflow tract [0-5]	1.9	(±1.95)	3.3	(±1.87)	**<0.001***	(-2.27 ; -0.67)
Inadequate cardiac scan §	29	(64.4%)	14	(31.8%)	**0.002***	
Incomplete cardiac scan ¶	21	(46.7%)	10	(22.2%)	**0.02***	

Table 3. *(Continued)*

	Undetected CHD (n=45)		Detected CHD (n=47)		p	95% CI of difference
Detectable						
CHD clearly visible **	14	(31.1%)	36	(83.7%)	**<0.001***	

Data are given as n (%) or mean (±SD).
* A p-value < 0.05 was considered statistically significant.

a. Unfavorable fetal position: all positions in which the fetal spine is lying towards the probe (on the opposite site of the maternal spine), i.e. from 10 to 2 o'clock (clockwise), were classified as unfavorable.
† Scored on 5-point Likert scale as follows: 1, anhydramnios; 3, normal volume of amniotic fluid; and 5, polyhydramnios.
‡ Scored 1–5 for each plane; score = 0 if not obtained
§ Total quality score < 12
¶ ≥1 of the cardiac planes not obtained or saved
** CHD clearly visible: original images showed abnormal cardiac anatomy according to our fetal echo experts [MH, AT].

Table 4. Routine quality assessment of cardiac images from the SAS in uncomplicated pregnancies

	Performance of a sonographer					
	who missed a CHD[1] (n=42)		who was selected randomly[2] (n=120)		p	95% C.I.
Quality score						
Quality score [0-4] ‡	3.14	(±0.90)	3.20	(±0.87)	0.72	(-0.24 ; 0.27)
Four-chamber view [0-1]	0.93	(±0.26)	0.96	(±0.20)	0.45	(-0.37 ; 0.25)
Three vessel view [0-1]	0.86	(±0.35)	0.88	(±0.32)	0.66	(-0.11 ; 0.05)
Left ventricular outflow tract [0-1]	0.71	(±0.46)	0.68	(±0.47)	0.64	(-0.14 ; 0.09)
Right ventricular outflow tract [0-1]	0.64	(±0.48)	0.68	(±0.46)	0.63	(-0.13 ; 0.20)
Inadequate cardiac scan§	6	(14.3%)	25	(20.8%)	0.35	
Incomplete cardiac scan ¶	10	(23.8%)	31	(25.8%)	0.80	

Data are given as mean (± SD) or n (%).
Three scans included per sonographer.
Quality assessment data based on results of quality monitoring assessments in Leiden region in 2015.
‡ Maximum score of 1 for each plane.
§ Quality score of 0 for ≥ 2 planes.
¶ ≥ 1 plane not obtained or saved.
1. Assessment of the standard performance of sonographers, that missed a CHD in our cohort, in uncomplicated pregnancies
2. Assessment of the standard performance of sonographers, randomly selected from the same population, in uncomplicated pregnancies.

On univariate regression analysis, the volume of SAS performed per year by each sonographer and screening center had a small, but significant, influence on the quality of the cardiac scan in CHD cases. Multivariate regression analysis, however, showed that only an increase in the number of SAS performed by each sonographer significantly improved the overall score of the cardiac examination (Table 5).

Table 5. Analysis of the association between experience and quality of the cardiac examination in fetus with severe CHD (n=92)

Variable	Regression coefficient (95% CI)	SE	*P*
Univariate analysis			
Sonographer experience in years	0.07 (−0.410 to 0.548)	0.24	0.78
Volume of SAS performed in *n*/year			
Per sonographer	0.007 (0.001 to 0.013)	0.003	0.02
Per screening center	0.001 (0.000 to 0.003)	0.001	< 0.05
Multivariate analysis			
Volume of SAS performed in *n*/year			
Per sonographer	0.006 (0.000 to 0.012)	0.003	< 0.05
Per screening center	0.001 (0.000 to 0.002)	0.001	0.15

SE, standard error.

Analysis of undetected CHD cases revealed that the quality of the cardiac examination was inadequate and the defect was not clearly visible due to *lack of adaptational skills* in 22/45 cases (48.9%). In 14/45 undetected cases (31.1%), the heart defect was visible on the cardiac planes obtained and was therefore classified as *a lack of recognition*. In 9/45 cases (20.0%), the heart defect was not visible even though the quality of the images was adequate; these undetected cases were therefore classified as *inevitable*. Images of undetected cases belonging to either one of the three categories for a missed prenatal diagnosis are depicted in Figure 3.

Aortic coarctation, transposition of the great arteries and tetralogy of Fallot were diagnoses that were often not recognized. The inevitable group involved mainly CHD types that are speculated to be difficult to diagnose prenatally, such as aortic coarctation or total anomalous pulmonary venous return. This study shows that these diagnoses indeed show normal images in a considerable number of cases. Table 6 reports the cardiac diagnoses included in this study in relation to the respective proportion that was prenatally diagnosed, causes for a missed prenatal diagnosis and scores on each of the four cardiac planes.

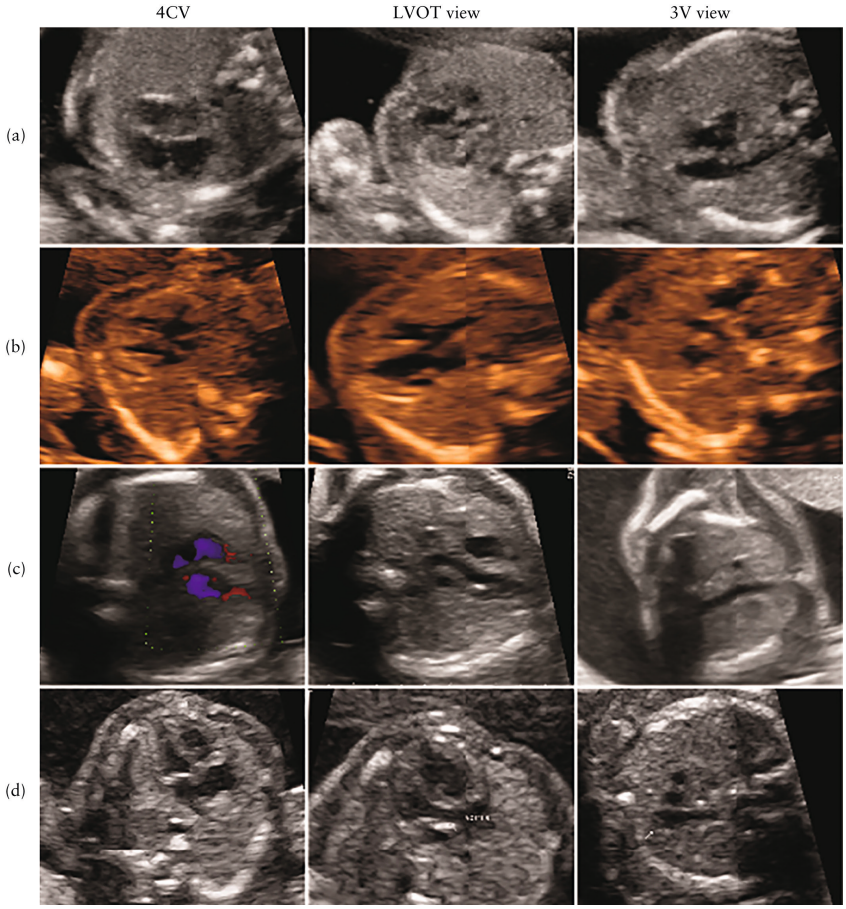

Figure 3. Ultrasound images of fetal heart in four-chamber (4CV), left ventricular outflow tract (LVOT) and three-vessel (3V) views in four cases with severe congenital heart disease at birth that was missed on prenatal ultrasound examination due to poor adaptational skills of sonographer (a), inability of sonographer to recognize defect (b,c) or defect not being visible despite adequate image quality (d).

(a) Case of atrioventricular septal defect missed prenatally due to poor adaptational skills of sonographer. In 4CV, atria are blurred, plane is not taken at proper level (too far towards diaphragm, showing atrioventricular valve annuli instead of valves). Only ventricles and septum are visible. LVOT view quality was scored 1 as aorta is barely recognizable.

(b) Case of tetralogy of Fallot that was not recognized by sonographer; although quality of planes is inadequate (total score of 6), ventricular septal defect can be identified with over-riding aorta. In 3V view, it is clearly visible that pulmonary artery is small and ascending aorta is relatively large. Right aortic arch is visible just anterior to spine.

(c) Case of transposition of great arteries that was not recognized by sonographer despite planes having adequate quality score; as only two vessels (right superior caval vein and ascending aorta arising from right ventricle) are visible in 3V view, which is typical for this diagnosis.

(d) Case of coarctation of aorta that was classified as inevitably missed, as quality of cardiac examination was adequate (total score of ≥ 12), and in particular, no discrepancies in size of ventricles or great arteries were evident on any cardiac images obtained.

Table 6. Overview of the types of CHD included in this study with the respective causes for a missed diagnosis and scores on the four cardiac plan

CHD diagnosis	Prenatal detection			US available	Causes for a missed diagnosis			4CV		3VV		LVOT		RVOT	
					Technical skills‡	§ Not recognized	Inevitable ¶	mean	score ≥3	mean	score ≥3	mean	score ≥3	mean	score ≥3
TAPVR	All	3	2.6%	3				3.3	66.7%	3.3	66.7%	1.0	33.3%	1.7	33.3%
	Undetected	3	100.0%	3	66.7%		33.3%	3.3	66.7%	3.3	66.7%	1.0	33.3%	1.7	33.3%
Ventricular septal defect*	All	21	18.4%	16				2.9	56.3%	2.6	43.8%	1.9	31.3%	1.4	25.0%
	Undetected	18	85.7%	13	76.9%		23.1%	2.8	53.8%	2.6	46.2%	1.8	30.8%	1.2	23.1%
Double aortic arch	All	4	3.5%	2				4.5	100.0%	4.0	100.0%	4.5	100.0%	4.5	100.0%
	Undetected	3	75.0%	2		50.0%	50.0%	4.5	100.0%	4.0	100.0%	4.5	100.0%	4.5	100.0%
Tetralogy of Fallot	All	4	3.5%	4				3.8	75.0%	4.0	100.0%	3.5	75.0%	3.3	75.0%
	Undetected	3	75.0%	3		66.7%	33.3%	3.7	66.7%	4.0	100.0%	3.3	66.7%	3.0	66.7%
Aortic coarctation	All	18	15.8%	13				2.5	46.2%	3.9	76.9%	2.5	53.8%	3.0	53.8%
	Undetected	12	66.7%	9	11.1%	77.8%	11.1%	2.1	33.3%	3.9	77.8%	2.0	33.3%	2.4	44.4%
Aortic stenosis	All	6	5.3%	6				3.2	66.7%	2.7	50.0%	2.2	50.0%	2.7	66.7%
	Undetected	4	66.7%	4	50.0%	50.0%		3.0	75.0%	2.8	50.0%	2.3	50.0%	2.3	50.0%
Pulmonary valve stenosis	All	3	2.6%	2				3.5	50.0%	3.5	100.0%	3.0	50.0%	3.0	50.0%
	Undetected	2	66.7%	1	100.0%			2.0	0.0%	3.0	100.0%	2.0	0.0%	2.0	0.0%
Atrioventricular septal defect	All	7	6.1%	5				3.8	60.0%	4.2	100.0%	2.4	40.0%	3.2	60.0%
	Undetected	2	28.6%	2	50.0%		50.0%	3.5	50.0%	4.0	100.0%	0.5	0.0%	3.5	50.0%
Shone's complex	All	3	2.6%	2				3.5	50.0%	4.5	100.0%	2.0	50.0%	4.5	100.0%
	Undetected	1	33.3%	0											

Table 6. *(Continued)*

CHD diagnosis	Prenatal detection			US available	Causes for a missed diagnosis			4CV		3VV		LVOT		RVOT	
					Technical skills‡	§ Not recognized	Inevitable ¶	mean	score ≥3	mean	score ≥3	mean	score ≥3	mean	score ≥3
Transposition of the great arteries	All	19	16.7%	18				3.8	82.4%	3.9	82.4%	3.8	76.5%	3.4	72.2%
	Undetected	3	15.8%	3	33.3%	66.7%		1.7	33.3%	1.7	33.3%	2.0	33.3%	0.3	0.0%
Double outlet right ventricle – ToF	All	7	6.1%	5				3.6	80.0%	4.2	100.0%	3.8	80.0%	3.4	80.0%
	Undetected	0	0.0%	0											
Interrupted aortic arch	All	2	1.8%	2				4.0	100.0%	3.5	50.0%	0.5	0.0%	1.5	50.0%
	Undetected	0	0.0%	0											
Common arterial trunk	All	2	1.8%	1				3.0	100.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%
	Undetected	0	0.0%	0											
Pulmonary valve atresia - IVS	All	2	1.8%	1				4.0	100.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%
	Undetected	0	0.0%	0											
Unbalanced AVSD	All	2	1.8%	2				4.0	100.0%	4.0	100.0%	2.0	50.0%	2.5	50.0%
	Undetected	0	0.0%	0											
Miscellaneous	All	11	9.6%												
	Undetected	6	54.5%												
Total	**All**	**114**	**100**	**92**	**48.9%**	**31.1%**	**20.0%**	**3.3**	**66.3%**	**3.4**	**68.9%**	**2.6**	**51.7%**	**2.6**	**54.4%**

Data are given as n or n, %. * requiring surgery in the first year of life
‡ Cardiac examination had inadequate quality score (< 12) and heart defect was not clearly visible.
§ Defect was clearly visible on images but was not recognized by sonographer, irrespective of examination quality.
¶ Defect was not visible despite adequate quality score (≥ 12).

3V, three-vessel view; 4CV, four-chamber view; AVSD, atrioventricular septal defect; CoA, coarctation of the aorta; DORV, double outlet right ventricle; LVOT, left ventricular outflow tract view; PA/IVS, pulmonary atresia with intact ventricular septum; PVS, pulmonary valve stenosis; RVOT, right ventricular outflow tract view; TAPVR, total anomalous pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

DISCUSSION

This study audited images obtained during the second-trimester SAS to identify potential causes for a missed prenatal diagnosis of CHD, by comparing ultrasound examinations between undetected and detected cases. Although sonographers practiced in a high-quality screening program, our results showed that the cardiac planes obtained during the SAS were of significantly better quality in detected compared with undetected CHD cases. Cardiac examinations appeared of better quality when performed by sonographers, who carried out a greater number of SAS per year.

Sonographers who missed a CHD diagnosis were not poorly trained, as all had passed the national quality assessment. At the initial assessment 25% of sonographers, however, did not obtain or save all cardiac planes, indicating that they either accepted technically incorrect planes or did not obtain and save all cardiac planes in a structured manner. The poorer performance in CHD cases may also be explained by slightly impaired motor skills when acquiring accurate planes in abnormal anatomy, combined with a lack of gut feeling for detection of abnormal cases. We hypothesize that the poorer performance in missed cases may be attributed to certain personality traits and lack of adaptational skills. The second reason for a missed prenatal diagnosis was failure to recognize the CHD despite being visible on the images, which involved mainly subtle signs, such as asymmetry in the 4CV and discrepancy between the size of aorta and the pulmonary trunk in aortic coarctation. Missed prenatal diagnosis was classified inevitable in 20% of undetected cases, which may be explained partly by development later in gestation.

Although further analysis revealed a small, but significant, positive association between sonographer volume (number of SAS performed per year) and quality of the cardiac planes, quality was not associated with sonographer experience in years. This indicates that a minimum number of examinations per year might be necessary to maintain skills and develop a 'gut feeling' for detection of abnormalities.[34] We speculate that sonographers performing a low volume of examinations may be more likely to question their own capability and accept technically incorrect cardiac planes, whereas those performing a high volume of examinations will rely on their technical skills to obtain the cardiac images properly, trust their 'gut feeling' that the images are abnormal due to differences in fetal anatomy and refer the case to a specialized fetal medicine unit. The screening center's size was also independently associated with superior quality of the cardiac planes. This might be explained by their increased exposure to abnormal scans, as high-volume sonographers will most likely work in large screening centers, which enables them to review difficult cases with fellow-sonographers. A French study

confirmed this by showing that meetings in which cases are discussed, contribute to increased DRs in conjunction with training.[34] The recording of videoclips during the SAS might also enhance screening results and aid the review of difficult cases. The fact that high volume was associated with better quality of the examination, but not with increased prenatal detection, may be explained by a lack of power, as 20% of the missed cases were inevitable and allocated to undetected cases by definition.

Cardiac images in undetected cases scored particularly low for the outflow tract planes, which was not the case in the detected group. Previous cohort studies have confirmed that assessment of the outflow tracts, including the 3VV or three vessels and trachea view (3VT), is valuable for prenatal detection.[31, 35, 36] The use of universal guidelines and increased effort to obtain these outflow tract views has therefore shown to increase prenatal DRs.[9, 18] Specific training programs, focused on achieving satisfactory views of the heart, were able to improve significantly DRs 60%.[28, 36-39] As DRs in our region are already above 60%[23], we hypothesize that monitoring, alongside training, is imperative to assure strict adherence to protocol and to reach higher DRs.

Our results also suggest that an increase in the annual volume of SAS performed by the sonographers, rather than their experience in years, can improve quality. Setting up large screening centers with sonographers performing a high volume of examinations might be the final step to reach DRs of the previously mentioned goal of 80%, because it will ensure sufficient exposure to abnormal cardiac images and create an environment that potentially counteracts the above described character traits. This is in line with the current opinion that centralization of care improves quality. Factors that possibly hamper proper cardiac assessment, such as maternal obesity or unfavorable fetal position, were not found to influence the prenatal detection of CHD, which is in accordance with previous reports.[24,25,40,41]

Although this topic can be studied only retrospectively, this design led to some inevitable limitations. First, it is not possible to determine if improved quality of images led directly to detection of the heart defect, rather than *vice versa*. Second, we had to obtain consent from the mothers in order to retrieve the images, which may have resulted in selection bias. The inclusion of only live cases should not affect the study's clinical value significantly, because the cases that resulted in termination of pregnancy or neonatal demise comprised mainly univentricular defects with DRs of nearly 100% in our country.[23] However, this did impede blinding of assessors to whether a heart defect was present, as we were unable to retrieve the original images from healthy fetuses. Finally, the distribution of diagnoses differed between the two groups. This, however, does not affect our primary results, as sonographers are still obliged to

acquire and save proper cardiac planes, even if they assume a structurally normal heart, as described in our national SAS protocol.[42]

In conclusion, the quality of the cardiac examination, at the time of second-trimester screening, appears to be the cornerstone in improving prenatal DRs for CHD in a low-risk population. Although it seems obvious that sonographers performing a high volume of scans are more likely to retain technical skills and remain qualified, this association has not been demonstrated previously. The volume of examinations performed by a sonographer, alongside training, was shown to be equally important in ensuring adequate examination of the fetal heart and recognition of abnormality. Future research should therefore consider performing more extensive audit studies and evaluating annual volume targets for sonographers who perform SAS, in order to maintain their competence.

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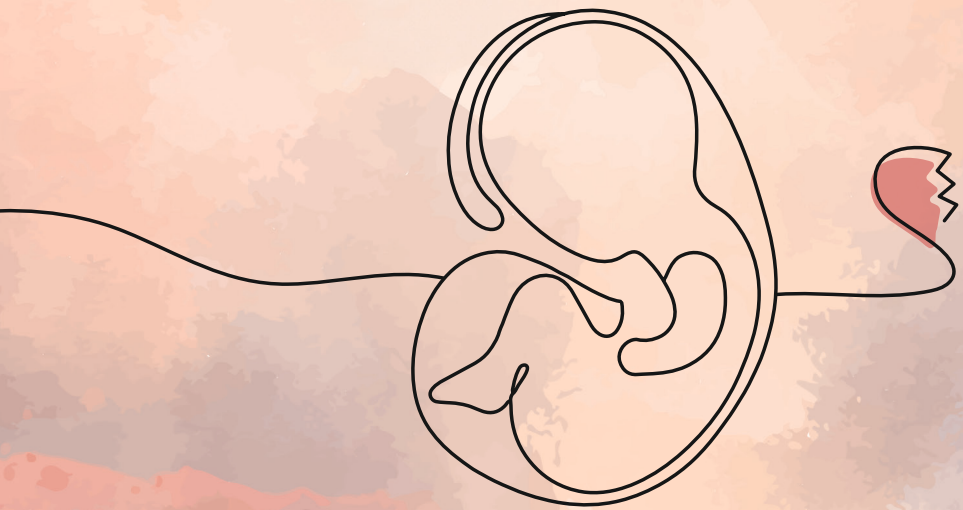

CHAPTER 3

The prevalence of genetic diagnoses in fetuses with severe congenital heart defects

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ABSTRACT

Purpose

Congenital heart defects (CHD) are associated with genetic syndromes. Rapid aneuploidy testing and chromosome microarray analysis (CMA) are standard care in fetal CHD. Many genetic syndromes remain undetected with these tests. This cohort study aims to estimate the frequency of genetic variants, in particular structural chromosome abnormalities and sequence variants, in fetuses with severe CHD at mid-gestation, to aid prenatal counselling.

Methods

Fetuses with severe CHD were extracted from the PRECOR registry (2012-2016). We evaluated pre- and postnatal genetic testing results retrospectively to estimate the frequency of genetic diagnoses in general, as well as for specific CHDs.

Results

919 fetuses with severe CHD were identified. After exclusion of 211 cases with aneuploidy, a genetic diagnosis was found in 15.7% (111/708). These comprised copy number variants in 9.9% (70/708). In 5.8% (41/708) sequence variants were found that would have remained undetected with CMA. Interrupted aortic arch, pulmonary atresia with ventricular septal defect and atrioventricular septal defect were most commonly associated with a genetic diagnosis.

Conclusion

In case of normal CMA results, parents should be offered exome sequencing sequentially, if time allows for it, especially if the CHD is accompanied by other structural malformations due to the large variety in genetic syndromes.

INTRODUCTION

Congenital heart defects (CHDs) are the most common congenital birth defects with a prevalence of 5-8 per 1.000 liveborns.[1] Approximately a third of these cases involve *severe* CHDs.[2] Although survival rates have increased significantly over the past decades, the extent of (disease-related) morbidity is considerable and life-expectance remains reduced.[3]

It is well known that aneuploidies (numerical chromosome abnormalities), such as trisomy 13, 18, 21 and Turner syndrome, are associated with CHDs.[4, 5] Submicroscopic deletions or duplications, commonly referred to as copy number variants (CNVs), have been reported in approximately 10-15% of children with CHD.[6, 7] As these CNVs can have a major impact on development, quality of life and life expectancy of children with CHDs[7, 8], chromosome microarray analysis (CMA) is the standard of care in the fetal setting in most developed countries.[9] Nevertheless, it is not uncommon in cases with CHD, that a genetic syndrome is diagnosed after birth or during childhood, as sequence variants remain undetected with CMA.

Although CHDs have been described as part of many genetic syndromes, there are only a few cohort studies available that report on genetic diagnoses (chromosome abnormalities or sequence variants) in fetuses diagnosed with a CHD. The studies that have been performed in fetal cohorts are outdated, as they mainly focus on aneuploidy.[10-13] This restricts prenatal counseling, because currently available data are mostly based on postnatal studies. The prevalence of genetic diagnoses is expected to be lower in the postnatal cohorts, as cases with termination of pregnancy (TOP), intra uterine fetal demise or early neonatal death are often not included in these cohorts.[5] More importantly postnatal sequencing is mostly requested due to the evolving clinical phenotype, which makes it difficult to estimate a true prenatal prevalence of genetic syndromes at mid-gestation.

Antenatal knowledge on the prevalence of genetic variants, which may either be considered the genetic cause for the CHD or a secondary finding, can improve prenatal counseling in cases with severe CHDs. Accurate figures on the occurrence of these genetic diagnoses will aid professionals to make decisions regarding the use of currently available additional tests, such as exome sequencing (ES) or genetic testing for specific genetic syndromes.

This study aims to provide a conservative estimate of the prevalence of chromosome abnormalities and sequence variants in fetuses with severe CHDs, in particular after an aneuploidy is excluded, by assessing results from both pre- and postnatal genetic testing. The potential diagnostic yield of ES for fetal CHDs, and factors that potentially increase the chance of an underlying genetic diagnosis, will be evaluated as well.

MATERIALS AND METHODS

In the northwestern region of the Netherlands, the care for children with CHDs is centralized in three tertiary referral centers in Amsterdam and Leiden. All fetuses and infants diagnosed with a *severe* CHD within this region have been registered in the PRECOR registry since 2002. Severe CHD was defined as the need for surgery or therapeutic intervention in the first year of life. Data collection for this registry has been previously described.[14] The Leiden University Medical Center has a general privacy statement informing patients that their data can be used for (retrospective) scientific research.

From this registry, all cases with a diagnosis of a severe CHD, born between 2012 and 2016, were extracted. We chose this period, as CMA became a routine diagnostic test from 2012 onwards, if parents opted for invasive testing in pregnancy. In order to ensure a reasonable follow-up period after birth, we included cases born before 2017 and followed them until September 2019. From a clinical perspective, we chose not to exclude cases in which genetic testing was not performed pre- or postnatally to avoid a substantial selection bias, and consequently an overestimation of prevalence of genetic diagnosis. All numbers reported in this cohort are therefore conservative estimates of the prevalence of chromosome abnormalities and sequence variants. Cases with an aneuploidy were not included in subsequent analyses, as the prevalence of aneuploidy in fetal CHD cases has already been well-documented in the literature.[12] To estimate the minimum prevalence of chromosome abnormalities and sequence variants in fetuses with CHDs, results from pre- and postnatal genetic testing, postnatal clinical evaluation and postmortem reports were assessed.

Antenatal characteristics

The obstetric databases were evaluated to collect information on maternal and fetal characteristics, including maternal age, obstetric history, obesity, smoking, alcohol use, positive family history of CHDs, self-reported consanguinity, multiple pregnancy, the presence of additional fetal extra-cardiac malformations (ECM) and prenatal genetic testing. Routine prenatal genetic testing, which involves Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) analysis and chromosome microarray analysis (CMA), was offered in all cases with a prenatal diagnosis of a CHD. Genetic testing for specific genetic diagnoses, not detectable with routine prenatal genetic testing, was only performed in fetuses with features that raised suspicion of a specific syndrome. The CHD was classified as either isolated or non-isolated, as it can be expected that the presence of additional structural malformations in the fetus would increase the probability of an underlying genetic cause. *Isolated* was defined as the absence of

significant structural ECMs or intra-uterine growth restriction (according to consensus-based definition[15]) prenatally, whereas *non-isolated* comprised cases diagnosed with one or multiple structural ECMs. Minor antenatal extra-cardiac abnormalities, also referred to as 'soft markers', such as an echogenic bowel or single umbilical artery, were considered non-significant ECMs.

Postnatal course

Data on pregnancy and neonatal outcome, such as gestational age at birth, birth weight, presence of dysmorphic features postnatally, mortality and need for medication were retrieved from electronic patient records. Until the end of the study period we assessed whether signs of developmental delay, such as neurocognitive or speech and language disorders, were reported in the patient records. Clinical records were evaluated for results of CMA, genetic testing for specific syndromes (e.g. if CHARGE, Noonan, Kabuki or Alagille syndrome was suspected) and focused or exome-wide analysis of ES, if parents chose to delay (additional) genetic testing until after birth. If additional signs for a genetic syndrome, such as dysmorphic features or developmental delay, were apparent but less specific and CMA results were normal, ES was considered. ES was only performed in postnatal cases, as this was not yet available in a prenatal setting during the study period. The specific ES based CHD gene panel used in our facilities for focused analysis of sequence data, consisted of a panel of 129 genes that are known to be associated with CHD.[16] A clinical geneticist was consulted in both pre- and postnatal cases before genetic testing for a specific syndrome or exome sequencing was performed.

Classification of all genetic variants

Structural chromosome abnormalities and sequence variants were classified using the guidelines for interpretation of CNV and sequence variants, developed by The American College of Medical Genetics and Genomics (ACMG).[17, 18] This classification allocates abnormal results into the following five categories, based on their expected clinical relevance: pathogenic, likely pathogenic, uncertain significance, likely benign and benign. Likely pathogenic was only used when there was a certainty of at least 90% that a variant was disease-causing.[17] Pathogenic and likely pathogenic variants, later referred to as 'genetic diagnoses', will therefore be reported together in the subsequent analysis.

The Affymetrix Cytoscan HD array or Agilent CGH 180K oligo array (Amadid 023363) platforms were used for CMA, as described earlier by Jansen *et al*.[19] ES was performed using the Agilent SureSelectXT Human all Exon v5 or Clinical Research Exome v2 capture library kit (Agilent, Santa Clara, USA) accompanied by paired end Sequencing on an Illumina sequencing platform (Illumina, San Diego, USA), generating reads with

at least 80x median coverage. The sequence analysis pipeline and tool for annotation of variants has extensively been reported on before.[20] Trio samples of the fetus and both parents were assessed for sequencing to optimize variant filtering, when available. Reported variants were submitted to the DECIPHER database.

Statistical analysis

A conservative estimate of the prevalence of chromosomal abnormalities and sequence variants in mid-gestation fetuses diagnosed with a CHD was determined by complementing genetic diagnoses made in pregnancy with those detected after birth, based on postnatal clinical evaluation and follow-up assessment. We assessed the proportion of sequence variants, not detectable with CMA, as this is currently recommended for all fetuses with CHDs.[9] Information on the presence of clinical features for a genetic syndrome is limited on prenatal ultrasound. This reduces the applicability of genetic testing for a specific syndrome in utero. We therefore evaluated the potential diagnostic yield of ES, either analyzed using our specific CHD gene panel[16] or exome-wide, for the detection of (likely) pathogenic sequence variants in fetal CHD cases. The probability of structural chromosome abnormalities and sequence variants, after aneuploidy is excluded, was determined separately for each heart defect that encompassed at least 1% of this cohort. We also calculated whether the probability of genetic diagnoses in fetuses with CHDs is affected by maternal age, obstetric history, family history of CHDs, consanguinity, multiple pregnancy and additional structural fetal malformations. The clinical impact of structural chromosome abnormalities and sequence variants was assessed by comparing neonatal outcome of fetuses with and without abnormal genetic testing results.

Numeric variables were studied for significant differences using an independent t-test, whereas a χ^2-test was used to test associations between categorical variables. A Fisher's exact test was used, if the expected number was < 5. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics 25.0 (IBM, Armonk, NY, USA). This study was approved by the Leiden University Medical Ethics Committee.

RESULTS

A total of 919 cases, diagnosed with a severe CHD between January 2012 and December 2016, were extracted from the PRECOR registry. Parents chose invasive prenatal testing in 542/919 cases (59.0%), whereas in 185/919 cases (20.1%) genetic testing was performed after birth. Aneuploidy testing was performed in all these cases. Genetic testing was not performed pre- or postnatally in 192/919 cases (20.9%). In these cases parents either declined genetic testing or it was not indicated, because dysmorphic features were absent and the CHD was expected not to be associated with genetic syndromes. After clinical assessment by a geneticist, signs of a genetic syndrome were absent after birth in the majority of these cases (173/192, 90.1%), whereas 9.9% (19/192) did have additional ECMs (Figure 1).

An aneuploidy was found in 211/919 (23.0%) cases. As we were mainly interested in the prevalence of structural chromosome abnormalities and sequence variants, rather than aneuploidy, only the remaining 708 euploid cases were included in further analyses. Genetic testing in these cases involved CMA in 64.7% (458/708), genetic testing for specific syndromes in 13.3% (94/708) and focused (7.2%, 51/708) or exome-wide analysis of exome sequencing data (8.1%, 57/708). Baseline characteristics of these cases are enclosed as Supplemental Material (Table S1).

Prevalence genetic variants

An estimate of the prevalence of chromosome abnormalities and sequence variants amongst fetuses with a CHD in this cohort was determined by complementing all genetic variants reported (including 'uncertain significance') in pregnancy with those detected after birth. An overview of all genetic variants encountered in this cohort, is shown in Table 1.

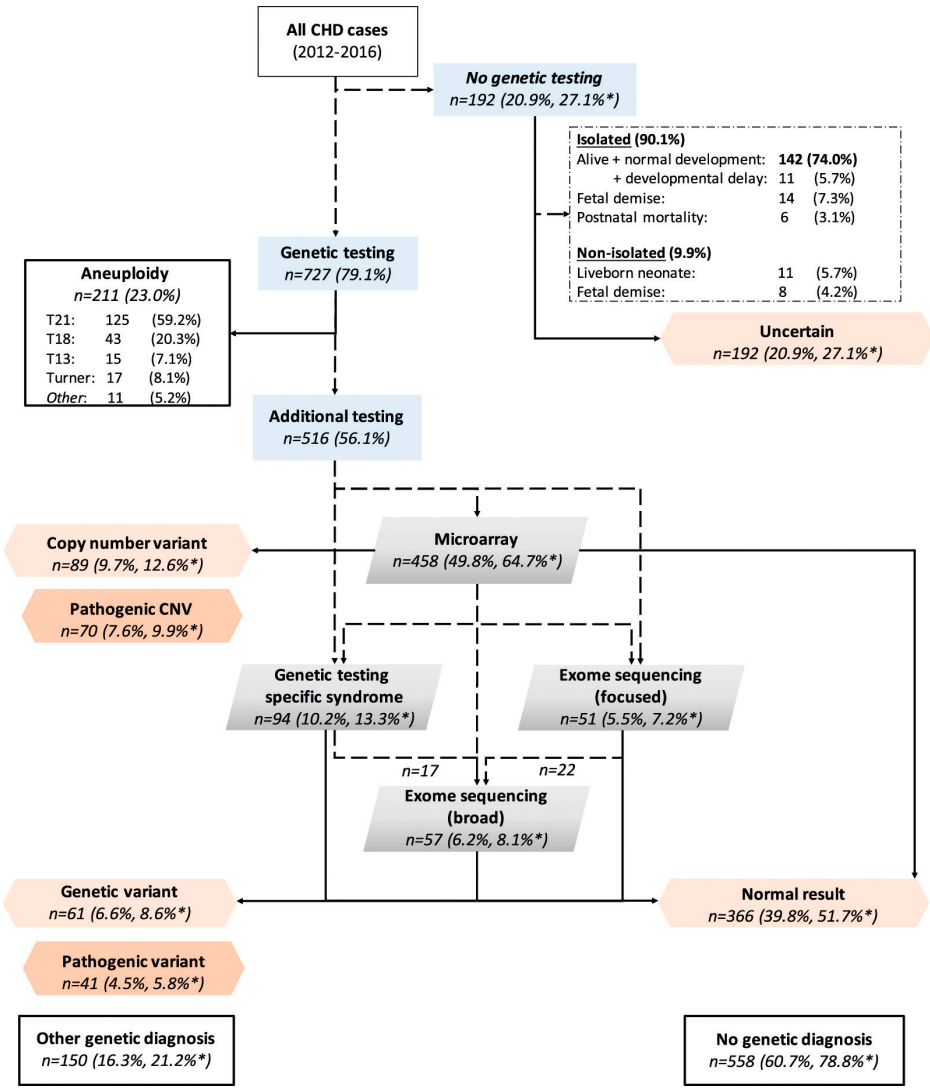

Figure 1. flow chart case selection

*adjusted prevalence after exclusion of aneuploidy cases
CHD congenital heart defect, CNV copy number variant, T21 trisomy 21, T18 trisomy 18, T13 trisomy 13.

Table 1. Abnormal results chromosome microarray analysis & exome sequencing

Structural chromosome anomalies (CMA)			**Sequence variants (ES)**		
Pathogenic	**63**	**(8.9%)**	**Pathogenic**	**33**	**(4.7%)**
22q11.2 syndrome		30	CHARGE syndrome		5
1q21.1 deletion syndrome		3	Kabuki syndrome		3
16p11.2 deletion syndrome		2	Noonan syndrome		3
Beckwith-Wiedemann syndr.		1	Tuberous sclerosis complex		3
Cri du chat syndrome		1	Alagille syndrome		2
Phelan-McDermid syndrome		1	CM-AVM syndrome		1
Ringchromosome 20 syndr.		1	Cornelia de Lange syndrome		1
Waardenburg syndrome		1	Jeune syndrome		1
Williams syndrome		1	Rubinstein-Taybi syndrome		1
6p25 microdeletion syndr.		1	Schaaf-Yang syndrome		1
8p23.1 microdeletion syndr.		1	Smith-Lemli-Opitz syndrome		1
13q deletion syndrome		1			
15q11-13 duplication syndr.		1			
18p deletion syndrome		1			
Others		17	*Others*		11
Likely pathogenic	**7**	**(1.0%)**	**Likely pathogenic**	**8**	**(1.1%)**
Uncertain significance	**19**	**(2.7%)**	**Uncertain significance**	**20**	**(2.8%)**
Likely benign	23	(3.2%)	*Likely benign*	0	(0.0%)
Benign	3	(0.4%)	*Benign*	0	(0.0%)
Total *(without (likely) benign)*	**89**	**(12.6%)**	**Total** *(without (likely) benign)*	**61**	**(8.6%)**

Data are given as n (%). Syndr. syndrome, CM-AVM Capillary malformation-arteriovenous malformation syndrome.
The American College of Medical Genetics and Genomics' and the Association for Molecular Pathology's guideline for interpreting copy number variations and sequence variants was used to categorize genetic variants into the five categories displayed above.

Genetic diagnoses were encountered in 111/708 (15.6%) euploid cases with severe CHD. These comprised copy number variants (CNVs) diagnosed with CMA in 70/708 cases (9.9%), of which 63/70 were classified as pathogenic and 7/70 likely pathogenic. In 2.7% of cases (19/708) a CNV of uncertain significance was found. The 22q11.2 deletion syndrome was the most common microdeletion syndrome encountered, with a prevalence of 4.2% (30/708), encompassing 42.9% (30/70) of all (likely) pathogenic CNVs detected.

In 41/708 (5.8%) fetuses with a CHD, a potentially causal sequence variant was detected with additional testing. These variants were classified pathogenic in 33/41 and likely pathogenic in 8/41. A sequence variant of uncertain significance was found in 2.8% of cases (20/708). Frequently encountered genetic syndromes included CHARGE (5/41, 12.2%), Kabuki (3/41, 7.3%) and Noonan syndrome (3/41, 7.3%).

Diagnostic yield additional testing

In 65.9% (27/41) of (likely) pathogenic sequence variants, genetic testing for a specific syndrome (e.g. Kabuki or Noonan syndrome) had been performed based on clinical suspicion. These variants were diagnosed postnatally in the majority of cases (16/27, 59.3%). The remaining 34.1% (14/41) of (likely) pathogenic sequence variants were detected with either focused or exome-wide analysis of exome sequencing data.

To compare the strengths and limitations of a gene panel approach to an exome-wide analysis of ES data, we analyzed the diagnostic yield in relation to all reported sequence variants (including 'uncertain' significance). In 24.6% (15/61) of all sequence variants, a (likely) pathogenic variant was encountered that did not involve genes known to be related with CHDs. Significant variants, such as a RASA1 sequence variant causing capillary malformation-arteriovenous malformation syndrome or a variant in the TSC1 gene leading to tuberous sclerosis (not suspected prenatally), will thus remain undetected if ES data are only analyzed using a targeted CHD gene panel. The disadvantage of exome-wide analysis of ES data is the increased identification of variants of 'uncertain significance', as identified in 19.7% (12/61) of all sequence variants.

Genetic cause for CHD

Eighty-five percent (94/111) of the encountered structural chromosome abnormalities and sequence variants were considered a definite explanation for the development of the heart defect. This means that in 13% (94/708) of euploid CHD cases, a genetic cause for the heart defect was found, which was 18% (94/516) for those who underwent genetic testing. Secondary findings involved genetic causes for severe hemophilia, Charcot-Marie-Tooth Disease and psychomotor retardation.

Structural chromosome abnormalities and sequence variants that were classified pathogenic or likely pathogenic, were both considered a pathogenic diagnosis in subsequent analyses, and therefore reported together. The remaining cases without genetic testing results were regarded as having a normal test result, whereas those with a variant of uncertain significance were reported separately.

Specific heart defects

Heart defects that were most frequently accompanied by a genetic diagnosis included an interrupted aortic arch (IAoA), pulmonary atresia with a ventricular septal defect (PA-VSD), (un)balanced atrioventricular septal defect (AVSD), truncus arteriosus and Tetralogy of Fallot. Genetic diagnoses were, on the other hand, barely encountered amongst fetuses with a diagnosis of transposition of the great arteries with intact ventricular septum (simple TGA), tricuspid atresia, double inlet left ventricle or total anomalous pulmonary venous connection. The genetic diagnoses encountered in two cases with simple TGA were both secondary findings that did not explain the heart defect. In one case hemophilia type A was diagnosed, and the mother turned out to be a carrier, whereas the other comprised a de novo distal 16q11.2 deletion which was very unlikely to be associated with the heart defect. For each common CHD, and isolated cases separately, the probability for genetic diagnoses is presented in Table 2.

Risk factors for a genetic diagnosis

The probability of a genetic diagnosis was significantly lower for prenatally isolated (11.6%, 63/541) compared to non-isolated cases (28.7%, 48/167) ($p<0.001$). Self-reported consanguinity (35.5% vs 2.9%, $p=0.002$) and a positive parental history of a CHD (32.3% vs 3.8%, $p=0.03$) were also significantly associated with genetic diagnoses. Other variables of interest, such as maternal age, gravidity, obesity or multiple pregnancy were not significantly correlated with genetic diagnoses (Table 3).

Table 2. Prevalence structural chromosome abnormalities and sequence variants for specific diagnoses

	Genetic diagnosis							Uncertain significance	
	Yes		No		OR	95% CI	p		
Interrupted aortic arch [b]	10	71.4%	4	28.6%	13.7	4.22 - 44.57	**<0.001**[a]	0	0.0%
isolated	8	66.7%	4	33.3%				0	0.0%
Pulmonary atresia with VSD	5	41.7%	7	58.3%	3.7	1.16 - 11.92	**0.03**[a]	0	0.0%
isolated	1	16.7%	5	83.3%				0	0.0%
AVSD, unbalanced	3	33.3%	6	66.7%	2.6	0.63 - 10.38	0.18	0	0.0%
isolated	1	20.0%	4	80.0%				0	0.0%
AVSD, balanced	8	28.6%	19	67.9%	2.2	0.94 - 5.17	0.11	1	3.6%
isolated	4	21.1%	14	73.7%				1	5.3%
Isolated right aortic arch	2	28.6%	5	71.4%	2.0	0.39 - 10.60	0.33	0	0.0%
isolated	1	16.7%	5	83.3%				0	0.0%
Truncus arteriosus	4	25.0%	11	68.8%	1.9	0.58 - 5.95	0.29	1	6.3%
isolated	4	36.4%	7	63.6%				0	0.0%
Tetralogy of Fallot	12	21.1%	43	75.4%	1.5	0.74 - 2.85	0.28	2	3.5%
isolated	8	17.0%	38	80.9%				1	2.1%
Valvular aortic stenosis	5	20.0%	20	80.0%	1.3	0.47 - 3.46	0.59	0	0.0%
isolated	2	10.0%	18	90.0%				0	0.0%
Ventricular septal defect	19	17.8%	84	78.5%	1.2	0.68 - 2.01	0.58	4	3.7%
isolated	6	8.5%	63	88.7%				2	2.8%
Left isomerism	2	16.7%	10	83.3%	1.0	0.22 - 4.65	1.00	0	0.0%
isolated	1	25.0%	3	75.0%				0	0.0%
DORV-Fallot type	5	16.7%	23	76.7%	1.1	0.41 - 2.95	0.80	2	6.7%
isolated	2	12.5%	14	87.5%				0	0.0%
DORV-Taussig Bing	3	13.0%	18	78.3%	0.8	0.24 - 2.88	1.00	2	8.7%
isolated	2	10.5%	16	84.2%				1	5.3%
Hypoplastic aortic arch	1	12.5%	6	75.0%	0.8	0.10 - 7.02	1.00	1	12.5%
isolated	1	20.0%	4	66.7%				1	16.7%

Table 2. *(Continued)*

	Genetic diagnosis							Uncertain significance	
	Yes		No		OR	95% CI	p		
Hypoplastic left heart syndrome	6	11.5%	44	84.6%	0.7	0.28 - 1.61	0.36	2	3.8%
isolated	5	11.6%	38	84.4%				2	4.4%
Aortic coarctation	5	7.7%	55	84.6%	0.4	0.17 - 1.10	0.07	5	7.7%
isolated	3	5.8%	49	86.0%				5	8.8%
Valvular pulmonary stenosis	2	6.7%	22	73.3%	0.4	0.10 - 1.93	0.40	6	20.0%
isolated	2	8.7%	21	77.8%				4	14.8%
Hypoplastic right heart syndrome	1	6.7%	12	80.0%	0.4	0.05 - 3.21	0.71	2	13.3%
isolated	0	0.0%	10	83.3%				2	16.7%
TGA (with significant VSD or PS)	1	5.0%	18	90.0%	0.3	0.04 - 2.06	0.34	1	5.0%
isolated	1	5.9%	16	94.1%				0	0.0%
TGA (simple)	2	4.3%	44	93.6%	0.2	0.05 - 0.90	**0.02**[a]	1	2.1%
isolated	2	4.4%	43	93.5%				1	2.2%
Tricuspid valve atresia	1	4.0%	23	92.0%	0.2	0.03 - 1.58	0.16	1	4.0%
isolated	1	4.8%	20	90.9%				1	4.5%
Double inlet left ventricle	0	0.0%	6	85.7%	n/a		0.60	1	14.3%
isolated	0	0.0%	6	85.7%				1	14.3%
TAPVC	0	0.0%	10	90.9%	n/a		0.38	1	9.1%
isolated	0	0.0%	8	88.9%				1	11.1%
Miscellaneous	*14*	*15.9%*	68	77.3%				6	6.8%
Total	**111**	**15.7%**	**558**	**78.8%**				**39**	**5.5%**

Data are given as n (%). a: p-value < 0.05 was considered statistically significant. b. 8/10 with a pathogenic variant was diagnosed with 22q11 deletion syndrome. VUS variant of uncertain significance
VSD ventricular septal defect. AVSD atrioventricular septal defect. DORV double outlet right ventricle. TGA transposition of the great arteries. VSD ventricular septal defect. PS pulmonary valve stenosis. TAPVC total anomalous pulmonary vein connection

Impact on outcome

The effect of genetic diagnoses on the pregnancy outcome of all included cases is depicted in Table 3. The postnatal outcome was compared between cases with and without a genetic diagnosis. First of all, parents opted for TOP significantly more often when the heart defect was accompanied by a genetic diagnosis compared to those without a genetic diagnosis (36.9% vs 24.4%; p=0.01). The detection of variants of 'uncertain significance' did not lead to an increase in TOPs, as the proportion of parents that terminated pregnancy was even lower in these cases compared to those with normal genetic testing results (10.3% vs 24.4%; p=0.04). This indicates that the specific diagnosis rather influences the parental decision for TOP than the detection of variants of 'uncertain significance'. The proportion of cases with a birth weight < 3rd centile was higher amongst CHD cases with a genetic diagnosis (20.6% vs 6.5%; p=0.01). Postnatal mortality was also increased in cases with a genetic diagnosis (32.8% vs 9.0%, p<0.001). Signs of developmental delay were significantly more often present in children with (75.6%) compared to those without a genetic diagnosis (9.7%) (p<0.001). Cases with genetic diagnoses required medication for other reasons than the cardiac defect itself, more often (30.0% vs 5.4%, p=0.001), as well as (medical) support, such as speech therapy, physical therapy or special education (79.5% vs 9.5%, p<0.001).

Table 3. Genetic variants: risk factors & outcome

	Pathogenic variants							Uncertain Significance	
	Yes (n=111)		No (n=558)		*OR*	*95% CI*	*p*		
Non-isolated CHD	48	28.7%	107	19.2%	0.3	0.20 - 0.48	**<0.001**[a]	12	30.8%
Isolated CHD	63	11.6%	451	80.8%				27	69.2%
Dysmorphic features	36	28.8%	73	13.1%	6.9	3.95 - 12.07	**<0.001**[a]	16	41.0%
Age mother (yr)	31.0	5.38	30.9	4.94			0.76		
Gravida	2.6	2.10	2.2	1.43			0.07		
Consanguinity, self-reported	11	35.5%	16	2.9%	3.7	1.68 - 8.25	**0.002**[a]	4	10.3%
Positive history of CHD, 1st degree	10	32.3%	21	3.8%	2.4	1.09 - 5.22	**0.03**[a]	1	2.6%
Multiple pregnancy	4	8.5%	41	7.3%	0.5	0.16 - 1.34	0.15	2	5.1%
Pregnancy outcome									
Termination of pregnancy	41	36.9%	136	24.4%	1.8	1.18 - 2.80	**0.01**[a]	4	10,3%
Intrauterine fetal demise [b,c]	6	5.4%	23	4.1%	1.1	0.30 - 3.74	1.00	1	2.6%
Live births	64	57.7%	399	71.5%	0.5	0.36 - 0.83	**0.004**[a]	34	87.2%
Gestational age at birth [d]	38.0	2.83	38.7	2.57			**0.03**[a]	37.9	2.63
< 37 weeks	13	20.6%	50	9.0%	1.8	0.91 - 3.53	0.09	10	25.6%
Birth weight (g) [d,e]	2870.2	761.82	3165.7	710.09			**0.003**[a]	2821.7	689.80
< 3rd centile	13	20.6%	36	6.5%	2.5	1.23 - 4.98	**0.01**[a]	7	17.9%
Mortality [d]	21	32.8%	50	9.0%	3.4	1.87 - 6.21	**<0.001**[a]	9	23.1%
<1 year	20	95.2%	48	8.6%				7	17.9%
Developmental delay	31	75.6%	54	9.7%	15.4	7.15 - 33.37	**<0.001**[a]	11	28.2%
Medication use [f]	12	30.0%	30	5.4%	4.1	1.89 - 8.89	**0.001**[a]	4	10.3%
Therapy modalities for delay [g]	31	79.5%	53	9.5%	19.0	8.27 - 43.66	**<0.001**[a]	11	28.2%
Follow-up (yr) [d]	3.4	2.79	4.4	2.28			**0.02**[a]	4.0	2.73

All data are given in n (%) or mean [SD]. a. p-value < 0.05 was considered statistically significant, b. included 9 cases that deceased during labour (3 with and 6 without genetic diagnosis), c. OR and p-value calculated for continuing pregnancies (TOP cases not included), d. Live births, e. 1 gram equals 0.04 ounces, f. Other than heart medication, g. E.g. speech therapy, physical therapy, psychological therapy or special education. TOP termination of pregnancy.

Prevalence including aneuploidy

The minimum prevalence of (likely) pathogenic CNVs in all fetuses with a CHD appears 7.6% (70/919). For sequence variants, not detectable with standard micro-array testing, the prevalence of (likely) pathogenic variants is estimated at 4.5% (41/919) of all fetuses with a CHD (Figure 1). A genetic cause for the CHD was encountered in 33% (305/919) of all CHD cases, which was 42% (305/708) for those who underwent genetic testing (Supplemental Material, Figure S1).

DISCUSSION

This is the largest cohort study on the total prevalence of genetic diagnoses in fetuses with a severe CHD that included results from genetic testing for specific syndromes and ES as well. In the setting of prenatal counseling concerning a fetal heart defect, a 15.7% probability should be counseled for clinically significant genetic diagnoses, other than aneuploidy. These involved CNVs in 9.9%, whereas 5.8% had a sequence variant not detectable with QF-PCR and CMA. These numbers, however, comprise conservative estimates, as not all patients underwent genetic testing and exome sequencing was not performed in the majority of cases. More importantly, a genetic diagnosis worsened the prognosis significantly, both on surgical outcome and, not unexpectedly, (neuro-) development[21].

Our results show that a genetic diagnosis has a substantial impact on neonatal outcome in fetuses with a severe CHD. Not only does it significantly increase the risk of mortality, but also morbidity, as these cases showed more often developmental delay (75.6% vs 9.7%) and required more (medical) support and medication, other than for the heart defect itself. The estimated prevalence of pathogenic CNVs in 7.6% of our entire cohort corresponds to three previously described cohorts of fetuses with CHDs, as these report pathogenic CNVs in 8-11%.[22-24] The proportion of CNVs appeared slightly lower in postnatal cohorts that reported CNVs in 5-8% of neonates with CHDs.[25, 26] One recent fetal cohort reported a prevalence of 16% pathogenic CNVs.[27] This proportion, however, reflects a selected population, as they only included those referred for invasive genetic testing.

As CMA is routinely offered in pregnancy, there remains a residual probability of at least 6.6% (41/616) to identify (likely) pathogenic sequence variants after birth. Due to the large variety of genetic syndromes, which can occur in less than 1:1000 CHD cases, exome sequencing should be considered in fetuses with CHDs. Especially, as it may change the prognosis considerably, which is why parents opt for invasive genetic testing in the first place. Our results suggest that the diagnostic yield of ES will be no less than 6.6% if ES is offered routinely for all fetal CHDs, and even higher for those with additional structural malformations. This association with ECMs is in line with previous literature[14, 22, 23], and therefore an important factor for prenatal counseling and the decision to perform additional genetic testing in some cases. Another recently published fetal cohort found diagnostic sequence variants in as many as 13.6% of fetuses with any cardiac malformation with normal results after aneuploidy testing and CMA.[28] These findings can again be explained by the selection of their cohort; ES

was not offered to all women and only those with ES results were eligible for inclusion. The estimated diagnostic yield encountered in our cohort is therefore conservative.

The prevalence of sequence variants appeared also higher in fetuses (6.6%) than neonates, as one postnatal cohort study reported genetic syndromes in 5.1% of neonates with normal chromosomes[26]. This risk is probably higher in fetal cohorts compared to cohorts that focus exclusively on postnatal cases, as cases with TOP, intra uterine fetal demise or early neonatal death are often not included in postnatal cohorts. It is therefore important that our data are evaluated from a prenatal perspective to enable prenatal counseling at mid-gestation. This is confirmed by the fact that parents in this cohort opted for TOP more often, if a genetic cause was identified, which is similar to our previous findings in prenatal exome sequencing.[20]

This study shows that if a focused approach is chosen as the method of advanced genetic testing in the setting of normal CMA, 36.6% (15/41) of (likely) pathogenic variants would remain undetected. This method is less preferable in a prenatal setting, as essential clinical symptoms may be impossible to detect in the fetus, which may lead to analysis of the 'wrong' gene panel. Exome-wide analysis of ES data can detect changes in the entire exome, but at the expense of the turnaround time or costs. Sequence variants in genes not associated with CHDs particularly, may also increase the risk of additional morbidity instead of being an explanation for the development of the heart defect itself. This might be important for prenatal counseling, as it can affect prognosis and neonatal management significantly. Exome-wide analysis of sequence data is also imperative to identify novel pathogenic genes and consequentially improve currently available gene panels for CHDs. It did lead to the detection of variants of uncertain significance in 19.7% (12/61) of all sequence variants, which may complicate prenatal counseling and parental decision making. We believe, however, that the advantages of an exome-wide analysis of exome sequencing data for fetal CHDs may outweigh its difficulties[20], particularly in the presence of additional structural malformations.

A limitation of this study is that in 20.9% of our entire cohort, genetic testing was not performed. The majority of these cases, however, comprised isolated cases without dysmorphic features after birth that showed a normal development, as all the children do have follow-up visits in our center due to relatively short travel distances and a very low threshold for genetic testing after birth. We therefore chose not to exclude cases, to avoid substantial selection bias, as this might lead to an overestimation of the prevalence.

Due to the large sample size and completeness of our regional CHD registry, we were able to stratify the probability of genetic diagnoses according to the specific heart defect, using not only results from karyotyping and FISH for 22q11.2, but CMA,

genetic testing for specific syndromes and exome sequencing in selected cases as well. Although several recent cohorts have studied aneuploidy or 22q11.2 deletion syndrome in CHD cases[22, 23, 26, 29], evidence on the prevalence of other structural chromosome abnormalities and sequence variants for specific heart defects is limited. IAoA, PA-VSD and AVSD were most associated with the presence of genetic diagnoses. The particularly strong correlation between IAoA and submicroscopic genetic changes (69% probability in IAoA cases) was also demonstrated by a large study that evaluated the results of karyotyping and fluorescence in situ hybridization (FISH) for 22q11.2 in a population of infants with CHDs.[30] Although we evaluated results from additional diagnostic modalities as well, genetic diagnoses were encountered in a similar proportion (71%) of IAoA cases. This stresses that IAoA is mainly associated with 22q11.2 deletion syndrome. One study on CMA in fetuses with a VSD reported pathogenic CNVs in 12%.[13] This might be an overestimation, as our cohort comprised twice as many VSD cases with pathogenic CNVs in 9.3% (10/107). A cohort of Tetralogy of Fallot infants found genetic diagnoses in 25% of cases, after exclusion of aneuploidy, which comprised a heterogenous set of genetic syndromes.[31] This is consistent with the 21.1% probability of pathogenic CNVs or sequence variants in our cohort.

In conclusion, this cohort study shows that, after an aneuploidy is excluded, structural chromosome abnormalities and sequence variants are identified in a substantial proportion of cases with severe CHDs. In 5.8% of euploid fetuses with a CHD, the genetic diagnosis would not have been found, if only CMA had been performed. ES should therefore be considered for fetal CHDs, especially if accompanied by other structural malformations, because genetic diagnoses can affect neonatal outcomes significantly. Future research, which offers ES to all fetuses with a CHD, is however needed to obtain more reliable estimates.

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SUPPLEMENTAL MATERIAL

Table S1. Patient characteristics of all euploid cases (n=708)

Maternal age (years)	30.9	[5.02]
Multigravida	405	63.5%
Obesity a	180	41.4%
Intoxications		
Alcohol use during pregnancy	5	1.0%
Smoking during pregnancy	55	10.4%
Medical history parents		
CHD	38	5.9%
Genetic abnormality	64	9.0%
Consanguinity	31	4.4%
Pre- or postnatal genetic testing	516	72.9%
Gender. male	401	56.5%
Multiple pregnancy	47	6.7%

Data are given in n(%) or mean [SD].
a. Obesity was defined as a body mass index (BMI) ≥25. CHD: congenital heart defects

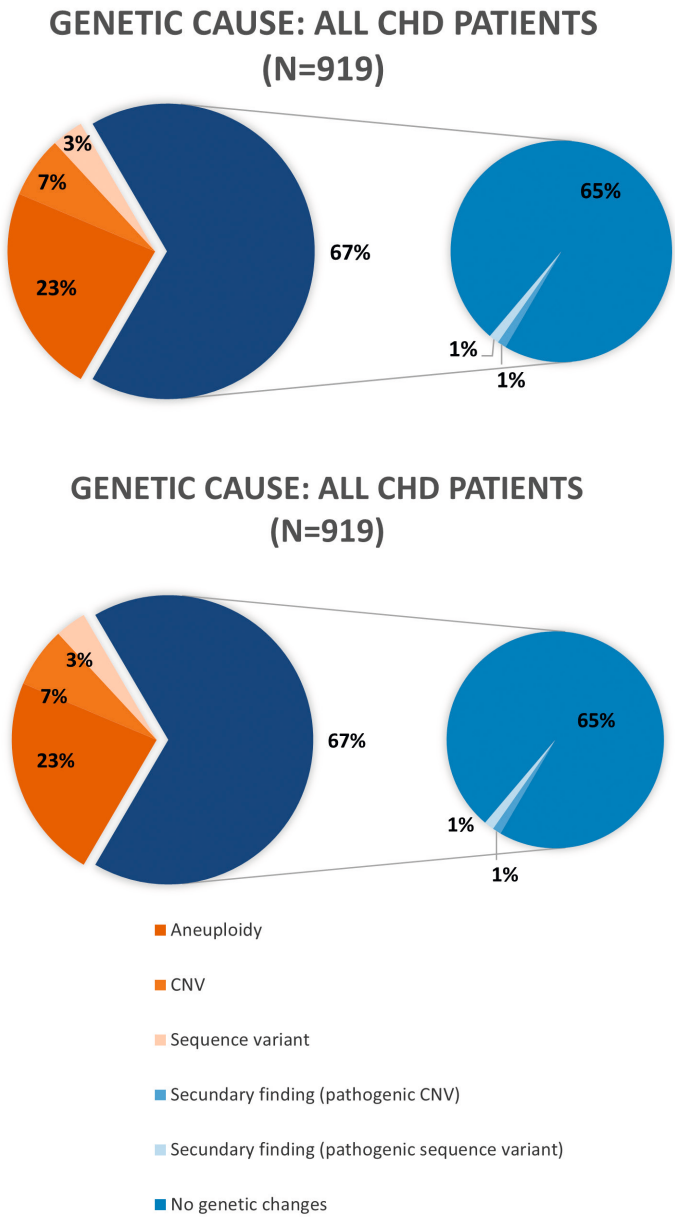
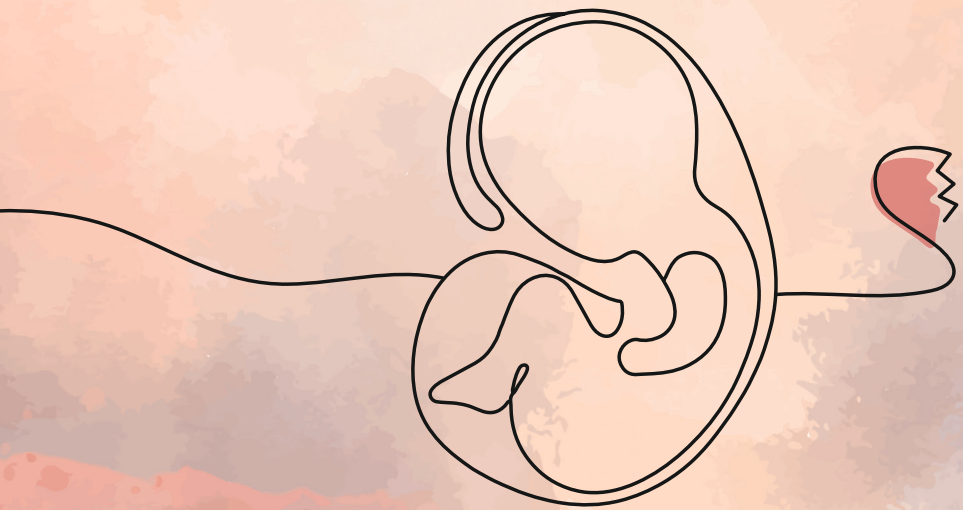


Figure S1. Genetic cause for chd



CHAPTER 4

Response to Thibodeau and Langlois

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LETTER TO THE EDITOR

We thank Thibodeau and Langlois for their interest in our work and the valuable suggestions, which gives us the opportunity to elaborate on our data.[1] The authors particularly acknowledge the fact that genetic counselling for prenatally detected congenital heart defects (CHDs) remains a challenge, as results of genetic studies in these fetuses are highly heterogeneous. The few studies that assess the diagnostic yield of exome sequencing for fetal CHDs thereby often not separately describe their findings in isolated CHD cases nor specify the CHD subtypes included. The authors request additional information on the genetic syndromes associated with each CHD diagnosis, to differentiate between those detectable with chromosome microarray analysis (CMA) and exome sequencing. They suggest that this information would enhance the scientific contribution of our paper to daily clinical practice.[2]

We completely agree with the authors that this information can aid clinicians to determine when exome sequencing should be offered in a prenatal setting. We have specified the associated genetic syndromes for each CHD diagnosis with the diagnostic modality to detect these genetic variations. To show the potential yield of sequencing in a prenatally detected *isolated* CHD, these cases are separately described (Table 1).

After exclusion of aneuploidy cases, a genetic diagnosis was found in 28.7% of non-isolated and 11.6% of isolated cases with a *severe* CHD in our cohort. A severe CHD was defined as a cases that demised or required surgery before the age of 1. Two-third of these genetic diagnoses involved copy number variations (CNVs), detectable with routine CMA. CNVs appeared particularly associated with aortic valve and arch anomalies, such as interrupted aortic arch, isolated right or hypoplastic aortic arch and an aortic valve stenosis, which was mainly attributable to their association with 22q11.2 deletion syndrome. Other CHDs with a >10% incidence of (likely) pathogenic CNVs involved pulmonary atresia with a ventricular septal defect, Tetralogy of Fallot (ToF), (atrio-)ventricular septal defect and persistent left superior vena cava.

Exome sequencing was however necessary to diagnose the remaining one-third of affected cases, representing 6.3% of all prenatally detected CHD cases, and 4.3% of cases that appeared isolated in the prenatal setting. Interestingly, isolated CHD subtypes that were particularly often accompanied by sequence variants comprised the conotruncal heart defect, such as a critical pulmonary valve stenosis with intact ventricular septum, ToF, double outlet right ventricle (ToF or Taussig Bing) and complex transposition of the great arteries. Tuberous sclerosis attributed to the high diagnostic

yield in isolated left isomerism and rhabdomyomas, whereas several pathogenic variants were found in cases with cardiomyopathy.

In our cohort we did not encounter genetic diagnoses, but variants of unknown significance (VUS), in cases with Ebstein anomaly (20.0%; 1/5), total anomalous pulmonary vein connection (9.1%, 1/11), aortopulmonary window (25.0%, 1/4), pulmonary atresia with an intact ventricular septum (18.2%, 2/11) and double inlet left ventricle (14.3%, 1/7).

Heart defects that were never accompanied by eiter a genetic diagnosis or VUS in this cohort were tricuspid valve dysplasia or insufficiency, mitral valve insufficiency, partial anomalous pulmonary vein connection, double aortic arch, congenitally corrected transposition of the great arteries, right isomerism and anomalous left coronary artery from the pulmonary artery.

Exome sequencing for CHD has recently become available in a prenatal setting. Prenatal counseling for fetal CHD however remains a challenge, as limited studies evaluate the diagnostic yield of this modality and full phenotyping with fetal ultrasound in not possible. With this letter we provide additional details on genetic syndromes associated with different CHD diagnoses, and specifically those not detectable with routine CMA. As sequence variants were identified in 4.3% of CHDs that appeared isolated, we believe exome sequencing should be considered in a prenatal setting, especially in those with conotruncal anomalies, left isomerism and rhabdomyomas.

On behalf of all authors,
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Table 1. Genetic diagnoses in a retrospective cohort of 708 euploid fetuses with a severe congenital heart defect (2012-2016)

Congenital heart defect	Genetic testing	All cases		*Isolated*		Genetic syndrome	Other genetic diagnosis
Septal defects							
AVSD, *balanced*	***Microarray***	17.9%	5/28	*15.8%*	*3/19*	1q21.1 DS (2)	unbalanced translocation (1;17)(p36.3;q25), unbalanced translocation (15;16)(q26.3;p13.2), unbalanced translocation (12;17)(p13.33p13.32;q12q25.3) - (3)
	Sequencing	10.7%	3/38	*5.3%*	*1/19*	Charge (1/2) and Noonan syndrome (1)	-
VSD	***Microarray***	10.3%	11/107	*5.6%*	*4/71*	22q11 DS (3), 16p11.2 DS (1/2), Primary microcephaly type 1 (homozygous 8p23.2p23.1 deletion) (1), 13q deletion syndrome (1)	unbalanced translocation (3;6)(p26.3:p22.3) (1), 4q trisomy (1), 2q35-36.2 deletion (1), 8q24.3 deletion (1)
	Sequencing	7.5%	8/107	*2.8%*	*2/71*	Noonan (1), Schaaf-Yang syndrome (1), Jeune (1), CM-AVN (1), Smith-Lemli-Opitz (1)	Variant COL1A1 gene (1), ZMYM2 gene (1), BCOR gene (1)
Valvular anomalies							
AoS	***Microarray***	12.0%	3/25	*5.0%*	*1/20*	22q11 DS (2), Williams syndrome (1)	-
	Sequencing	8.0%	2/25	*5.0%*	*1/20*	-	Variant in GATA5 gene (1), ELN gene without Williams syndrome (1)
PS	***Microarray***	6.7%	2/30	*7.4%*	*2/27*	22q11 DS (1)	18p deletion (1)
	Sequencing	0.0%	0/30	*0.0%*	*0/27*	-	-
MS	***Microarray***	0.0%	0/1	-	*0/0*	-	-

Table 1. *(Continued)*

Congenital heart defect	Genetic testing	All cases		*Isolated*		Genetic syndrome	Other genetic diagnosis
	Sequencing	100.0%	1/1	-	*0/0*	Cornelia de Lange (1)	-
Venous return anomalies							
PLSVC[a]	***Microarray***	33.3%	2/6	*50.0%*	*1/2*	Beckwith-Wiedemann syndrome (1)	7q21.3-31.1 deletion (1)
	Sequencing	0.0%	0/6	*0.0%*	*0/2*	-	-
Aortic arch anomalies							
Interrupted AoA	***Microarray***	64.3%	9/14	*66.7%*	*8/12*	22q11 DS (8), 1q21.1 DS (1)	-
	Sequencing	7.1%	1/14	*0.0%*	*0/12*	Charge syndrome (1)	-
Right AoA [b]	***Microarray***	28.6%	2/7	*16.7%*	*1/6*	22q11 DS (1)	unbalanced translocation (4;6)(q31.23;q26) (1)
	Sequencing	0.0%	0/7	*0.0%*	*0/6*	-	-
Shone syndrome	***Microarray***	0.0%	0/6	*0.0%*	*0/6*	-	-
	Sequencing	16.7%	1/6	*16.7%*	*1/6*	-	variant NKX2-6 gene (1)
Hypoplastic AoA	***Microarray***	12.5%	1/8	*16.7%*	*1/6*	22q11 DS (1)	-
	Sequencing	0.0%	0/8	*0.0%*	*0/6*	-	-
CoA	***Microarray***	4.6%	3/65	*3.5%*	*2/57*	22q11 DS (1), 15q11-13 duplication syndrome (1)	unbalanced translocation (7;19) (p22.3;q13.41) (1)
	Sequencing	3.1%	2/65	*1.8%*	*1/57*	Kabuki syndrome (1/2)	-
Conotruncal anomalies							
PA+VSD	***Microarray***	33.3%	4/12	*16.7%*	*1/6*	22q11 DS (1/2)	unbalanced translocation (2;9)(p15;p22), unbalanced translocation (7;9) - (2)
	Sequencing	8.3%	1/12	*0.0%*	*0/6*	Alagille syndrome (1)	-

Table 1. *(Continued)*

Congenital heart defect	Genetic testing	All cases		*Isolated*		Genetic syndrome	Other genetic diagnosis
CAT	***Microarray***	25.0%	4/16	*36.4%*	*4/11*	22q11 DS (4)	-
	Sequencing	0.0%	0/16	*0.0%*	*0/11*	-	-
APVS	***Microarray***	25.0%	1/4	*25.0%*	*1/4*	22q11 DS (1)	-
	Sequencing	0.0%	0/4	*0.0%*	*0/4*	-	-
ToF	***Microarray***	15.8%	9/57	*10.6%*	*5/47*	22q11 DS (2/5), Cri du chat syndrome (1)	7p22 deletion (1), homozygous 15q15.3 deletion (1), 17q12 duplication (1)
	Sequencing	5.3%	3/57	*6.4%*	*3/47*	Alagille syndrome (1)	variant NKX2-5 gene (1), TBCE + MYH7 gene (1)
DORV-Fallot	***Microarray***	10.0%	3/30	*6.3%*	*1/16*	-	19p13.2 duplication + 19p13.12 deletion (1), mosaic trisomy 9 (1), 9p24.3q21.11 tetrasomy (1)
	Sequencing	6.7%	2/30	*6.3%*	*1/16*	Rubinstein-Taybi syndrome (1), Charge syndrome (1)	-
DORV-TGA	***Microarray***	4.3%	1/23	*5.3%*	*1/19*	22q11 DS (1)	-
	Sequencing	8.7%	2/23	*5.3%*	*1/19*	Charge syndrome (1)	variant ACVR1 gene (1)
TGA (complex)	***Microarray***	0.0%	0/20	*0.0%*	*0/17*	-	-
	Sequencing	5.0%	1/20	*5.9%*	*1/17*	-	variant CDK13 gene (1)
TGA (simple)	***Microarray***	2.1%	1/47	*2.2%*	*1/46*	-	16p11.2 deletion (1)
	Sequencing	2.1%	1/47	*2.2%*	*1/46*	-	Hemophilia type A (1)

Table 1. *(Continued)*

Congenital heart defect	Genetic testing	All cases		*Isolated*		Genetic syndrome	Other genetic diagnosis
Univentricular heart defects							
AVSD, *unbalanced*	***Microarray***	22.2%	2/9	*20.0%*	*1/5*	8p23.1 microdeletion syndrome (1)	9p24.3-p11.2 tetrasomy (1)
	Sequencing	11.1%	1/9	*0.0%*	*0/5*	-	variant ZIC3 gene (1)
HLHS	***Microarray***	3.8%	2/52	*2.2%*	*1/45*	6p25 microdeletion syndrome (1), Waardenburg syndrome (1)	-
	Sequencing	7.7%	4/52	*8.9%*	*4/45*	Kabuki syndrome (1)	variant NOTCH 1 gene (1), TKT gene (1), Primary ciliary dyskinesia (1)
HRHS	***Microarray***	6.7%	1/15	*0.0%*	*0/12*	-	10q21q22 deletion (1)
	Sequencing	0.0%	0/15	*0.0%*	*0/12*	-	-
TV atresia	***Microarray***	4.0%	1/25	*4.5%*	*1/22*	Charcot-Marie Tooth syndrome type 1A (1)	-
	Sequencing	0.0%	0/25	*0.0%*	*0/22*	-	-
Absent left AV-C	***Microarray***	100.0%	1/1	*100.0%*	*1/1*	Ringchromosome 20 syndrome (1)	-
	Sequencing	0.0%	0/1	*0.0%*	*0/1*	-	-
Atrial isomerism							
Left isomerism	***Microarray***	8.3%	1/12	*0.0%*	*0/4*	18p deletion syndrome (1)	-
	Sequencing	8.3%	1/12	*25.0%*	*1/4*	Tuberous sclerosis complex (1)	-

Table 1. *(Continued)*

Congenital heart defect	Genetic testing	All cases		*Isolated*		Genetic syndrome	Other genetic diagnosis
Miscellaneous							
Rhabdomyomas	***Microarray***	0.0%	0/3	*0.0%*	*0/1*	-	-
	Sequencing	100.0%	3/3	*100.0%*	*1/1*	Tuberous sclerosis complex (1/2)	Spondyloepiphyseal dysplasia congenita (1)
Cardiomyopathy	***Microarray***	0.0%	0/3	*0.0%*	*0/2*	-	-
	Sequencing	66.7%	2/3	*100.0%*	*2/2*	-	variant MYBC3 + ACTC1 gene (1), MIPEP gene (1)
DCRV	***Microarray***	50.0%	1/2	*50.0%*	*1/2*	Phelan-McDermid syndrome (1)	-
	Sequencing	0.0%	0/2	*0.0%*	*0/2*	-	-
Left-/right discrepancy	***Microarray***	0.0%	0/6	*0.0%*	*0/1*		-
	Sequencing	16.7%	1/6	*0.0%*	*0/1*	Noonan (1)	-
Complex CHD[c]	***Microarray***	20.0%	1/5	-	*0/0*	-	unbalanced translocation (13;17) (q14.13;q25.3) (1)
	Sequencing	0.0%	0/5	-	*0/0*	-	-
Total	***Microarray***	11.1%	71/639	*8.6%*	*42/487*		
	Sequencing	6.3%	40/639	*4.3%*	*21/487*		

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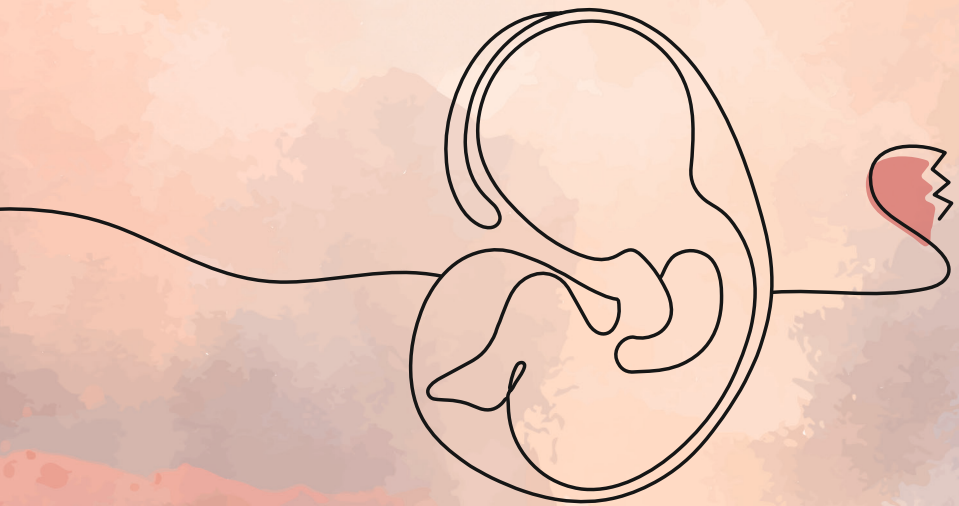

CHAPTER 5

Impact of extracardiac pathology on head growth in fetuses with congenital heart defects

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ABSTRACT

Objective

Neurodevelopmental delay is frequently encountered in children with congenital heart defects (CHD). Fetuses with major CHD have a smaller head circumference (HC), irrespective of altered cerebral flow or brain oxygenation. This cohort study compared head circumference in cases with isolated and non-isolated CHD to evaluate the effect of additional pathology on head growth in these fetuses.

Methods

All prenatally diagnosed CHD cases were selected from our regional PRECOR registry (2002-2014). Cases of multiple pregnancy, and those affected by maternal diabetes, severe fetal structural brain anomalies or functional CHD were excluded. Subjects were divided into an isolated and non-isolated group. The non-isolated group was subdivided into three groups: cases with genetic anomaly, extracardiac malformation or placental pathology. In both isolated and non-isolated CHD groups, CHDs were also grouped according to their potential effect on aortic flow and oxygen saturation. Mean HC Z-scores at 20 weeks and the in- or decrease (Δ) of HC Z-scores over the course of pregnancy were compared between isolated and non-isolated groups, using mixed linear regression models.

Results

We included 916 prenatally diagnosed CHD cases, of which 378 (41,3%) were non-isolated (37 with placental pathology, 217 with genetic anomaly and 124 with extracardiac malformation). At 20-weeks, non-isolated cases had significantly lower HC z-scores compared to isolated cases (Z = –0.70 vs –0.03; p<0.001) and head growth over the course of pregnancy showed a larger decrease in this group (Δ HC Z-score= –0.03 vs –0.01 per week; p=0.01). Cases with placental pathology had the smallest HC z-score of –1.29 at 20 weeks and the largest decrease in head growth (–0.06 per week). In CHD subjects with a genetic diagnosis (Z = -0.73; Δ HC Z-score = -0.04 per week) and in those with an extracardiac malformation (Z = -0.49; Δ HC Z-score = -0.02 per week),

HC Z-scores were also lower compared with those in subjects with isolated CHD. CHDs that result in low oxygenation or flow to the brain were present more frequently in isolated than non-isolated cases.

Conclusion

Smaller HC in fetuses with CHD appears to be associated strongly with additional pathology. Placental pathology and genetic anomaly in particular seem to be important contributors to restricted head growth. This effect appears to be irrespective of altered hemodynamics caused by the CHD. Previously reported smaller HC in CHD should, in our opinion, be attributed to additional pathology. Neurodevelopment studies in infants with CHD should, therefore, always differentiate between isolated and non-isolated cases.

INTRODUCTION

Congenital heart defects (CHD) occur in 5-8 per 1000 live births.[1] Neurodevelopmental impairment (NDI) occurs in a significant number of these children and was originally attributed to cardiothoracic surgery.[2-5] Abnormalities at neurological imaging prior to surgery, however, suggested that pre-operative factors may influence brain development in neonates with CHD.[6-8] This raised the question whether circulatory changes in utero, caused by the CHD, could be responsible for the neurological abnormalities on pre-operative scans.

To study brain development in utero, several cohorts reported on head circumference (HC), as a proxy for neurocognitive outcome[9-21], as fetal head size is directly related to brain volume. These studies have reported a lower mean HC in fetuses and neonates with CHD, particularly in hypoplastic left heart syndrome (HLHS) and transposition of the great arteries (TGA).[10, 13, 18, 20] A large recent cohort, comprising only *isolated* heart defects, with repetitive antenatal HC measurements, could not replicate these results.[12] That study found only small differences in fetal head growth, as HC values remained within the normal range and were irrespective of alterations in aortic flow or saturation.[12] More recent large cohort studies opposed the hypothesis of decreased oxygenation as an explanation for smaller HC as well, and showed very small differences in HC between normal and CHD fetuses.[15, 17] A remarkable finding was that small changes in HC size were also encountered in cases with CHD types that do not result in fetal circulatory changes.[15]

As these latter studies were unable to confirm the hypothesis of diminished fetal head growth as a result of altered fetal hemodynamics, we hypothesize that genetic effects that remained undetected in pregnancy or placental factors could play a role. To test this hypothesis, all non-isolated fetuses were retrieved from our regional registry PRECOR, as suggested in a referee commentary on our previous study.[22] The aim of this study was to compare head growth patterns in fetuses with isolated and non-isolated heart defects[12], to explore if additional morbidity could explain the reduced head size found in neonates with CHD.

METHODS

This cohort study used data from three tertiary care centers in Amsterdam and Leiden: Amsterdam University Medical Centers, location AMC and VUMC Amsterdam and Leiden University Medical Center, Leiden. These three centers collaborate in the care for children with CHD within 'CAHAL' (CAHAL: in Dutch 'Center for Congenital Heart Disease Amsterdam-Leiden'). Cases were extracted from CAHAL's fetal and neonatal registry PRECOR. The data collection for this registry has been described previously.[23] We used this registry to extract all prenatally diagnosed CHD cases from January 2002 to July 2014, which corresponds with the assessed timeframe of the isolated cases in our previous study.[12] Not eligible for inclusion were subjects with functional CHDs, primary arrhythmias with normal cardiac anatomy, multiple pregnancies or co-existing factors that are a clear cause of altered fetal head growth such as trisomy 13 and 18 or that show an increase in fetal growth in general, like maternal diabetes. Subjects with severe structural brain anomalies that influence fetal head size in itself, such as hydrocephaly or holoprosencephaly, were not included. If fetal HC measurements were not available, cases were excluded as well.

Data retrieval

Fetal databases and pediatric files in the three centers were used to retrieve data for all isolated and non-isolated CHD cases. We collected data regarding fetal biometry, pre- and postnatal cardiac findings, extra-cardiac abnormalities, results of genetic tests (duplications, deletions, specific gene panels), maternal information (medical and obstetric history, body mass index) and pregnancy outcome. Gestational age was determined at a first-trimester dating scan. Biometry measurements (HC and abdominal circumference, AC) were entered into the fetal databases prospectively, as they were part of standard fetal monitoring and therefore measured routinely. All measurements were performed according to the guidelines of the Dutch Society for Obstetrics and Gynecology [24], which is in concordance with those described by the International Society of Ultrasound in Obstetrics and Gynecology.

Data regarding postnatal cardiac diagnosis and follow-up of these CHD cases were gathered from the pediatric files. Confirmation of the CHD was based on postnatal echocardiography or post-mortem examination. In case of pregnancy termination without permission for autopsy, the cardiac diagnosis was based on prenatal echocardiography. Earlier reports demonstrated a high compliance between pre- and postnatal diagnosis in these centers, as a result of close collaboration between fetal specialists and pediatric cardiologists.[25] We retrieved complete follow-up for all liveborn cases until at least the age of 1 year. Genetic alterations or results of the assessment

of a clinical geneticist were noted in our database. Extra-cardiac anomalies diagnosed postnatally were added to the registry as well.

Clustering

All cases were either allocated to the isolated or non-isolated CHD group according to the existence of additional morbidity. Isolated CHD was defined as the absence of genetic anomaly, extracardiac malformation and intrauterine growth restriction (IUGR)[12]. If genetic testing was not performed, but cases did not present with additional structural malformations or signs of placental insufficiency, they were allocated to the isolated group. Minor additional findings, such as soft markers, amniotic-fluid pathology, mild pericardial effusion and/or single umbilical artery were not considered to be a significant structural malformation[12]. As the original isolated cohort described by Jansen *et al*.[12] did not include subjects from the Amsterdam University Medical Centers in the last 2.5 years, and these cases became available by data extraction from PRECOR, we supplemented the original isolated cohort of 436 cases with these subjects. Both isolated and non-isolated subjects were clustered according to the expected effect of their CHD on both aortic flow and oxygenation to the brain, based on theoretical hemodynamics, as described in our previous study[12]. A list of diagnoses assigned to each category is given in Supplemental material S1.

The non-isolated group was also further subdivided into three groups: cases with specific genetic alterations or evident dysmorphic features ('genetic-diagnosis group'); cases with significant extracardiac malformations but without a genetic diagnosis after consultation with a clinical geneticist ('extracardiac-malformation group'); and cases with maternal complications associated with placental pathology, including IUGR, pre-eclampsia, hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome and hemolytic uremic syndrome (HUS) ('placental-pathology group'). Cases with combined pathology, which could be allocated to more than one group, were excluded from the subgroup analysis. For example, a case with 22q11 syndrome and mild pre-eclampsia was considered non-isolated, but was not allocated to any of the three subgroups. IUGR was defined as postnatal birth weight < 3rd percentile, based on national postnatal birth-weight charts in term subjects.[26] Preterm infants were considered IUGR if either the fetal AC or the estimated fetal weight (EFW) was < 3rd centile or the AC or EFW was < 10th centile in combination with abnormal Doppler measurements in the umbilical artery at the last ultrasound scan prior to birth. These cut-offs were chosen as the postnatal birth-weight charts in our country do not exclude children who underwent planned preterm delivery due to IUGR or pre-eclampsia, resulting in charts with overrepresentation of pathology. Pre-eclampsia was defined as gestational blood pressure elevation (> 140 mmHg systolic or > 90 mmHg diastolic)

combined with proteinuria (> 0.3 g/24 h, 30 mg/dL or + 1 on dipstick). The Tennesee classification was used to define HELLP syndrome.[27] To analyze centiles for both AC and EFW with advancing gestation, we used the growth curves of Verburg *et al*.[24] and Hadlock *et al.* [28].

Data analysis

The distribution of several factors that have the ability to affect fetal growth, such as maternal obesity and smoking, were compared at baseline in the isolated and non-isolated CHD groups. These two groups were also evaluated for differences in the distribution of types of CHD, with regards to their expected effect on aortic flow and saturation. We compared mean HC *Z*-scores at 20 weeks' gestation and fetal head growth with advancing gestation (slope of the regression) between isolated and non-isolated CHD subjects. When there were significant differences between the two groups present at baseline, mean HC and AC *Z*-scores were corrected for these factors. These outcome parameters were also evaluated for all non-isolated subgroups separately, compared to reference curves of the standard population[24]. The independent effect of type of comorbidity and other variables of interest on HC *Z*-scores was assessed amongst non-isolated CHD subjects by performing a multivariate regression analysis. We examined these data at around 20 weeks' gestation, as biometric data were available in most cases around this time.

To evaluate the effect of alterations in the intrauterine environment on head growth in CHD cases, we also estimated the expected mean HC *Z*-score at 36 weeks, as any effect is likely to be most evident in the last few weeks prior to birth. This was corrected for maternal age and based on the mean HC *Z*-score at 20 weeks and fetal head growth with advancing gestation. AC was also evaluated to relate fetal head size to intrauterine body growth.

Differences in characteristics at baseline were tested with an independent *t*-test for numerical data, while a χ^2-test was performed for all categorical variables. Biometric data (HC and AC) were converted into *Z*-scores to adjust for the effect of gestational age on fetal growth and to be able to relate the values observed in the dataset to those of the normal population. The growth charts by Verburg *et al*. were used to calculate *Z*-scores, as they included a large Dutch cohort selected over a similar period of time and these charts have been validated for the Dutch population.[24, 29]

In order to evaluate HC *Z*-scores amongst isolated and non-isolated subjects according to advancing gestation and to account for the dependency between repeated measurements, we used a mixed linear regression model with a random intercept

and, if data allowed, random slope. This was necessary, as fetal biometry was measured multiple times within the same cases and the interval between the measurements could differ between cases. If data on a variable of interest were missing for >10% of the cases, the variable was not included in the multivariate analysis. $P < 0.05$ was considered to be statistically significant. IBM SPSS statistics version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

RESULTS

Case selection

In total, we extracted from the PRECOR registry 1387 fetuses diagnosed with CHD over the study period. We excluded 64 cases with functional cardiac disease or normal cardiac anatomy on the postnatal scan, 113 cases of multiple pregnancy, 26 with maternal diabetes, 26 with structural congenital brain anomalies and 182 with trisomy 13 or 18 or triploidy. A further 60 were excluded because the HC measurements had not been recorded, of which 44 underwent termination of pregnancy (TOP) before 17 weeks, seven underwent TOP immediately after fetal echocardiography without routine obstetric measurements in the second trimester, eight were referrals near term in which HC measurements were technically impossible due to the head being deeply engaged and one case with ventricular septal defect was referred back to a local hospital after a single fetal echo without obstetric measurements. Thus, after exclusion of 471 cases, 916 cases were eligible for analysis (Figure 1).

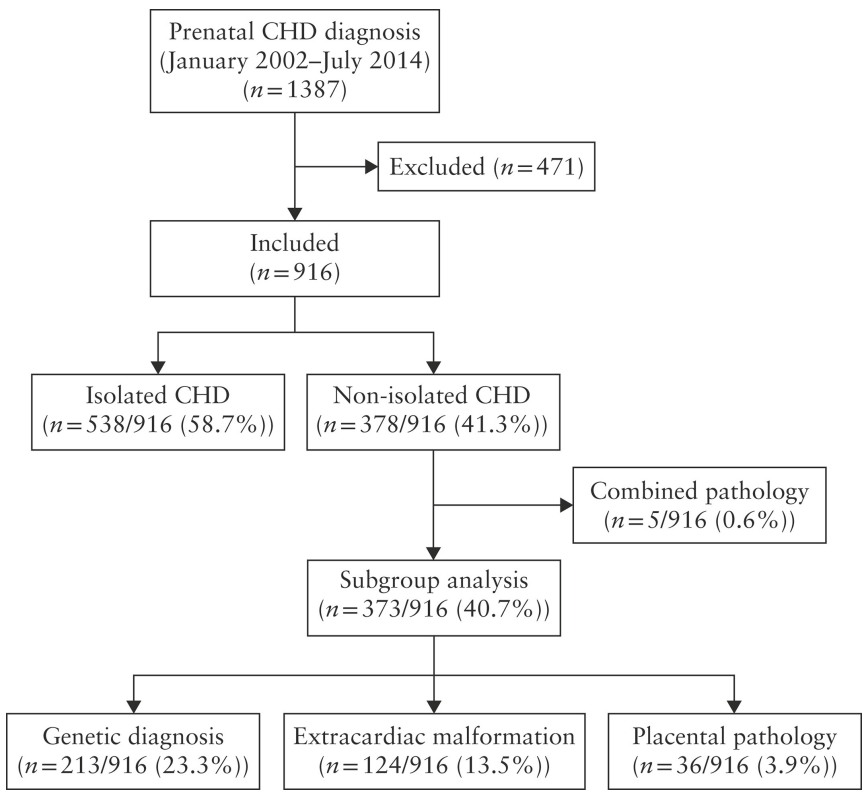

Figure 1. Flowchart summarizing case selection and inclusion from a cohort of fetuses with congenital heart defects.

Characteristics of study subjects

Of the 916 cases analyzed, 538 had no additional pathology and were classified in the isolated CHD group; of these, 436 have been described previously.[12] In 59.9% of all isolated CHD cases, pre- or postnatal karyotyping was performed, confirming the absence of chromosomal abnormalities. In most cases, clinical genetic assessment or type of heart defect was the reason for karyotyping. The remaining 378 subjects were assigned to the non-isolated groups: genetic diagnosis (n=213), extra-cardiac malformation (n=124) or placental pathology (n=36). Five cases with combined pathology, who could be allocated to both the 'genetic diagnosis' and 'placental pathology' group, were excluded from further subgroup analysis. These comprised three cases with (mild) pre-eclampsia and a 22q11 syndrome, one with (mild) pre-eclampsia and mosaic trisomy 12 and one case with severe HELLP syndrome and fetal trisomy 21. There were 177 subjects with a genetic diagnosis and coexisting extracardiac malformation, all of which were included in the genetic-diagnosis group, because the extracardiac malformations were always part of the genetic diagnosis.

Maternal age and BMI differed significantly between the isolated CHD and non-isolated CHD groups; mothers of subjects in the non-isolated CHD group were older (difference between means = 0.9 years) and more of them had BMI > 25 kg/m2 (difference = 10%) (Table 1). We corrected for maternal age in all subsequent analyses. As BMI was not available in 44% of the subjects, we decided not to correct for BMI. A list of the CHD diagnoses in each of the two groups is given in Supplemental material S2.

Table 1. Baseline characteristics

Characteristics	Isolated (n=538)	Non-isolated (n=378)	P
Fetal HC measurements			
Total (n)	1556	1044	
Mean (n) per case	2.9	2.8	
Maternal age (years)	30.4 ± 5.04	31.3 ± 5.70	0.02*
Parity			
Nulliparous	235 (43.9)	145 (38.4)	0.09
Parous	300 (56.1)	233 (61.6)	
Maternal BMI			
≤25 kg/m^2	210 (66.9)	115 (56.9)	0.02*
>25 kg/m^2	104 (33.1)	87 (43.1)	

Table 1. *(Continued)*

Characteristics	Isolated (n=538)	Non-isolated (n=378)	P
Smoking			
Non-smoker	420 (90.5)	303 (88.6)	0.38
Smoker	44 (9.5)	39 (11.4)	
Pregnancy outcome			
Livebirth	373 (69.3)	169 (44.7)	
Termination of pregnancy	153 (28.5)	164 (43.4)	
Fetal demise	12 (2.2)	45 (11.9)	

Data are given as n/N (%) or mean ± SD unless stated otherwise.
* p-value <0.05 is considered statistically significant.
BMI, body mass index; HC, head circumference;

The CHDs in the isolated compared with the non-isolated CHD group comprised more defects that, theoretically, result in low saturation levels (8.4% vs 1.3%; P < 0.001) (Table 2). The proportion of CHDs that lead to reversed (17.5% vs 11.9%; P = 0.02) or obstructed (17.7% vs 10.6%; P = 0.003) aortic flow was also higher in the isolated compared with the non-isolated CHD cases. CHDs that cause intracardiac mixing, but do not lead to obstructed aortic flow, such as tetralogy of Fallot and pulmonary atresia with ventricular septal defect, were encountered more often amongst the non-isolated CHD cases (57.9% vs 33.5%; P < 0.001) (Table 2), as these defects are known to be associated with genetic defects.

The mean HC Z-score was significantly lower in the non-isolated CHD cohort (Z-score = –0.70 (95% CI, –0.84 to –0.55)) compared with the isolated CHD cohort (Z-score = –0.03 (95% CI, –0.15 to 0.10)) at 20 weeks (P < 0.001) (Table 3). The reduction in head growth with advancing gestation was significantly greater in the non-isolated CHD cases (change in HC Z-score of –0.03 vs –0.01 per week; P = 0.01). The estimated expected mean HC Z-score at 36 weeks was –1.22 (95% CI, –1.45 to –0.98) for non-isolated CHD cases and –0.19 (95% CI, –0.36 to –0.01) for isolated CHD cases, which means both estimates still lay within the limits of normality. AC Z-scores were also significantly lower in non-isolated CHD fetuses, with a mean AC Z-score of –0.47 compared with –0.02 in isolated CHD cases (P < 0.001), and a change in AC Z-score of −0.02 per week in non-isolated vs change of + 0.01 per week in isolated cases at 20 weeks (P = 0.001) (Figure 2).

Table 2. Distribution of congenital heart defects (CHD) in study population, with regards to their expected effect on aortic flow and oxygenation to brain, for isolated and non-isolated cases

Cerebral hemodynamics	**Total**	**Isolated CHD**	**Non-isolated CHD**	**Difference* (95% CI)**	***P***
Oxygenation					
Low	50 (5.5%)	45 (8.4%)	5 (1.3%)	7.1% (4.35 to 9.79)	**<0.001**
Mixed	595 (65.0)	314 (58.4%)	281 (74.3%)	–16.0% (–21.88 to –9.79)	**<0.001**
Normal	271 (29.6%)	179 (33.3%)	92 (24.3%)	8.9% (2.96 to 14.69)	**0.004**
Aortic flow					
Reversed	139 (15.2%)	94 (17.5%)	45 (11.9%)	5.6% (0.86 to 10.06)	**0.02**
Obstructed	135 (14.7%)	95 (17.7%)	40 (10.6%)	7.1% (2.47 to 11.46)	**0.003**
Normal	642 (70.1%)	349 (64.9%)	293 (77.5%)	–12.6% (–18.33 to –6.70)	**<0.001**
Combined	**Total**	**Isolated CHD**	**Non-isolated CHD**	**Difference* (95% CI)**	***P***
Aortic flow reversed					
Oxygenation mixed	139 (15.2%)	94 (17.5%)	45 (11.9%)	5.6% (0.86 to 10.06)	**0.02**
Aortic flow obstructed					
Oxygenation mixed	57 (6.2%)	40 (7.4%)	17 (4.5%)	2.9% (–0.26 to 5.97)	**0.07**
Oxygenation normal	78 (8.5%)	55 (10.2%)	23 (6.1%)	4.1% (0.46 to 7.62)	**0.03**
Aortic flow normal					
Oxygenation low	50 (5.5%)	45 (8.4%)	5 (1.3%)	7.1% (4.35 to 9.79)	**<0.001**
Oxygenation mixed	399 (43.6%)	180 (33.5%)	219 (57.9%)	–24.5% (–30.69 to –17.99)	**<0.001**
Oxygenation normal	193 (21.1)	124 (23.1)	69 (18.3)	4.8 (–0.59 to 9.97)	0.10

P < 0.05 considered statistically significant.
* Isolated minus non-isolated result.

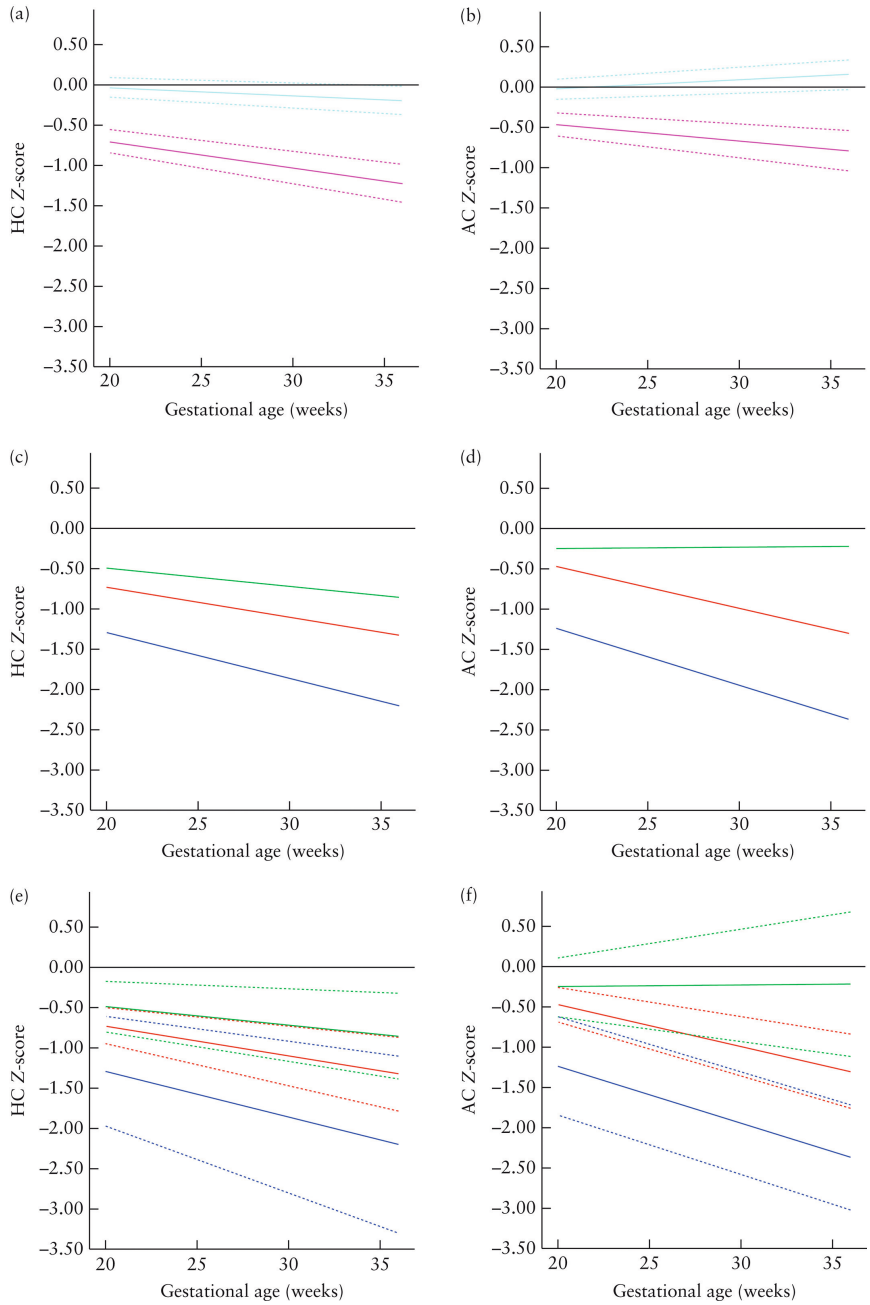

Figure 2. Head (a,c,e) and abdominal (b,d,f) growth trajectories of fetuses with congenital heart defect (CHD): (a,b) in all cases, according to whether CHD was isolated (cyan) or non-isolated (magenta); (c–f) in non-isolated CHD cases, subdivided according to additional pathology (red, genetic anomaly; green, extracardiac malformation; blue, placental pathology). Solid lines are mean and dotted lines are 95% CI. AC, abdominal circumference; HC, head circumference.

Subgroup analysis

We allocated 213 of the 378 non-isolated subjects that were diagnosed with chromosomal or genetic disorders, such as trisomy 21 or 22q11 syndrome, to the 'genetic diagnosis group'. In 143/213 cases chromosomal anomalies were diagnosed, comprising trisomy 21 (n=102), Turner syndrome (n=20), mosaic variegated aneuploidy syndrome (n=3), unbalanced translocations (n=14) and other chromosomal anomalies (n=4). The other 70 subjects had a genetic diagnosis, including 22q11.2 microdeletion syndrome (n = 27), CHARGE syndrome (n = 4), VACTERL association (n = 4), Noonan syndrome (n = 3), another miscellaneous genetic syndrome (n = 15), duplication (n = 8), deletion > 5 Mb on array comparative genomic hybridization (n = 7), or a strong clinical suspicion for a specific genetic syndrome without further diagnostic tests (n = 2). Significant extracardiac abnormalities were encountered in 124 subjects without a genetic diagnosis or dysmorphic features (extracardiac-malformation group). The 36 subjects presenting with placental pathology resulting in impaired fetal growth (placental-pathology group) included subjects with pre-eclampsia (n = 8), HELLP (n = 2), HUS (n = 1) and IUGR without maternal disease (n = 25).

We evaluated the HC Z-scores, corrected for maternal age, for these three subgroups separately (Table 3). All three subgroups showed a reduction in HC compared to the normal growth charts[24]. Subjects with a genetic anomaly had a mean HC Z-score at 20 weeks of –0.73 ($P < 0.001$) and an estimated Z-score at 36 weeks of –1.32. The extracardiac-malformation group had a mean HC Z-score at 20 weeks of –0.49 ($P = 0.002$) and an estimated Z-score at 36 weeks of –0.85. The placental-pathology group showed the greatest negative effect on fetal HC; the mean HC Z-score at 20 weeks in this group was –1.29 ($P < 0.001$), which decreased further to an estimated HC Z-score of –2.20 at 36 weeks. The mean AC Z-scores for each subgroup are presented in Table 3.

Table 3. Head circumference (HC) and abdominal circumference (AC) *Z*-scores in isolated and non-isolated cases with a fetal congenital heart defect (CHD)

HC z-score	**20 weeks GA§**					**36 weeks GA†**	
	Mean	**(95% CI)**	**P**	**Slope (SD/ week)**	**P**	**Mean**	**(95% CI)**
Isolated	-0.03	(-0.15 ; 0.10)	<0.001 α	-0.010	0.01 *α*	-0.19	(-0.36 ; -0.01)
Non-isolated	-0.70	(-0.84 ; -0.55)		-0.032		-1.22	(-1.45 ; -0.98)
Genetic diagnosis ‡	-0.73	(-0.95 ; -0.50)	<0.001	-0.037		-1.32	(-1.79 ; -0.86)
Extracardiac malformation ‡	-0.49	(-0.80 ; -0.17)	0.002	-0.023		-0.85	(-1.38 ; -0.32)
Placental pathology ‡	-1.29	(-1.97 ; -0.61)	<0.001	-0.057		-2.20	(-3.30 ; -1.10)
AC z-score							
Isolated	-0.02	(-0.15 ; 0.10)	<0.001 *α*	0.012	0.001 *α*	0.16	(-0.03 ; 0.34)
Non-isolated	-0.47	(-0.61 ; -0.32)		-0.020		-0.79	(-1.04 ; -0.54)
Genetic diagnosis ‡	-0.47	(-0.69 ; -0.26)	<0.001	-0.016		-1.30	(-1.76 ; -0.84)
Extra-cardiac malformation ‡	-0.25	(-0.62 ; 0.11)	0.17	0.002		-0.22	(-1.11 ; 0.68)
Placental pathology ‡	-1.24	(-1.84 ; -0.63)	<0.001	-0.071		-2.37	(-3.03 ; -1.72)

All values depicted are corrected for maternal age.
P < 0.05 considered statistically significant.
§ Mean HC z-scores and slopes calculated from biometric data.
†Mean Z-scores and slopes estimated using mixed linear regression model with gestational age (GA) centered at 20 weeks.
‡ Mean HC z-score in the subgroups was compared separately to normal growth reference (mean z=0)
α Isolated vs non-isolated groups.

Multivariate analysis

The multivariate analysis to evaluate the influence of type of comorbidity, maternal age, smoking and being parous on mean HC Z-score and intrauterine fetal head growth at 20 weeks in all non-isolated CHD subjects is summarized in Table 4. Corrected for the other variables of interest, the presence of placental pathology, smoking and being parous appeared to be significant independent risk factors for a lower HC Z-score at 20 weeks' gestation. Additional pathology and being parous also tended to have a negative effect on head growth progression (slope) at 20 weeks.

Table. 4. Multivariate effect estimation of influence of extracardiac pathology and possible confounders

	Fetuses (n=373)	HC measurements (n=1031)	Mean HC Z-score	(SD)	P	HC Slope (SD/week)	(95% CI)	P-value
Type comorbidity*					0.05			0.59
Extra-cardiac malformation	124	337	Reference			Reference		
Genetic syndrome	213	531	- 0,33	±0,10	0.13	- 0,02	(- 0,07 ; 0,03)	0,43
Placental insufficiency	36	163	- 0,77	±0,02	**0.02***	- 0,05	(- 0,12 ; 0,01)	0,11
Variables of interest								
Maternal age	373	1031	+ 0,02	±0,32	0.27	+ 0,001	(-0,003 ; 0,004)	0,73
Smoking (yes)	39	99	- 0,64	±0,02	**0.03***	+ 0,01	(-0,05 ; 0,08)	0,69
Parous (yes)	233	647	- 0,56	±0,01	**0.04***	- 0,02	(-0,06 ; 0,03)	0,44

Mean HC z-scores and slopes are estimated with a mixed-linear regression model with GA centered at 20 weeks.
* p-value <0.05 is considered to be statistically significant.

DISCUSSION

Fetuses with non-isolated CHD had a significantly smaller HC at mid-gestation and more constrained head growth towards the end of pregnancy, compared with fetuses with isolated CHD. Although the mean HC *Z*-score prior to delivery was –1.2 amongst CHD cases with additional pathology, compared with –0.2 in isolated CHD cases, both estimates still lie within the limits of normality. The decrease in HC appeared most prominent amongst subjects with placenta-related pathology.

Most studies that have explored fetal HC in CHD compare their findings to head size of normal fetuses[9, 14, 15, 21, 30-32] and did not strictly exclude non-isolated cases.[11, 16, 21, 30, 33, 34] As our cases originate from a large regional cohort with follow-up, we were able to analyze differences between non-isolated and isolated cases, and to test specific subgroups separately, with clustering of specific CHD types. All non-isolated CHD subgroups showed a significant decrease in head growth compared with normal charts. The largest effect was encountered in fetuses affected by placental pathology, followed by those with a genetic diagnosis and those with an extracardiac malformation. The progressive decline in (head) growth towards the end of pregnancy, encountered in all three subgroups, is a feature of placental insufficiency and characterized by a decrease in the ability to reach a certain growth potential with advancing gestation[35]. This implies that cases with a genetic diagnosis or extracardiac malformation as well as truly isolated cases did not reach their genetic growth potential, despite measurements lying within the limits of normality[36].

The types of CHD differed significantly between the isolated CHD and the non-isolated CHD groups. The isolated CHD group, in which HC growth was decreased only minimally, included significantly more CHDs that result in low cerebral oxygenation, such as transposition of the great arteries. CHDs that cause decreased flow towards the brain (e.g. aortic coarctation and hypoplastic left heart syndrome) were also encountered more frequently amongst cases of isolated CHD. Non-isolated CHD subjects, in which impaired head growth was most pronounced, had mainly CHDs without any hemodynamic effect on aortic flow (e.g. tetralogy of Fallot). If our previously raised hypothesis[12], that diminished fetal head growth in CHD is a result of altered fetal hemodynamics, is correct, it should have been head growth in the isolated group that was the more severely decreased, but our current findings indicate the opposite. Reduced head size cannot, therefore, be attributed to altered cerebral hemodynamics.

Recent studies support that both growth restriction (17-18%) and placenta-related complications, such as pre-eclampsia, occur more frequently in CHD cases.[15, 36-41]

However, as previous studies reporting on HC in CHD did not look at birth weight, growth trajectories or additional genetic pathology, their findings may, in our opinion, be explained by additional pathology.

As all three non-isolated CHD subgroups in our study showed some form of growth restriction, it is possible that a common factor, involving both the development of CHD and the placenta, could play a role. The levels of (anti-)angiogenetic markers, including placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF) and other factors associated with chronic hypoxia, are altered (increased or decreased) in CHD subjects.[42, 43] These factors have been proven to be related to embryonic and cardiac development as well[44-49], and overexpression has been shown to result in abnormal heart development.[47, 50] Lower oxygen saturation levels in the umbilical vein[42, 43] and increased resistance in the umbilical artery[19] , which are additional signs of impaired placental function, have also been reported in CHD pregnancies. This angiogenic imbalance is also encountered in placental tissue derived from IUGR fetuses.[51] Altered levels of PlGF and sFlt-1 have been shown to be associated with a higher risk for IUGR and developing pre-eclampsia[52-54]. This similarity of antiangiogenic environment in subjects with CHD and those with IUGR implies that the pathophysiology of both diseases might share a common pathway.[36,47]

Furthermore, it seems that (head) growth restriction is linked to specific CHDs.[12, 15, 36, 55, 56] Differences in the VEGF signalling pathway have been shown to affect endocardial cushion formation and septation of the cardiac chambers, and may result in aberrant aortic arch artery patterning and outflow tract anomalies.[47, 57,58] Future research should assess large cohorts and, if available, biobanks, to explore this further. The findings in our cohort highlight that future studies on fetal (brain) development should not be undertaken without analysis of fetal growth and additional morbidity.

As head growth is multifactorial and the direct relationship with neurodevelopmental outcome has not yet been studied in strictly isolated cases[11, 21], it is debatable whether HC should be used as a proxy. It was found that decreased somatic growth, rather than head growth, might be an important predictor for NDI in a group with specific types of CHD.[11,21] Results of a recent Dutch cohort support this, as a smaller AC and lower EFW were risk factors for NDI, whereas HC alone was not.[59]

A limitation of our study is the fact that the biometric data were not distributed equally throughout pregnancy in non-isolated compared with isolated CHD cases, due to the higher incidence of TOP and intrauterine fetal demise in the non-isolated CHD group. Furthermore, genetic testing was not performed in several cases for the same reason.

Although completeness of data is rarely possible in retrospective cohorts, information was available in a significant proportion of cases, as follow-up was complete until at least 1 year of age. The current literature lacks information on the impact of additional pathology, as this has not, to our knowledge, been studied before in such a large cohort.

Our results show that the presence of additional pathology has a large effect on head growth in fetuses with CHD, which is more pronounced than the effect of aortic flow or saturation in these cases. The factor contributing most seems to be abnormal placentation, although genetic anomaly also plays an important role. Pathways involved in both the development of CHD and fetal growth appear to influence head growth more than does the CHD itself. Future research on brain development in CHD fetuses and infants should, therefore, relate to overall growth and additional pathology.

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SUPPLEMENTAL INFORMATION

Distribution of diagnoses, based on their effect on oxygen delivery to the brain and flow through the aortic arch, in isolated and non-isolated cases.

Supplemental information 1. List of primary diagnoses per subgroup

Isolated		**Oxygen delivery to the brain**			
		low	**mixed**	**normal**	**Total**
Flow through the aorta	reversed	0	Cat. 4: 94	0	94
	obstructed	0	Cat. 5: 40	Cat. 2+3: 55	95
	normal	Cat. 1: 45	Cat. 6: 180	Cat. 7: 124	349
Total		45	314	179	538

Non-isolated		**Oxygen delivery to the brain**			
		low	**mixed**	**normal**	**Total**
Flow through the aorta	reversed	0	Cat. 4: 45	0	45
	obstructed	0	Cat. 5: 17	Cat. 2: 23	40
	normal	Cat. 1: 5	Cat. 6: 219	Cat. 7: 69	293
Total		5	280	93	378

Diagnoses are based on a combination of pre- and postnatal assessment.

Category 1. Transposition of the great arteries

Oxygen-poor blood filling the aorta, with normal aortic arch flow.

Defects	N
TGA	39
TGA with small VSD	11
Total	**50**

Category 2. Aortic obstruction

Biventricular heart defects. Right-to-left shunt at atrial level might be reduced, but the mitral valve is normal.

Defects	N
Aortic valve stenosis	13
Aortic arch hypoplasia / coarctation	42
Interruption of the aortic arch	9
Total	**64**

Category 3. Small left heart syndrome

Biventricular heart defects, with left ventricle outflow tract obstruction, presenting with antenatal forward aortic arch flow. The mitral valve is small and the atrial right-to-left shunt is restricted. The preload of the LV is reduced.

Category 2 and 3 have been combined into one category due to the small sample sizes and the overlapping hemodynamic characteristics.

Defects	N
Shone syndrome	5
Persistent left superior caval vein with LV inflow obstruction	4
Premature closure of foramen ovale	3
ccTGA with coarctation of the aorta	1
Polyvalvular disease with aortic arch hypoplasia	1
Total	**14**

Category 4. Reversed aortic arch flow

Severe aortic obstruction, presenting with antenatal reversed aortic arch flow. Univentricular heart defects. Left-to-right shunt at atrial level (mixed blood reaches the brain through the duct and reversed aortic arch flow).

Defects	N
Hypoplastic left heart syndrome (with reversed flow)	125
Unbalanced AVSD with aortic atresia	8
ccTGA, RV hypoplasia and aortic arch hypoplasia	2
Left Isomerism with AVSD and hypoplastic LV	3
Interruption of the aortic arch, hypoplasia ascending aorta, large VSD	1
Total	**139**

Category 5. Intracardiac mixing with aortic obstruction "any level"

Biventricular heart defects with abnormal connection of the great vessels and a large VSD, or univentricular heart defects, or (un)balanced AVSDs.
Intracardiac mixing occurs at atrial (a) or ventricular (v) level.

Either of above, presenting with antenatal aortic obstruction, with forward aortic arch flow (aortic arch hypoplasia, coarctation, or aortic valve stenosis).
Postnatal intervention necessary to palliate or repair aortic obstruction.

Defects	N
Absent left A-V connection, DORV (a)	14
Double inlet left ventricle	4
Tricuspid atresia	4
Complex TGA with VSD/DORV and/or ventricular hypoplasia	9
Complex ccTGA with VSD/DORV and/or ventricular hypoplasia	1
Unbalanced AVSD	19
Left Isomerism with unbalanced AVSD and hypoplastic aortic arch	6
Total	**56**

Category 6. Intracardiac mixing with unobstructed aortic flow

Biventricular heart defects with abnormal connection of the great vessels and a large VSD, or univentricular heart defects, or (un)balanced AVSDs.
Intracardiac mixing occurs at atrial (a) or ventricular (v) level.

Either of above, presenting with antenatal normal aortic calibre and flow.

Defects	N
Absent left A-V connection, DORV (a)	19
Double inlet left ventricle	5
Tricuspid atresia	29
HRHS: pulmonary atresia or critical stenosis	32
Tetralogy of Fallot / PA with VSD / Fallot-like DORV	106
Complex TGA with DORV and/or ventricular hypoplasia	32
Complex ccTGA with VSD and/or ventricular hypoplasia	11
Truncus arteriosus / AP window	25
(Un)balanced AVSD	114
Left Isomerism with unbalanced AVSD (normally sized aortic valve and arch)	14
Right Isomerism with large (A)VSD and/or (c)TGA (normally sized aortic valve and arch)	7
Ventricular septal defect, large	4
Ebstein's anomaly with large VSD	1
Total	**399**

Category 7. No mixing, normal aortic flow

Biventricular heart defects, presenting with antenatal normal aortic flow

Defects	N
Small VSD	84
Ebstein's anomaly (no hydrops/normal cardiac output)	16
Tricuspid dysplasia	5
Pulmonary stenosis (not critical)	4
Absent pulmonary valve	2
Persistent left superior caval vein without LV inflow obstruction	35
Right aortic arch	9
ccTGA without additional cardiac defects	6
Aneurysm of the interventricular septum or cardiac diverticulum	4
Rhabdomyomata	10
Miscellaneous (scimitar, ASD, PAPVR, dextroposition)	18
Total	**194**

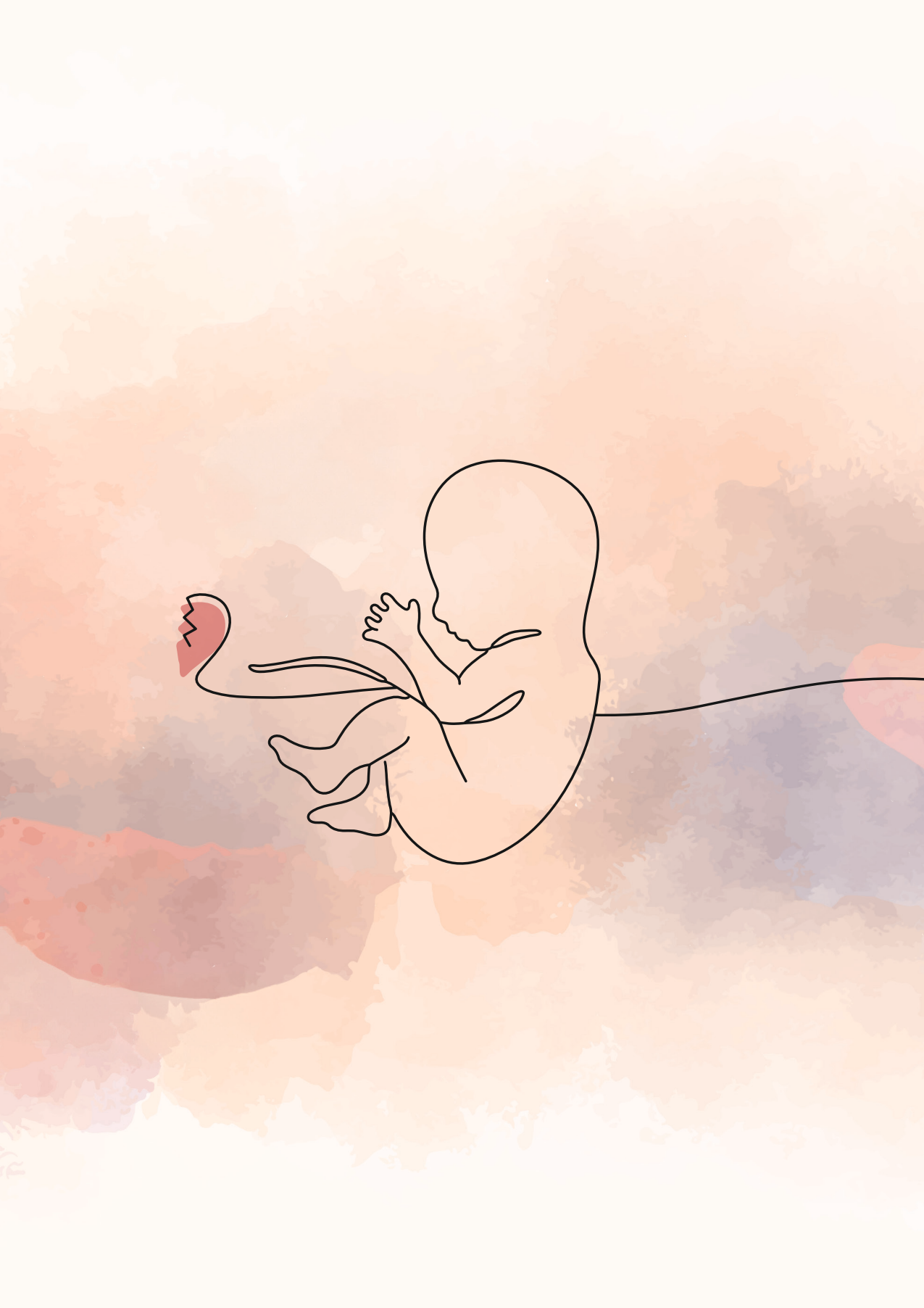
Abbreviations: ASD atrioseptal defect, AV atrioventricular, AVSD atrioventricular septal defect, ccTGA congenitally corrected transposition of the great arteries, DORV double outlet right ventricle,
HLHS hypoplastic left heart syndrome, HRHS hypoplastic right heart syndrome, LV left ventricle, PAPVR partially aberrant pulmonary vein return, RV right ventricle, TGA transposition of the great arteries, VSD ventricular septal defect.

Supplemental information 2. List of primary diagnoses per group, based on their proportion within this cohort and risk for additional pathology

	Isolated		Non-isolated		Total		OR	95% C.I.	p
Congenitally corrected transposition of the great arteries	20	3.7%	0	0.0%	20	2.2%	n.a.		**<0.001***
Double outlet right ventricle with mitral valve and LV hypoplasia	19	3.5%	3	0.8%	22	2.4%	4.6	1.34 ; 15.58	**0.01***
Transposition of the great arteries	74	13.8%	17	4.5%	91	9.9%	3.4	1.96 ; 5.84	**<0.001***
Coarctation of aorta	25	4.6%	8	2.1%	33	3.6%	3.0	1.23 ; 7.44	**0.01***
Persistent left superior caval vein	29	5.4%	10	2.6%	39	4.3%	2.3	1.09 ; 4.99	**0.03***
Tricuspid valve atresia	23	4.3%	8	2.1%	31	3.4%	2.1	0.91 ; 4.67	0.08
Hypoplastic left heart syndrome	88	16.4%	38	10.1%	126	13.8%	1.7	1.15 ; 2.58	**0.01***
Unbalanced atrioventricular septal defect	23	4.3%	21	5.6%	44	4.8%	0.8	0.43 ; 1.51	0.50
Hypoplastic right heart syndrome	14	2.6%	13	3.4%	27	2.9%	0.8	0.34 ; 1.67	0.49
Tetralogy of Fallot	42	7.8%	45	11.9%	87	9.5%	0.6	0.40 ; 0.98	**0.04***
Ventricular septal defect	40	7.4%	48	12.7%	88	9.6%	0.5	0.34 ; 0.82	**0.004***
Left isomerism	9	1.7%	14	3.7%	23	2.5%	0.4	0.19 ; 1.03	0.05
Pulmonary atresia with Ventricular septal defect	8	1.5%	13	3.4%	21	2.3%	0.5	0.19 ; 1.14	0.09
Common arterial trunk	9	1.7%	15	4.0%	24	2.6%	0.4	0.18 ; 0.95	**0.03***
Atrioventricular septal defect	14	2.6%	81	21.4%	95	10.4%	0.1	0.05 ; 0.17	**<0.001***
Ebstein malformation	14	2.6%	4	1.1%	18	2.0%			
Aortic valve stenosis	11	2.0%	2	0.5%	13	1.4%			
Absent left A-V connection	10	1.9%	2	0.5%	12	1.3%			

Supplemental information 2. *(continued)*

	Isolated		Non-isolated		Total		OR	95% C.I.	p
Interrupted aortic arch	4	0.7%	6	1.6%	10	1.1%			
Right aortic arch	9	1.7%	0	0.0%	9	1.0%			
Double inlet left ventricle	9	1.7%	0	0.0%	9	1.0%			
Right isomerism	1	0.2%	6	1.6%	7	0.8%			
Hypoplastic aortic arch	2	0.4%	4	1.1%	6	0.7%			
Dysplastic tricuspid valve	4	0.7%	1	0.3%	5	0.5%			
Shone	4	0.7%	1	0.3%	5	0.5%			
Absent pulmonary valve syndrome	2	0.4%	3	0.8%	5	0.5%			
Pulmonary valve stenosis	3	0.6%	1	0.3%	4	0.4%			
Mitral valve stenosis	1	0.2%	0	0.0%	1	0.1%			
Total abnormal pulmonary venous connection	1	0.2%	0	0.0%	1	0.1%			
Pulmonary vein stenosis	0	0.0%	1	0.3%	1	0.1%			
Aortopulmonary window	1	0.2%	0	0.0%	1	0.1%			
Miscellaneous	*25*	*4.6%*	4	*3.4%*	*38*	*4.4%*			
Total	**538**		**378**		**916**				



CHAPTER 6

Postnatal outcome of fetal isolated ventricular size disproportion in the absence of aortic coarctation

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ABSTRACT

Objectives

Cardiac ventricular size disproportion is a fetal marker for aortic coarctation (CoA), but approximately 50% of fetuses do not have CoA after birth. The aim of this study was to evaluate the postnatal outcome of cases with fetal ventricular size disproportion in the absence of CoA after birth.

Methods

All cases with fetal isolated ventricular size disproportion diagnosed between 2002 and 2015 were extracted from a regional registry with prenatal congenital heart defects. Cases were stratified according to presence (CoA) or absence (non-CoA) of aortic arch anomalies after birth. Postnatal outcome of non-CoA cases was evaluated by assessing the presence of cardiac and other congenital malformations, genetic syndromes and other morbidity after birth. Non-CoA cases were further classified according to whether they had cardiovascular pathology requiring medication or intervention.

Results

Seventy-seven cases with fetal ventricular size disproportion were identified, of which 46 (60%) did not have CoA after birth. Of these, 35 did not require cardiovascular intervention or medication, whereas 11 did. Of the 46 non-CoA cases, six presented with clinical pulmonary hypertension requiring treatment after birth, 24 cases had cardiac defects and four presented syndromic features. Overall, 43% of all non-CoA children were still under surveillance at the end of the study period.

Conclusions

The postnatal course of cases with fetal ventricular size disproportion is complicated by prenatally undetected congenital defects (46%) and pulmonary or transition problems (35%) in a significant number of cases that do not develop CoA. Proper monitoring of these cases is therefore warranted and it is advisable to incorporate the risks for additional morbidity and neonatal complications in prenatal counseling.

INTRODUCTION

Aortic coarctation (CoA) accounts for approximately 4-6% of all congenital heart defects and frequently requires surgery in the first year of life.[1,2] Prenatal diagnosis of CoA is important as timely management with prostaglandins after birth can prevent clinical deterioration of the neonate before surgery, resulting in a lower mortality rate.[3] Ventricular and semilunar valve-size disproportion, with a smaller left side on fetal ultrasound are considered predictors of CoA.[4] However, given the moderate sensitivity and low specificity of these ultrasonographic signs[4-9], the prenatal diagnosis remains challenging. The low specificity can be attributed to the difficulty in differentiating between physiological enlargement of the right ventricle and pathological ventricular size disproportion.[10]

Over the past decade, most studies in this area have focused on the improvement of prenatal detection of CoA.[10-14] Despite a false-positive rate of around 50% for the finding of fetal cardiac disproportion[12-13], hardly any studies have assessed the postnatal course and long-term outcome of fetuses with ventricular size disproportion for whom aortic surgery was not required.

We hypothesize that ventricular size disproportion is accompanied by, or is the result of, an altered fetal circulation that may have an effect on cardiac and pulmonary development. The main objective of this study was to explore the postnatal outcome, with a focus on pulmonary complications in particular, of cases diagnosed prenatally with isolated fetal ventricular size disproportion in the absence of CoA postnatally.

METHODS

The tertiary care centers of the regions Amsterdam and Leiden, including the Leiden University Medical Center and the Amsterdam University Medical Centers, collaborate in the care for children with congenital heart defects. This collaborations is named Center for Congenital Heart Defects Amsterdam Leiden (CAHAL) and covers an area of approximately 40% of all live births in the Netherlands, which is equivalent to 72 000 infants per year. Since 2002, all fetuses and infants diagnosed in CAHAL with a *severe* congenital heart defect (CHD) have been registered. A severe CHD is defined as cases born with a CHD requiring a therapeutic cardiac intervention or cardiac surgery in the first year of life. All pregnant women are seen by both a pediatric cardiologist and a fetal medicine specialist, who collaborate closely in the care of fetuses with CHD in these centers. The prevalence of severe congenital heart defects in this registry is 2,0 per 1000 live births, which corresponds with the generally accepted prevalence of severe CHDs.[1,2] Data collection for the CAHAL regional cohort registry has been described previously.[15]

All cases with fetal ventricular size disproportion, diagnosed between 2002 and 2015, were extracted from this registry. We included all cases diagnosed with isolated ventricular size disproportion in which parents were counseled about the possibility that the neonate might develop CoA after birth and postnatal intensive care admission was initiated to monitor ductal closure. Since only cases with isolated ventricular size disproportion were included, no other antenatally detected cardiac or non-cardiac defects were present. Cases with ventricular disproportion with persistent left superior vena cava (PLSVC) were included if it was decided prenatally to admit the neonate for ductal closure monitoring.[16] Cases born before 36 weeks' gestational age were excluded, as it is difficult to distinguish whether morbidity is due to prematurity or ventricular disproportion in these cases.

Cases were classified into those that developed CoA or hypoplastic aortic arch requiring aortic arch surgery within the first year postpartum (CoA group) and those that did not develop aortic arch anomaly (non-CoA group). For the cases that did not develop CoA postnatally, the aortic valve (AoV) and pulmonary valve (PV) diameters were extracted from the database. If these measurements could not be retrieved from the patient file, original images or videoclips were obtained and the measurements were performed on these. The AoV/PV ratio was calculated to rule out the effect of both gestational age and small- or large-for-gestational age, and the measurements were also converted to *Z*-scores based on gestational age.[17] In cases with serial measurements, the last complete echocardiogram before delivery was used.

According to postnatal outcome, non-CoA cases were subsequently stratified according to whether they required cardiovascular intervention or medication (treatment) or not (non-treatment). The treatment group included cases that required medication or an intervention to treat cardiovascular diseases that were not or could not have been detected prenatally.

Pediatric charts were assessed to retrieve data on postnatal outcome. We assessed baseline characteristics such as age and sex in both the non-CoA and the CoA groups. To evaluate the outcome in the non-CoA group in particular, we assessed the postnatal presence of cardiac and extracardiac (congenital) malformations, syndromes or other chromosomal anomalies, the presence of neonatal pulmonary hypertension, drug administration, number of interventions and number of admissions to the hospital in the first year after birth.

Pulmonary hypertension (PH) was defined as failure of the normal postnatal decline of pulmonary vascular resistance that may be associated with oxygenation failure or right ventricular dysfunction. The severity of PH was based on its duration. PH was defined as need for respiratory support or vasodilators, with or without use of diuretics. The duration of PH was scored as either self-limiting (< 6 weeks) or persistent (> 6 weeks). If PH developed at a later stage as the consequence of a cardiac malformation, rather than as a result of alterations in hemodynamics during transition, it was scored as 0. The following grading system for PH was used: 0 = absence of PH; 1 = self-limiting PH; 2 = persistent PH.

Baseline characteristics in both groups were analyzed using SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). An independent *t*-test was used to test numeric variables for significance. The chi-square test was used to compare categorical variables and calculate odds ratios. A *P*-value of < 0.05 was considered statistically significant. For all other variables, descriptive statistics were used to outline the expected heterogeneity in possible outcomes.

RESULTS

Between 1 January 2002 and 31 December 2015, 100 women were referred to one of the three centers because of a prenatally diagnosed ventricular left-right disproportion. Additional cardiac and non-cardiac abnormalities were present in 11 cases (non-isolated), disproportion normalized during pregnancy in 8 cases and a premature birth (<36 weeks) occurred in 4 cases. Thus, 23 cases were excluded, leaving 77 cases available for analysis (Figure 1).

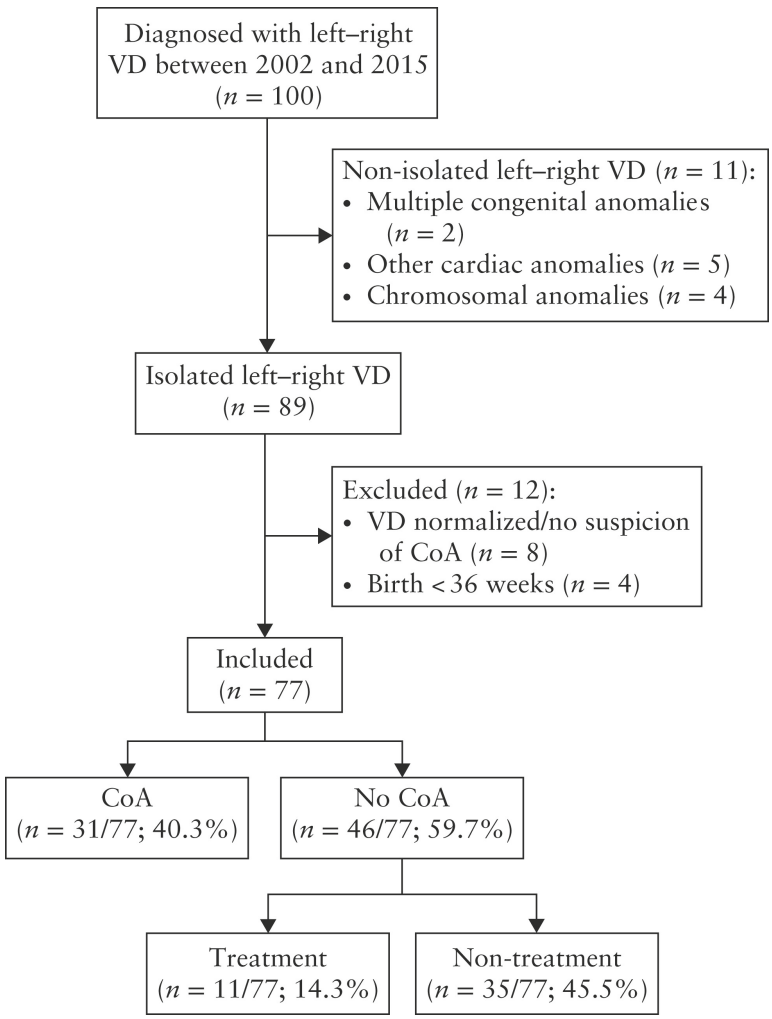

Figure 1: Flowchart showing inclusion in study and postnatal outcome of pregnancies diagnosed prenatally with left–right ventricular size disproportion (VD).

CoA, aortic coarctation.

All 77 cases with isolated prenatal ventricular size disproportion were initially admitted to the neonatal ward for observation upon ductal closure. Forty-six cases (60%) did not develop an aortic arch anomaly that required surgery (non-CoA group), whereas 31 cases (40%) required surgical intervention for aortic arch anomalies (CoA group). The prenatal presence of the findings PLSVC (12/77, 16%) and restricted or closed foramen ovale (9/77, 12%) did not differ significantly between both groups, as shown in Table 1. A significant difference was encountered in the incidence of postnatally diagnosed bicuspid aortic valve between the non-CoA (3/46, 7%) and CoA group (10/31, 32%), resulting in an odds ratio of 6,8 for CoA in the presence of a bicuspid aortic valve.

Table 1. Baseline characteristics of fetus diagnosed with left-right ventricular disproportion (n=77), divided by postnatal diagnosis

	Non-CoA (n=46)	**CoA (n=31)**	***P (95% CI)***	***OR (95% CI)***
Male sex	26 (56,5)	18 (58,0)	*0,89*	
Age at end of study period (years)	4.9 (0.3–12.0)	4.7 (0.3–14.3)	0.82 (–1.95 to 1.54)	
Additional prenatal findings				
Prematurely restricted or closed FO	6 (13,0)	3 (9,7)	0,65	0,74 (0,17 - 3,10)
PLSVC	9 (19,6)	4 (12,9)	0,44	0,61 (0,17 - 2,19)
Additional postnatal finding				
Bicuspid aortic valve	3 (6,5)	10 (32,3)	**0,003***	6,8 (1,7 - 27,5)

Data are given as n (%) or mean (range). * p-value <0,05

Non-CoA: group without aortic arch anomalies postpartum, CoA: group with aortic arch anomalies postpartum,
OR odds ratio, FO: foramen ovale, PLSVC: persistent left superior vena cava.

Non-CoA group

The AoV and PV diameters could be obtained in 45 of the 46 non-CoA cases. In 40 (89%) cases, both AoV and PV were retrieved from the fetal database and in five (11%) cases the AoV or the PV was measured on stored images. In the other case, AoV and PV diameters were not noted and original images were lost due to a change of storage systems. The AoV was smaller than the PV in all cases, with a mean AoV/PV ratio of 0.66 (95% CI, 0.36–0.96). The computed mean Z-scores for AoV and PV were 0.59 and 1.32, respectively.

Amongst non-CoA cases, undetected cardiovascular anomalies were encountered in 19 (41%) cases. These included valve anomalies (9/46), septal defects (4/46), anomalous pulmonary venous return (APVR) (2/46), other vascular anomalies or pathology (2/46) and decreased cardiac function (2/46), requiring medication (angiotensin converting enzyme (ACE) inhibitor/diuretics).

Genetic or syndromic features were seen in four cases, including one case with Down syndrome and three with dysmorphic features, such as syndactyly with undergrowth of digit IV, humeral exostosis and several facial dysmorphic features. Overall, 43% of all non-CoA children were still under surveillance at the end of the study period (December 2015).

Non-treatment group

Of the 46 cases that did not develop aortic arch anomalies postnatally, 35 did not require cardiovascular treatment. Of these, 18 (39%) cases did not have structural cardiac anomalies and 17 had minor cardiac or extracardiac abnormalities but intervention or medication for cardiovascular pathology was not required. In the latter subgroup, abnormal morphology of the semilunar valves was found in six cases, three had isolated PLSVC, one a small ventricular septal defect (VSD) together with PLSVC and one an isolated atrial septal defect type II. Furthermore, genetic syndromes or dysmorphic features were diagnosed in four cases and other anomalies in two (Table 2). All cases with PLSVC were identified prenatally. The mean observation period in this group was 7 days and only five of these cases needed readmission to the hospital. Follow-up visits were ongoing in nine cases, of which five were still under the age of 2 years at the end of the study period, and 26 cases did not have follow-up visits planned after the second year after birth.

Treatment group

The pathology encountered in 11 cases in the non-CoA group that required cardiovascular intervention or medication is outlined in Table 3. Ten neonates received medication, including ACE-inhibitors, diuretics and anticoagulants, because of renal vein and sagittal sinus thrombi. Five cases required cardiac surgery, including surgical correction of a total APVR (TAPVR) or partial APVR (PAPVR), aortic valve stenosis, mitral valve stenosis and multiple VSDs. The mean duration of initial hospital admission in this group was 35 days, varying from 2 to 189 days, and 18 re-admissions were required.

Table 2. Outcome of 'non-CoA' cases that did not require cardiovascular treatment postnatally (n=35).

Diagnosis	Value
No structural abnormality	18
Abnormal morphology of aortic,pulmonary or tricuspid valve*	6
Isolated PLSVC	3
VSD and PLSVC	1
Isolated ASD-II	1
Genetic disorder/dysmorphic features	
Down syndrome†	1
Facial dysmorphic features withthoracic scoliosis	1
Syndactyly with undergrowth ofdigit IV‡	1
Humeral exostosis	1
Other	2
Hospital visits	
Number of admissions	43
Postnatal duration of admission (days)	
Mean	6.5 (2–37)
Total	229
Follow-up visits§	
Yes	10
No	25
Pulmonary support	
Postnatal respiratory support	10
PH	
No PH	33
Clinical PH¶ (self-limiting)	2

Data are given as n or mean (range).
* In four cases, ASD-II occurred together with other anomalies.
† Pulmonary valve stenosis was also present in this case.
‡ VSD was also present in this case.
§ Consultation with medical specialist scheduled, as estimated at time of end of study period (December 2015).
¶ Defined as desaturation requiring respiratory support, vasodilators or other drugs supporting cardiac function.
ASD-II, atrial septal defect type-II; PH, pulmonary hypertension; PLSVC, persistent left superior vena cava; VSD, ventricular septal defect.

Table 3. Outcome of 'non-CoA' cases that did require cardiovascular treatment postnatally (n=11).

Patient	PH score*	Diagnosis	Treatment	Hospital visits			
				Admissions (*n*)	Stay (days)†	Consultation‡	Age at last visit
1	1	TAPVR	Intubation, surgical correction of TAPVR and ASD-II	2	39	Yes	6 mo
2	2	PAPVR, VSD	Surgical correction of PAPVR, ASD and VSDs, sildenafil, diuretics	1	47	Yes	3 yrs
3	0	NCCM	Heparin, enalapril, carvedilol, salbutamol	6	51	Yes	2 yrs
4	0	Aortic valve stenosis, cardiac failure due to post-intervention aortic insufficiency (need for intervention 5 mo after birth)	Balloon valvuloplasty, Ross procedure, elanapril, diuretics	4	65	Yes	1 yr
5	0	Mitral valve stenosis (developed 3 mo after birth)	Mitral valve replacement, pacemaker implantation, diuretics	4	87	Yes	8 mo
6	0	Multiple VSDs, persistent and recurrent vomiting, growth deficiency	Pulmonary artery banding, sildenafil, diuretics, enteral tube feeding	4	76	Yes	4 mo
7	0	Borderline increased LV dimension with high LA pressure (normalized within 2 mo)	Diuretics	1	20	Yes	3 yrs

Table 3. *(Continued)*

Patient	PH score*	Diagnosis	Treatment	Hospital visits			
				Admissions (*n*)	Stay (days)†	Consultation‡	Age at last visit
8	2	Pulmonary hypertension, cor pulmonale, severe AITP and AIN	Diagnostic heart catheterization (NO), respiratory support, bosentan, sildenafil, IVIG, filgrastim	2	190	Yes	10 mo
9	1	Isolated increased pulmonary pressure, hearing loss	Respiratory support, diuretics	2	16	No	6 yrs
10	0	Isolated increased severe pulmonary pressure	Diuretics	1	6	No	5 yrs
11	0	RVT and CVST, hypertension, hearing loss, delayed language development	Postnatal respiratory support, left kidney nephrectomy, tinzaparine, elanapril, special educational needs	5	23	Yes	6 yrs

* Pulmonary hypertension (PH) scored according to grading system: 0 = absence of PH; 1 = self-limiting PH; 2 = persistent PH.
† Total number of days in hospital during all admissions combined.
‡ Consultations with medical specialist scheduled, as estimated at time of end of study period (December 2015).

AIN, autoimmune neutropenia; AITP, autoimmune thrombocytopenia; ASD(-II), atrial septal defect (type II); CVST, cerebral venous sinus thrombosis; IVIG, intravenous immunoglobulin; LA, left atrial; LV, left ventricular; mo, months; NCCM, non-compaction cardiomyopathy; NO, nitric oxide; PAPVR, partial anomalous pulmonary venous return; RVT, renal vein thrombosis; TAPVR, total anomalous pulmonary venous return; VSD, ventricular septal defect; yrs, years.

Pulmonary hypertension

A high rate of need for respiratory support was observed. PH requiring respiratory support or vasodilators was present in 6/46 (13%) non-CoA cases (Table S1). Even in the group without need for cardiovascular intervention or medication, two cases required respiratory support for the treatment of PH because of ductal right–left shunting. Temporary respiratory support on the first day was given in 10 cases within this subgroup (Table 2). Amongst the cases in the treatment group, PH was present in four cases. This was persistent in two, including one case with PAPVR and one with cor pulmonale (Table 3). If the case with TAPVR had been diagnosed prenatally, and therefore had not been included in this cohort, the number of cases with clinical PH would have corresponded to 5/45 (11%) of all non-CoA cases.

DISCUSSION

This study shows that prenatal counseling in ventricular disproportion should include the significant risk of additional pathology, as 46% of cases that did not develop aortic arch anomalies were diagnosed with cardiovascular diseases or dysmorphic features that were undetected prenatally. Previous studies have focused mainly on improving the identification of cases that require aortic arch surgery.[10-14]

We encountered a relatively high incidence (46%) of prenatally undetected congenital malformations amongst cases without CoA, which mainly comprised cardiac defects. The association of left–right ventricular disproportion with both cardiac and extracardiac pathology has been reported before by Hornung *et al*.[18] and Axt-Friedner *et al*.[19], who found a considerable incidence of other CHDs, such as VSD, PLSVC, pulmonary or aortic valve stenosis and chromosomal diseases in these patients. These studies, however, included cases of both isolated and non-isolated ventricular size disproportion, resulting in a considerably higher incidence of severe cardiac and extracardiac pathology and genetic syndromes, and a higher termination and mortality rate compared with our findings. The presented frequency and severity of pathology encountered in this cohort represents the incidence of pathology only amongst cases of prenatally isolated ventricular size disproportion.

Additionally, our data suggest that this prenatal finding can have a considerable impact on both the neonatal period and following years, as evidenced by the number of children requiring antihypertensive drugs, diuretics and long-term follow-up visits. This number could, however, have been slightly lower if TAPVR had been recognized prenatally as the cause of the ventricular disproportion in one case. This case was from the early years of the cohort (2009), but underlines the importance of careful examination of these fetuses, as this diagnosis should not be missed in left–right disproportion. Other cardiac anomalies involved PAPVR, non-compaction cardiomyopathy, mild aortic valve and mitral valve stenosis, VSDs, bicuspid aortic valve and tricuspid valve dysplasia. These are all conditions known to be difficult to diagnose in the prenatal period, and may be progressive with advancing gestation or become apparent after birth, which can lead to long-term consequences. Furthermore, our findings showed that a prematurely closed or restrictive foramen ovale or PLSVC was found more often in cases that did not develop aortic arch anomalies rather than in CoA cases. A bicuspid aortic valve occurred in 32% of cases of the CoA compared with 7% of the non-CoA group, which is in accordance with the known association between bicuspid aortic valve and CoA.[4,11,20]

Pulmonary pathology in general was also encountered frequently. This varied from need for respiratory support in the neonatal period in 30% to clinically important PH requiring vasodilators, diuretics and respiratory support in 13% of the non-CoA cases, of which 4% even required long-term PH treatment. Overall, 35% of all non-CoA cases coped with respiratory problems and only 41% did not need postnatal support of any kind or other clinical intervention during the neonatal observation. It is therefore recommended to alert parents, as early as during prenatal counseling, of potential transition problems and the occurrence of other minor or major defects, as they may experience the transient need for respiratory support as a stressful event. The fact that all neonates were admitted to a neonatal intensive care unit for observation of ductal closure might, however, have resulted in a slightly lower threshold to provide respiratory support in these cases.

Although the exact mechanism behind prenatal left–right ventricular disproportion in the development of CoA is unknown, one of the hypotheses is that CoA results from an imbalance between the flow over the left ventricular outflow tract and the ductus arteriosus, causing decreased flow through the aortic isthmus.[14] We hypothesize that the altered hemodynamics in fetal life, with right ventricular dominance and a cardiac output larger than usual entering the pulmonary circulation, might have an effect on pulmonary development. The increased flow towards the pulmonary vascular system could influence the pulmonary vascular development during fetal life, potentially causing both short-term, in the transition phase from fetus to newborn, and long-term effects. This theory is supported by the rare situation in which constriction of the fetal ductus arteriosus *in utero* can cause idiopathic pulmonary hypertension.[21,22] Uterine constriction of this fetal shunt results in a pulmonary overflow, which causes remodeling of pulmonary vasculature with vascular wall thickening and smooth muscle hypoplasia.[23] Fetal ventricular disproportion also reflects an imbalance in pulmonary and aortic flow, possibly leading to comparable, but less severe, symptoms. This hypothesis might explain the high percentage of cases needing respiratory support (30%), due to either transition problems or PH. The prevalence of PH in this group (13%) is very high, compared with the reported prevalence in unselected cohorts (1.9 per 1000 live births).[24,25]

Finally, 45% of all cases with prenatal ventricular size disproportion did not require medication or intervention for cardiovascular disease after observation. In this series, ventricular size disproportion, known to precede a postnatal diagnosis of CoA[26], did not result in a coarctation after ductal closure in 60% of cases, which is consistent with previous publications, reporting rates from 33% up to 65%. The range can be

explained by the subjectivity of the finding and differences in inclusion and exclusion criteria.[5-7,9,11,26-28]

A limitation of this study is the lack of specific measurements of the cardiac ventricles to calculate ratios or *Z*-scores and define cases suspected for CoA more objectively. It is thereby important in cases with ventricular disproportion to measure cardiac structures and not only assume the left side is small. A large prospective cohort study, including the outcome of non-CoA cases, based on specific measurements, may be beneficial to support these findings with additional details.

In conclusion, despite the absence of aortic arch anomalies in cases with prenatal left–right ventricular disproportion, the number of neonates requiring intensive care postnatally and the frequency of additional cardiac and extracardiac morbidity is considerable. Proper monitoring of these infants is therefore warranted and incorporation of the risk for additional morbidity and neonatal complications into prenatal counseling should be considered.

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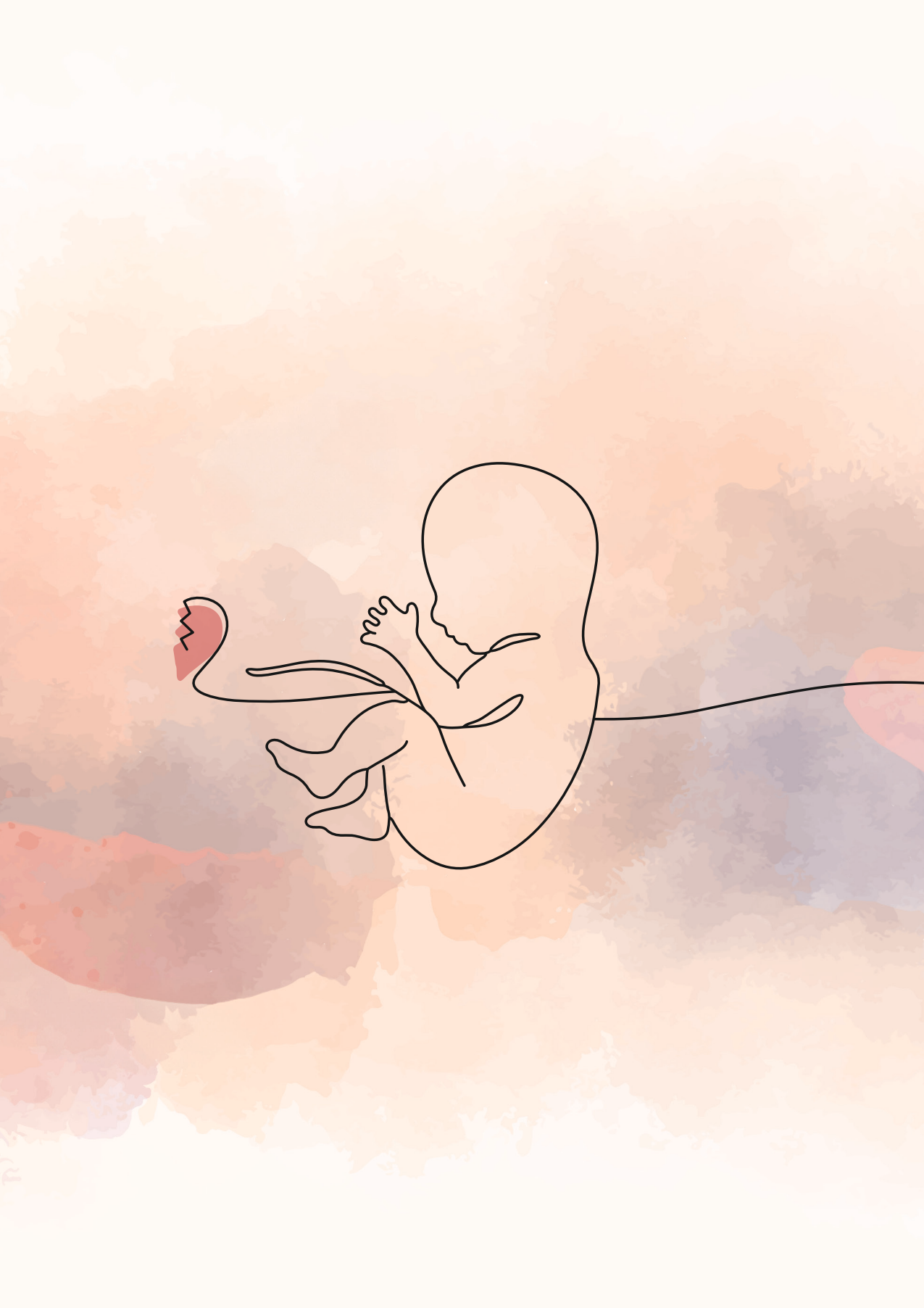
SUPPLEMENTAL INFORMATION

Table S1. Case description and treatment of six patients diagnosed prenatally with left–right ventricular size disproportion, who did develop aortic coarctation but required treatment for pulmonary hypertension (PH) postnatally

PH	Patient	Group	Diagnosis	PH treatment
Persistent	2	Treatment	PAPVR, ASD, VSD Left-to-right shunt (5:1) causing pulmonary flow hypertension	Sildenafil, diuretics and fluid restriction(Surgical correction PAPVR, ASD, VSDs)
	8	Treatment	Right heart failure Dilated RV with moderate systolic function, L-R systolic and R-L diastolic shunt over VSD, increased pressure RV	Respiratory support, bosentan, sildenafil(Diagnostic heart catheterization (NO))
Self-limiting	9	Treatment	Isolated increased pulmonary pressure Ductal R-L shunt and RV>LV	Respiratory support, diuretics
	1	Treatment	TAPVR RV dilatation, increased pressure RV, ductal R-L shunt	Intubation, inotropes, diuretics(Surgical correction TAPVR and ASD-II)
	X	No treatment	Isolated increased pulmonary pressure Dilated RV and RA, ductal systolic R-L shunt and diastolic L-R shunt	Respiratory support
	X	No treatment	Pulmonary valve stenosis with RV hypertrophy(Down syndrome)	Respiratory support

ASD, atrial septal defect; NO, nitric oxide; PAPVR, partial anomalous pulmonary venous return; TAPVR, total anomalous pulmonary venous return; VSD, ventricular septal defect.w



CHAPTER 7

The prognosis of common arterial trunk from a fetal perspective

A prenatal cohort study and systematic literature review

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ABSTRACT

Objective

The limited number of large fetal cohort studies on common arterial trunk (CAT) impedes prenatal counselling at mid-gestation. This study evaluates the prognosis of CAT from a fetal perspective.

Method

Fetuses with a prenatally diagnosed CAT were extracted from the PRECOR registry (2002-2016). We evaluated fetal and postnatal survival and the presence of additional morbidity at last follow-up. Literature databases were searched systematically for additional cases.

Results

Thirty-eight cases with a prenatal diagnosis of CAT were identified in our registry, of which 18/38 (47%) opted for pregnancy termination (TOP). Two cases resulted in spontaneous intra-uterine demise (10%, 2/20), six cases demised postnatally (33%, 6/18), leaving 60% (12/20) alive, after exclusion of TOP, at a mean age of six (range: 2-10 yr.).

Additional morbidity was found in 42% (5/12) of survivors, including 22q11.2 deletion syndrome, Adams-Oliver syndrome and intestinal atresia, whereas 8% (1/12) had developmental delay. The remaining 50% (6/12, and 30% of ongoing pregnancies) of survivors appeared isolated with normal development. All of whom required replacement of the initial right ventricle to pulmonary artery conduit.

Additionally, we reviewed 197 literature cases on short-term outcome.

Conclusion

The risk of fetal and neonatal demise, as well as significant morbidity amongst survivors, should be included in prenatal counselling for CAT.

INTRODUCTION

Common arterial trunk (CAT), also known as truncus arteriosus, is a rare congenital heart defect (CHD) that accounts for approximately 1% of fetuses diagnosed with a CHD.[1] It is characterized by a single arterial trunk, overriding the interventricular septum, which provides blood to the systemic and pulmonary circulation and coronary arteries. To describe the anatomical variations between CAT cases, three classification systems have been reported to date.[2-4]

Prenatal detection rates for conotruncal anomalies, including CAT, have increased substantially over the past years.[5-8] A prenatal diagnosis provides the opportunity for genetic analysis and advanced ultrasound examination, given its association with genetic syndromes and (extra-) cardiac malformations.[9-11] This is essential, as it enables parents to make an informed decision whether to continue the pregnancy and provides the opportunity for delivery in a specialized facility. Despite these clear benefits, evidence stating that a prenatal diagnosis would influence neonatal mortality and morbidity, is scarce.[12-17]

Parental counselling for fetuses with a CAT is, however, primarily based on postnatal cohort studies, due to the lack of large studies on prenatally detected cases. The majority of these postnatal cohorts focus on postoperative results or neonatal outcome, which may only reflect a selected population of CAT cases.[18-20] To provide evidence on the prognosis of CAT from a fetal perspective and improve prenatal counselling at mid-gestation, this study will focus on outcome of *fetuses* with a prenatal diagnosis of CAT. A systematic analysis of the literature is performed to assemble evidence from currently available studies.

METHODS

All fetuses and neonates with a diagnosis of a congenital heart defect (CHD) in the region Amsterdam-Leiden (40.000 births/year) are referred to a tertiary care center. Since 2002 these centers have together collected all CHD cases in our population-based registry 'PRECOR'. Data collection for this registry has explicitly been described before.[21] We used this registry to identify all fetuses with a prenatal diagnosis of CAT from 2002 to 2016. The standard mid-trimester anomaly scan was introduced as part of the Dutch national screening program in 2007. Our cohort has reported one of the highest prenatal detection rates since, including a 85% prenatal detection rate for CAT[21], which has only increased over time. As the majority of prenatally detected cases in this cohort originate from 2007-2016, we expect that our cohort is representative for all fetuses with CAT.

Postnatal echocardiography and post-mortem reports were assessed to ascertain the diagnosis in all cases. If pregnancy was terminated or spontaneous intra-uterine fetal demise occurred without parental consent for autopsy, cases were not excluded to avoid selection bias.

The fetal ultrasound databases were evaluated for data on structural malformations, genetic testing and pregnancy outcome. Patient records were studied to assess postnatal mortality, (age at) surgery, neurodevelopment at post-surgical outpatient consultations and verify the extracardiac malformations (ECMs) detected with prenatal ultrasound.

Patient characteristics and respective outcome parameters will be presented for each case individually. This study has been approved by the Leiden University's medical ethics committee.

Systematic review

Our systematic review of the literature is reported following the PRISMA statement[22] and has been submitted for registration in the PROSPERO database on 11 September 2019. We explored the PubMed, Embase, Web of Science, Academic Search Premier and Cochrane Library databases for articles on outcome of fetal CAT in September 2019. The entire search strategy is enclosed as supplementary material (Supplemental material S1).

Criteria for inclusion in the systematic review were; (1) case series (≥ 3 cases minimum) ór cohort studies (any number of CAT cases) that report on (2) pregnancy or postnatal

outcome of (3) prenatally diagnosed case(s) with CAT. Fetal studies focusing on cohorts with 22q11.2 deletion syndrome (DS) were not considered eligible for inclusion to avoid a potential selection bias. If information on pregnancy outcome was missing from the abstract or full-text, authors were contacted for additional information to enable inclusion of these studies in the review.

Two researchers [AvN, LH] independently screened the literature search results for eligible articles. Discordances were discussed and, if necessary, a third reviewer [MH] was consulted. The same authors [AvN, LH] studied the full-text of selected articles to extract data on pregnancy and postnatal outcome in fetuses with a prenatal diagnosis of CAT. Pregnancy outcome was considered our primary outcome, as most studies focused on perinatal parameters. Secondary parameters included: neonatal surgery, neonatal mortality (<28 days of age), survival at the end of the study period and the presence of a genetic diagnosis or additional malformations. If multiple studies reported on the same cases, the most eligible study was chosen.

The Quality in Prognostic Studies (QUIPS) tool[23] was used to evaluate the quality of selected articles was evaluated [AvN and LH, independently] and identify major risks of bias. This assessment was merely used for interpretation of results and did not determine inclusion in the review.

Descriptive statistics were used to display the results of all included articles separately, with regard to pregnancy outcome, postnatal course and the presence of additional morbidity. To estimate the prognosis of fetal CAT in a large cohort of prenatally diagnosed fetuses, we attempted to summarize the raw data from all included articles and combine these with our own original data, when possible.

RESULTS

We identified 43 fetuses with a prenatal diagnosis of CAT in the PRECOR registry. Consent for autopsy was obtained in 30% (6/20) of demised fetuses, which all confirmed the prenatal diagnosis. Postnatal echocardiography confirmed the diagnosis in 78% (18/23) of liveborn cases, resulting in an 83% (24/29) overall diagnostic accuracy. After exclusion of these five misdiagnosed cases with pulmonary atresia and a ventricular septal defect (PA-VSD), 38 cases were included in this study. The majority of fetuses originated from 2007-2016 (87%, 33/38).

Structural malformations

Fetuses with CAT had additional morbidity in 61% (23/38) of the cases, involving genetic syndromes (39%, 15/38) and/or structural extracardiac malformations (ECMs) (53%, 20/38). Karyotyping or aneuploidy testing was performed in all cases (38/38), whereas some received additional testing for genetic syndromes as well: 39% (15/38) FISH for 22q11.2 DS, 39% (15/38) chromosome microarray analysis and 18% (7/38) exome sequencing, respectively. Although 22q11.2 deletion syndrome (21%, 8/38) was diagnosed particularly often, less common syndromes, such as CHARGE, Adams-Oliver and Cri-du-Chat syndrome, were also found in a significant proportion of fetuses (18%, 7/38). The ECMs diagnosed on prenatal ultrasound were all confirmed postnatally, and none of the fetuses that appeared isolated on prenatal ultrasound showed ECMs after birth.

Additional cardiac anomalies were present prenatally in 37% (14/38) of all fetuses with CAT. These mainly comprised truncal valve regurgitation (moderate to severe) or stenosis (21%, 8/38) and interruption of the aortic arch (IAoA; 8%, 3/38). Other significant CHDs, including polyvalvular disease (3%, 1/38), anomalous pulmonary venous return (3%, 1/38), mitral valve stenosis (3%, 1/38) and unroofed coronary sinus (3%, 1/38), occurred in non-isolated cases only (Table 1).

Isolated CAT cases (39%, 15/38), without a (prenatally suspected) genetic diagnosis or ECMs, presented with significant prenatal truncal valve regurgitation or stenosis in 33% (5/15) or an interrupted aortic arch in 7% of cases (1/15), respectively. However, the majority (60%, 9/15) did not show other significant cardiac anomalies (right aortic arch or aberrant right subclavian artery not considered) (Table 1).

Termination of pregnancy

Parents opted for pregnancy termination (TOP) in 47% (18/38) of cases with a prenatally diagnosed CAT, of which 5% (2/38) comprised selective multifetal pregnancy reductions. The majority of terminated cases had additional morbidity (72%, 13/18) or significant truncal valve regurgitation (11%, 2/18) and only 17% (3/18) appeared isolated. The proportion of TOPs for CAT decreased over time: from 57% in 2002-2009 to 41% in 2010-2016.

Mortality

Intra-uterine fetal demise (IUFD) occurred in 10% (2/20) of continuing pregnancies. The remaining 90% (18/20) resulted in a liveborn neonate at a median gestational age of 39 weeks (Table 1). Four neonates (22%, 4/18 liveborns) died within the first week of life. Two had spontaneous pre-term pre-labor rupture of membranes (PPROM) and were not actively treated after birth. Both of whom had a very poor prognosis and expected quality of life, based on the combination of (extreme) prematurity and significant additional morbidity (case 22 and 24). The remaining two were actively treated, but died either pre- or postoperatively. The first (case 23) comprised a case with CHARGE syndrome and multiple congenital anomalies that was delivered at 34 weeks of gestation due to PPROM. She died the first day despite ventilation and intubation. The second case (case 21) with 22q11.2 deletion syndrome and IAoA underwent surgery at day 7, but died the same day due to severe postoperative complications.

We encountered two infant deaths (11%, 2/18 liveborns) at 5 and 18 months of age. One infant (case 25) was born dysmature at 31 weeks of gestation and had a complex CAT with an atrioventricular septal defect, severe left atrioventricular valve incompetence and mild-to-moderate truncal valve regurgitation. She underwent banding of the pulmonary arteries at three weeks of age (body weight: 1900 gram) and presented with poor right ventricular function at 5 months of age. Although corrective surgery was planned immediately, a cardiac arrest occurred during preoperative preparations and she eventually died of multi-organ failure. The second case (case 26) with CAT type 2, complicated by bilateral pulmonary artery stenosis, received corrective surgery and replacement of the Gore-Tex patch with a pulmonary homograft at 16 months of age. Two months later, the child suddenly deteriorated at home and a cardiac arrest followed shortly after, most likely provoked by a respiratory tract infection causing increased right ventricular pressures.

Table 1. Outcome and associated anomalies in 38 cases with a prenatal diagnosis of CAT

Case	Sex	GA dx	Birth year	CAT conf.	Outcome				Associated anomalies		
					Pregnancy	*GA at birth*	*Age at surgery*	*Devel. delay*	*Cardiac, prenatal*	*Extra-cardiac, prenatal*	*Genetic diagnosis*
1	F	19+0	2003	-	TOP	-	-	-	0	Cleft lip	22q11.2 DS
2	M	20+5	2006	+	TOP	-	-	-	0	MCA[1]	0
3	M	20+3	2006	+	TOP	-	-	-	0	0	22q11.2 DS
4	M	18+3	2006	-	TOP	-	-	-	0	MCA[2]	MODY type 3
5	M	20+4	2007	-	TOP	-	-	-	0	0	0
6	F	19+6	2007	-	TOP	-	-	-	RAA, PLSVC, ARSA	MCA[3]	0
7	F	21+5	2008	+	TOP	-	-	-	0	MCA[4]	0
8	M	20+1	2008	-	TOP	-	-	-	RAA	Cleft lip-palate	0
9	M	19+6	2008	-	TOP	-	-	-	Truncal valve regurg.	0	22q11.2 DS
10	M	19+5	2009	+	TOP	-	-	-	0	0	0
11	F	21+5	2009	-	TOP	-	-	-	0	0	0
12	F	20+6	2010	+	TOP	-	-	-	Truncal valve regurg., fibroelastosis	MCA[5]	Trisomy 9 mosaicism
13	M	19+1	2010	-	TOP	-	-	-	Polyvalvular disease	Cerebellar hypoplasia, Rocker bottom feet	Trisomy 13
14	F	20+0	2014	-	TOP	-	-	-	Truncal valve regurg./stenosis	0	0
15	F	21+0	2015	-	TOP	-	-	-	Truncal valve stenosis	0	22q11.2 DS

Table 1. *(Continued)*

Case	Sex	GA dx	Birth year	CAT conf.	Outcome				Associated anomalies		
					Pregnancy	***GA at birth***	***Age at surgery***	***Devel. delay***	***Cardiac, prenatal***	***Extra-cardiac, prenatal***	***Genetic diagnosis***
16	M	20+2	2016	-	TOP	-	-	-	Truncal valve regurg.	0	0
17	F	19+4	2009	-	MFPR	-	-	-	0	Abnormal aspect kidney + Urethral dilatation	PTHSL1
18	M	18+3	2014	-	MFPR	-	-	-	0	sIUGR (gratacos 3), SUA	0
19	M	20+3	2008	-	IUFD (29+0)		-	-	Truncal valve regurg.	Fetal hydrops	0
20	F	17+5	2009	+	IUFD (29+5)		-	-	Truncal valve regurg., IAoA	Fetal hydrops	0
21	M	20+5	2005	+	NND (day 7)	40+3	7	-	IAoA type B *[RAA]*	0	22q11.2 DS
22	M	21+0	2007	+	NND (day 1)	35+3 (PPROM)	-	-	0	IUGR	Cri-du-Chat syndrome
23	F	19+1	2011	+	NND (day 1)	34+1 (PPROM)	-	-	APVR	MCA[6]	CHARGE syndrome
24	F	17+1	2014	+	NND (day 4)	28+4 (PPROM)	-	-	MS, PLSVC, enlarged CS	IUGR	0
25	F	16+5	2016	+	InfD (5 mo.)	31+5	22	-	RAA *[AVSD]*	MCA[7]	0
26	M	20+5	2015	+	InfD (1.5 yr)	39+6	8	+	0	0	0

Table 1. *(Continued)*

Case	Sex	GA dx	Birth year	CAT conf.	Outcome				Associated anomalies		
					Pregnancy	*GA at birth*	*Age at surgery*	*Devel. delay*	*Cardiac, prenatal*	*Extra-cardiac, prenatal*	*Genetic diagnosis*
27	F	21+0	2007	+	Alive (4 yr.)	39+2	14	+	0	0	22q11.2 DS
28	F	34+1	2008	+	Alive (10 yr.)	38+1	96	0	0 *[RAA]*	MCA[8]	Adams-Oliver syndrome
29	M	20+2	2009	+	Alive (9 yr.)	40+1	16	+	*0*	MCA[9]	22q11.2 DS
30	M	21+1	2009	+	Alive (9 yr.)	39+1	13	0	0	0	0
31	F	22+3	2009	+	Alive (8 yr.)	39+3	18	0	RAA	0	0
32	F	20+4	2011	+	Alive (8 yr.)	37+0	11	0	IAoA type B	0	0
33	F	20+5	2011	+	Alive (7 yr.)	39+6	22	0	RAA	0	0
34	M	22+1	2012	+	Alive (6 yr.)	39+0	36	+	0	Bilateral hydronephrosis	22q11.2 DS
35	M	20+4	2014	+	Alive (4 yr.)	41+3	9	+	RAA	0	0
36	M	26+3	2015	+	Alive (4 yr.)	37+2	13	0	Truncal valve stenosis	0	0
37	M	19+1	2016	+	Alive (2 yr.)	37+2	125	0	Unroofed CS, PLSVC	Intestinal atresia	0
38	M	20+2	2016	+	Alive (3 yr.)	41+0	14	0	0	0	0

Table 1. *(Continued)*
Data presented between '[ ]' include associated anomalies that were not detected before birth. Outcome is assessed at last follow-up visit. Age at surgery reported in days. Devel. delay developmental delay (present at last follow-up visit), TOP termination of pregnancy, MFPR multifetal pregnancy reduction, IUFD intrauterine fetal demise, NND neonatal death (<28 days), InfD Infant death, 22q11.2 DS 22q11.2 deletion syndrome PTHSL1 Pitt-Hopkins-like syndrome-1, MODY Maturity-Onset Diabetes of the Young, RAA right aortic arch, regurg. Regurgitation, PLSVC persistent left superior vena cava, ARSA aberrant right subclavian artery (arteria lusoria), VSDs ventricular septal defects, IAoA interrupted aortic arch, APVR anomalous pulmonary venous return, MS mitral valve stenosis, CS coronary sinus, PPROM preterm pre-labor rupture of membranes, MCA multiple congenital anomalies, IUGR intrauterine growth restriction, sIUGR selective IUGR, + = present, 0 = not present, - = no information, yr. year, mo. Months
Cases with multiple congenital anomalies (MCA):
1. cheilognathopalatoschisis, diaphragmatic hernia, radial aplasia with ulnar shortening right, bilateral flexion contracture of the wrist, bilateral oligodactylia (two fingers and one thumb right hand, absent right foot), rocker-bottom foot left, thoracic kyphosis, hypospadias, possibly a diaphragmatic hernia with short ribs
2. holoprosencephaly, bilateral renal agenesis, single umbilical artery, oligohydramnios
3. multicystic dysplastic unilateral kidney, abdominal cyst, single umbilical artery, (uncertainty on diaphragmatic hernia)
4. abnormal sacral spine, dislocated/abnormal location kidneys, single umbilical artery, (oligohydramnios)
5. spina bifida (L3/L4 to sacrum), hydrochephaly, unilateral renal agenesis, unilateral foot deformity (or deviation), single umbilical artery, signs of fetal decompensation
6. unilateral schisis, unilateral renal agenesis, single umbilical artery
7. hemivertebra, rib malformation, polydactyly, unilateral club foot, single umbilical artery, absent growth at 31+5 due to maternal factors (preeclampsia, HELLP, placental insufficiency with abnormal peripheral Dopplers)
8. bilateral asymmetric dysplasia of feet with unilateral equinovarus deformity, bilateral flexion contracture wrist, IUGR with brain-sparing (increased end-diastolic flow MCA)
9. abnormal head shape, abnormal shape ear

Prenatal counselling

The classification by Collett & Edwards[2] was used to describe the type of CAT in 75% of cases (15/20). The CAT was classified type I in 27% (4/15) and type II in 73% (11/15) of fetuses. Fetuses with CAT type I and II showed a relatively similar survival rate (75%, 3/4 vs 63%, 7/11) and probability to present with additional malformations (75%, 3/4 vs 73%, 8/11).

Fetuses with additional morbidity (non-isolated) showed a 50% (5/10) mortality risk (TOPs not included), including all early neonatal deaths (40%, 4/10) and one infant death (10%, 1/10). All of whom had significant other cardiac anomalies, whereas none of the non-isolated survivors did.

Isolated cases had a 30% (3/10) probability of fetal (20%, 2/10) or postnatal demise (10%, 1/10). Significant truncal valve regurgitation was found in both IUFD fetuses, but in none of the survivors. The presence of an IAoA alone, apart from prenatal truncal valve regurgitation, was not associated with fetal or neonatal mortality. All isolated CAT survivors required replacement of the initial right ventricle to pulmonary artery (RV-PA) conduit (6/7) or RV-PA patch (1/7) and 43% (3/7) up to four surgical re-interventions, due to pulmonary stenosis or insufficiency (cardiac catheterizations not considered).

After exclusion of pregnancy terminations, 60% of fetuses with CAT (12/20) were alive at last follow-up visit (mean: 6 years, range: 2-10). Half of these survivors had a genetic diagnosis, significant ECMs or developmental delay, leaving 50% (6/12) isolated with normal development. This means that only 30% (6/20) of continuing pregnancies and a prenatal diagnosis of CAT were alive without additional morbidity or signs of developmental delay at 6 years of age (Figure 1).

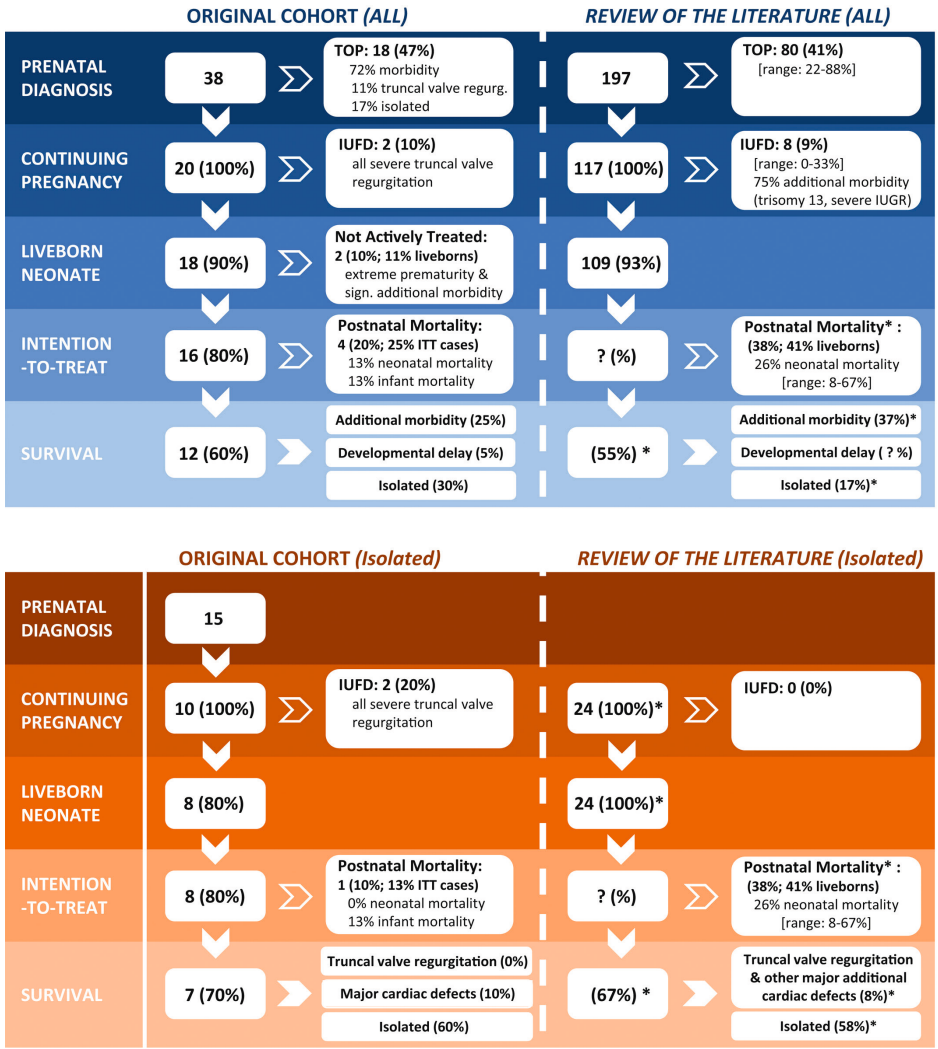

Figure 1. Outcome of (isolated) fetuses after a prenatal diagnosis of CAT

TOP termination of pregnancy, IUFD intrauterine fetal death, ITT Intention-to-treat, IUGR intrauterine growth restriction, Truncal valve regurg. Truncal valve regurgitation (> mild).
* Not all studies report on survival or the presence of additional morbidity

Systematic review

Our literature search identified 546 potentially relevant articles, of which 70 were assessed for eligibility based on title and abstract and 13 eventually met the inclusion criteria (Figure 2).[5-7, 24-33] Five studies focuses on CAT specifically[6, 7, 24, 30, 31], whereas the remaining 8 included other cardiac defects as well[5, 25-29, 32, 33]. Altogether, these studies described 197 fetuses with a prenatal diagnosis of CAT.

Figure 2. Flowchart systematic review of the literature

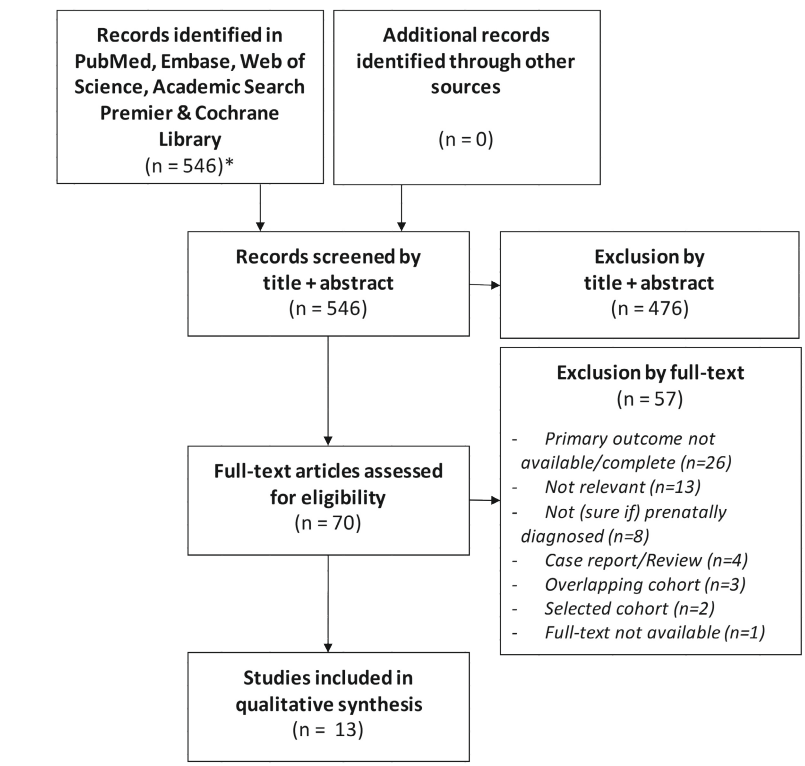

Figure 2. Flowchart systematic review of the literature

Additional morbidity

The available data on outcome and presence of additional morbidity in fetuses with CAT is reported for each study separately, and combined, in Table 2. A genetic syndrome was found in 30% (44/148) of all fetuses with CAT, which varied between 13% and 39% in large cohorts. Structural ECMs, such as holoprosencephaly, cleft lip, renal agenesis and esophageal or duodenal atresia, were present in 36% (61/170) of CAT cases. Associated cardiac anomalies were reported in five studies (39% of cases, 37/95)[7, 28, 30, 32, 33].

Table 2. Review of results on pregnancy outcome, postnatal course and additional mortality derived from included articles

Author, Year	Time	N.	Confirmation of diagnosis	Pregnancy outcome			Neonatal outcome		Survival		Associated anomalies		
				TOP	IUFD	Livebirth	Surgery	NND	All	*TOP excl.*	Genetic diagnosis	N Tested	Structural anomalies
Allan *et al.*,[30] 1984	< 1984	1	Yes (100%)	0% (0/1)	0% (0/1)	100% (1/1)	-	0% (0/1)	0% (0/1) alive; (1 InfD at 4 mo.)	0% (0/1) alive	0% (0/1)	-	0% (0/1) ECMs
Paladini *et al.*,[36] 1996	1990 - 1994	6	Yes (100%)	50% (3/6)	0% (0/6)	50% (3/6)	33% (1/3); (1 awaiting)	67% (2/3) (1 NNDpr, 1 NNDpo)	17% (1/6) alive and awaiting surgery;	33% (1/3)	17% (1/6): Trisomy 18	100% (6/6); karyo	17% (1/6) ECMs 0% (0/6) associated CVAs
Hafner *et al.*,[33] 1998	1992 - 1996	3	Yes	67% (2/3)	0% (0/3)	33% (1/3)	-	-	-	-	33% (1/3): Aneuploidy (47+fragment)	100% (3/3); karyo	33% (1/3) ECMs: spina bifida; *unknown for aneuploidy case*
Tometzki *et al.*,[22] 1999	1985 - 1997	3	Yes (100%)	33% (1/3)	33% (1/3)	33% (1/3)	100% (1/1)	0% (0/1)	33% (1/3) survival > 28 days;	50% (1/2)	67% (2/3): Trisomy 13, CHARGE syndrome	-	33% (1/3) ECMs: bilateral anophthalmos; *unknown for T13/ CHARGE cases*
Duke *et al.*,[21] 2001	1990 - 1999	17	Yes (100%)	24% (4/17)	0% (0/17)	76% (13/17)	62% (8/13)	54% (7/13) (5 NNDpr, 2 NNDpo)	29% (5/17) alive; (1 InfD > 3 mo.)	38% (5/13)	18% (3/17): 22q11.2 DS (3)	71% karyo, 59% 22q11.2 (FISH)	24% (4/17) ECMs: 1 hydrocephaly, 3 MCA
Volpe *et al.*,[8] 2003	1993 - 2002	23	Yes (100%)	35% (8/23)	9% (2/23)	57% (13/23)	62% (8/13) (2 awaiting)	- (3 prD, 2 poD¶)	35% (8/23) alive; (2 awaiting surgery)	53% (8/15)	35% (8/23): 22q11.2 DS (6), Trisomy 13, Trisomy 22	96% karyo, 83% 22q11.2 (FISH)	43% (10/23) ECMs: 4/10 MCA 30% (7/23) associated CVAs

Table 2. *(Continued)*

Author, Year	Time	N.	Confirmation of diagnosis	Pregnancy outcome			Neonatal outcome		Survival		Associated anomalies		
				TOP	IUFD	Livebirth	Surgery	NND	All	*TOP excl.*	Genetic diagnosis	N Tested	Structural anomalies
Galindo *et al.,*[6] 2009	1990 - 2005	13†	Yes (100%)	38% (5/13)	0% (0/13)	62% (8/13)	75% (6/8)	38% (3/8); (2 NNDpr, 1 NNDpo)	23% (3/13) alive; (2 InfDpo)	38% (3/8)	31% (4/13): Trisomy 13 (2), 22q11.2 DS (2)	-	54% (7/13) ECMs
Swanson *et al.,*[7] 2009	1992 - 2007	38	Yes, partly (only livebirths)	45% (17/38)	5% (2/38)	50% (19/38)	89% (17/19)	11% (2/19) (2 NNDpr, 4 poD¶)	34% (13/38) alive to 60 days;	62% (13/21)	-	-	32% (12/38) ECMs
Bourdial *et al.,*[31] 2012	2002 - 2007	16	Yes (not all fetal deaths)	88% (14/16)	0% (0/16)	22% (2/16)	-	-	-	-	25% (4/16): 22q11.2 DS (4)	-	-
Lee *et al.,*[34] 2013	2003 - 2012	12‡	Yes (100%)	33% (4/12)	0% (0/12);	67% (8/12)	88% (7/8)	25% (2/8) (1 NNDpr, 1 NNDpo)	50% (6/12) alive after surgery;	75% (6/8)	17% (2/12): unbalanced translocation, inversion	100% karyo, 75% 22q11.2 (FISH)	17% (2/12) ECMs 67% (8/12) associated CVAs
Traisrisilp *et al.,*[37] 2015	2004 - 2013	8§	Yes (100%)	75% (6/8)	0% (0/8)	25% (2/8)	-	-	-	-	13% (1/8): Trisomy 13	88% karyo	25% (2/8) ECMs 75% (6/8) associated CVAs
Gómez *et al.,*[32] 2016	2006 - 2013	8	Yes (100%)	88% (7/8);	0% (0/8)	12% (1/8);	100% (1/1)	0% (0/1)	13% (1/8) alive at 10 MoL.	100% (1/1)	38% (3/8): Trisomy 13 (2), Triploïdy	100% karyo / FISH for 22q11	50% (4/8) ECMs: 1 holoprosencephaly, 3 MCA 25% (2/8) associated CVAs
Morgan *et al.,*[35] 2019	1990 - 2014	49	Uncertain	22% (11/49)	2% (1/49)	76% (37/49)	*73% (27/37) primary BVR*	-	-	-	-	-	-

Table 2. *(Continued)*

Author, Year	Time	N.	Confirmation of diagnosis	Pregnancy outcome			Neonatal outcome		Survival		Associated anomalies		
				TOP	IUFD	Livebirth	Surgery	NND	All	*TOP excl.*	Genetic diagnosis	N Tested	Structural anomalies
Original data *This study*	2002 - 2016	38	Yes (63%)	47% (18/38)	5% (2/38)	47% (18/38)	83% (15/18)	22% (4/18) (3 NNDpr, 1 NNDpo)	32% (12/38) alive after surgery (2 InfDpo at 5 & 18 mo.)	60% (12/20)	39% (15/38): 22q11.2 DS (8), Aneuploidy (2), other genetic diagnosis (5)	100% karyo / FISH for 22q11	45% (17/38) ECMs 37% (14/38) associated CVAs
All included studies		**235**		**43%** (100/235) *[22-88%]*	**3%** (8/235) *[0-33%]*	**54%** (127/235) *[22-76%]*	**76%** (64/84) *[62-89%]*	**28%** (20/72) *[11-67%]*	**31%** (50/159) *[17-50%]*		**30%** (44/148) *[17-67%]*		**36%** (61/170) ECMs **39%** (37/95) associated CVAs
All included studies (TOP excl.)		**135**			6% (8/135) [0-50%]	94% (127/135) [50-100%]	70% (64/91) [53%-88%]	26% (20/77) [8-67%]		**55%** (50/91) *[33-75%]*			

Data are presented as % (n) or % (n) or % (n) [range]. Proportions reported for individual studies that are based on n=1, are not taken into account in the range.
TOP termination of pregnancy, IUFD intrauterine fetal demise, NND neonatal death (<28 days of life), BVR biventricular repair, pr preoperatively, po postoperatively, prD preoperative death (age at time of death unknown), poD postoperative death (age at time of death unknown), InfD infant death, mo. months of age, CVAs cardiovascular anomalies, ECMs extracardiac malformations, MCA multiple congenital anomalies, Karyo karyotyping, 22q11.2 DS 22q11.2 microdeletion syndrome.

† assessment of (neonatal) outcome/associated defects related to all CAT with definitive postnatal diagnosis (including those with different prenatal dx)
‡ assessment of associated defects related to postnatal confirmed CAT cases (excluding 2 fetal deaths without autopsy: 1 TOP, 1 IUFD)
§ only CAT type II and III was eligible for inclusion in this study
¶ not stated whether there were neonatal deaths amongst the cases that died pre- or postoperatively (Volpe. 2003), only that it happened <30 days after surgery (Swanson. 2009)

Outcome

Forty-three percent of pregnancies (100/235, range 22-88%) was terminated. IUFD occurred in 6% of continuing pregnancies (8/135, range 0-13% in larger cohorts), which means 94% (127/135) resulted in a liveborn neonate.

The probability of neonatal death, reported in 9 of the 14 available cohorts (including ours), appeared 28% (20/72) in liveborn neonates. Surgery was performed in 76% (63/83) of neonates, because 20% (17/83) died pre-operatively and 4% (3/83) were awaiting surgery. The study by Morgan *et al.*[31] only described the proportion of cases that underwent primary biventricular repair, which is the preferable surgical option for the correction of CAT in the majority of cases. As they did not specify the proportion of cases that died pre-operatively, were awaiting surgery or received alternative surgery, these cases were not included in the calculated proportion of cases that underwent surgery in all studies together. After exclusion of pregnancy terminations, 55% (50/91) of CAT fetuses were alive at the time each study was reported, based on the 10 studies that described survival.

Prenatal counselling

In 7 studies mortality was related to the presence of additional morbidity.[6, 8, 21, 22, 32, 34, 35] Genetic syndromes or ECMs were found in 75% of deceased cases (IUFD or neonatal death) versus 31% of surviving cases. Four studies reported on mortality for isolated CAT and its relation to associated cardiac anomalies.[7, 24, 28, 30] These studies together showed a postnatal mortality of 33% (8/24) (all with intention-to-treat). Prenatal truncal valve regurgitation or major additional cardiac defects were present in 63% (5/8) of demised cases compared to 13% (2/16) of survivors (data not presented). If data from our cohort were included as well, this was 64% (7/11) in non-survivors and 9% (2/23) in survivors, respectively.

To conclude, 54% (36/67) of CAT fetuses with complete data survived, of which 37% (25/67) occurred isolated and 17% (11/67) had additional morbidity (mainly genetic syndromes) (Figure 1, Supplemental material S2).

Quality assessment

The QUIPS tool[23] was used to identify major risks of bias for each of the 13 studies (Supplemental material S3). Most studies (10/13) scored low to moderate risk of bias on all six domains. Hafner *et al.*[29] scored high risk of bias on 'outcome measurement', because outcome was not clearly defined, not measured similarly in all patients and incomplete for pregnancy outcome. However, after we had contacted the authors, they supplied us with complementary data. Lee *et al.*[30] and Traisrisilp *et al.*[33] scored

high risk of bias on 'study attrition', because a significant proportion of cases were lost-to-follow-up or the number of cases excluded due to incomplete postnatal follow-up was not stated.

DISCUSSION

Our study shows a considerable risk of mortality in fetuses diagnosed with CAT. Demise mainly occurs during pregnancy or shortly after birth in cases with truncal valve incompetence or complications as a result of a genetic syndrome, in particular when delivered prematurely. Sixty percent of continuing pregnancies with intention-to-treat, calculated from mid-gestation, were alive after surgery and only 30% of cases showed no signs of additional morbidity or developmental delay at the age of six.

This is the first large cohort study that evaluates postnatal outcome, with regard to additional morbidity and neurodevelopment, in fetuses diagnosed with CAT. A systematic analysis of the literature to assemble evidence from currently available studies has to our knowledge never been performed either. First of all, we encountered a 10% IUFD risk in continuing pregnancies, which was slightly higher compared to the literature. This might be due to an underrepresentation of IUFD cases in reported studies, as some studies merely focus on cases with confirmation of the diagnosis on postnatal echocardiography or autopsy[5, 24, 28, 30], which can often not be performed after fetal demise. We expect that our findings approach the true risk of IUFD, as comparable results have been reported by two similar cohort studies.[6, 7]

Although the vast majority of continuing pregnancies appeared to result in a liveborn neonate, there remained a considerable risk of postnatal mortality (30%). Half of these cases did not undergo surgery, which all involved complex CAT cases with (extreme) prematurity. Active treatment after birth was not initiated in the majority of these preoperative deaths, as the prenatally expected prognosis and quality of life was poor. The postnatal mortality rate in all included studies combined appeared slightly higher, but still comparable.[5-7, 24, 30, 32] Unfortunately most of these cohorts merely mention case-specific, rather than general, causes for postnatal mortality and did not focus on potential prognostic factors apart from truncal valve pathology. Large postnatal cohorts that describe the outcome of CAT often solely include cases that underwent surgery.[9, 10, 34-37] This is important for prenatal counselling, because this selection explains why postnatal cohort studies overestimate the overall survival; these studies report 1-year survival rates between 79% and 89%, which is comparable to the 1-year postoperative survival of 87% in our cohort. From a fetal perspective, however, only 60% of reported fetuses with CAT were alive six years after surgery.

The presence of additional morbidity has shown to be an important predictor for mortality, as genetic syndromes or ECMs were found in 75% of non-survivors (IUFD and neonatal deaths) compared to 31% of survivors. Premature birth, which occurred only

in cases with additional morbidity, appeared equally important, as none of those that delivered prematurely survived until corrective surgery could be performed. In term neonates, the risk of postnatal mortality was still slightly higher in those with genetic syndromes or significant ECMs compared to those with isolated CAT and favorable cardiac anatomy. As it is likely that additional morbidity is directly related to preterm birth, and might reflect the more severely affected cases, we believe both aspects should be considered to estimate the prognosis. In isolated cases the presence of prenatal truncal valve regurgitation (greater than mild) was particularly associated with fetal and postnatal mortality. The finding that major additional cardiac anomalies (other than IAoA), beside truncal valve regurgitation, are a risk factor for postnatal mortality in isolated CAT, was not confirmed in our cohort.[7, 24, 30] Thus, despite the fact that most postnatal cohorts solely report on the need for truncal valve repair or additional cardiac defects as risk factors for mortality[9, 35, 36], these data show that genetic syndromes and significant ECMs are also important to consider.

The prognosis of fetal CAT is, however, not only influenced by the considerable risk of postnatal mortality, but significant morbidity among survivors as well. Genetic syndromes associated with neurodevelopmental delay or (postoperative) complications, such as 22q11.2 deletion and Adams-Oliver syndrome, were found in a third of fetuses that survived and have a significantly negative impact on the quality of life of these children. If advanced techniques, such as exome sequencing, are applied to rule out these genetic syndromes, counselling regarding the prognosis can be more specific and more optimistic, especially in isolated cases. This is important, as the proportion of isolated cases at mid-gestation increased over time, due to advances in prenatal detection of CAT. Accurate diagnosis of CAT at mid-gestation has, however, proven to remain a challenge, as a small proportion appeared to have a PA-VSD after birth.[5-7, 24]

An important limitation of the literature review is the fact that prenatally diagnosed cases with CAT originated from a long time-period (1990-2016) and studies mainly focused on short-term perinatal outcome. This complicates objective comparison of outcome data, as prenatal detection rates, surgical techniques and postnatal care management have changed significantly over time. Besides that, previous studies barely report on postnatal outcome beyond the neonatal period nor the presence of significant morbidity or developmental delay amongst survivors. In 4 of the 13 included studies[27, 29, 31, 33], data on postnatal course or survival were not even complete for all cases, which represent 32% of reported fetuses. As the vast majority originated from the large cohort by Morgan *et al.*[31], the authors were contacted and verified that all available data had been reported. Additionally, most studies did not perform genetic testing in all CAT cases[7, 24, 33] or did not report the proportion tested[6, 25-27, 29, 31, 32]. Lastly,

the presence of additional morbidity could not always be directly related to outcome, because it had either been described for all CAT cases together[6, 32] or the article lacked information on the postnatal course entirely[27, 29, 33]. Although this restricted our systematic review almost exclusively to short-term neonatal parameters, such an overview has never been presented before. Furthermore, it stresses the importance of large cohort studies with sufficient data on outcome and prognosis from a fetal perspective to improve prenatal counselling for CAT.

CONCLUSION

The survival rate for prenatally diagnosed CAT is low and depends highly on the presence of additional morbidity and occurrence of premature birth. As genetic syndromes, ECMs and developmental delay are present in half of the cases that do survive, microarray analysis with sequential exome sequencing should be considered in these cases. Large prospective cohort studies, that include extensive genetic testing for all cases, are needed to assess the prognosis with morbidity-free survival more precisely.

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SUPPLEMENTAL MATERIAL

Appendix S1. Search strategy systematic analysis of the literature

Access date: 1-9-2019

Pubmed

((“Prognosis”[Mesh] OR “prognosis”[tw] OR prognos*[tw] OR “Pregnancy Outcome”[Mesh] OR “outcome”[tw] OR “outcomes”[tw] OR “long-term”[tw] OR “longterm”[tw] OR “Follow-Up Studies”[Mesh] OR “Follow-Up”[tw] OR “Followup”[tw] OR “pregnancy outcome”[tw] OR outcome*[tw] OR “Live birth”[Mesh] OR “livebirth”[tw] OR “live birth”[tw] OR “livebirths”[tw] OR “live births”[tw] OR “Abortion, Induced”[Mesh] OR “abortion”[tw] OR “termination of pregnancy”[tw] OR “pregnancy termination”[tw] OR “demise”[tw] OR “Death”[Mesh] OR “Fetal Death”[mesh] OR “fetal death”[tw] OR “fetal deaths”[tw] OR “foetal death”[tw] OR “foetal deaths”[tw] OR “intrauterine death”[tw] OR “intrauterine deaths”[tw] OR “intra-uterine death”[tw] OR “intra-uterine deaths”[tw] OR “death”[tw] OR “deaths”[tw] OR “Mortality”[Mesh] OR “mortality”[Subheading] OR “mortality”[tw] OR “Fatal Outcome”[Mesh] OR “Morbidity”[Mesh] OR “morbidity”[tw] OR “Growth and Development”[Mesh] OR “growth and development”[Subheading] OR “development”[tw] OR “Neurobehavioral manifestations”[Mesh] OR “neurologic disorder”[tw] OR “nervous system disorder”[tw] OR “Nervous System Diseases”[Mesh] OR “nervous system disease”[tw] OR “neurologic disorders”[tw] OR “nervous system disorders”[tw] OR “nervous system diseases”[tw] OR “neurologic disease”[tw] OR “neurologic diseases”[tw] OR “neurodevelopment”[tw] OR neurodevelopment*[tw] OR “disability”[tw] OR “disabilities”[tw] OR “impaired”[tw] OR “impairment”[tw] OR “Quality of Life”[Mesh] OR “quality of life”[tw] OR “life quality”[tw] OR “HRQOL”[tw] OR “Genetic Diseases, Inborn”[Mesh] OR “genetic disease”[tw] OR “genetic diseases”[tw] OR “genetic disorder”[tw] OR “genetic disorders”[tw] OR “hereditary disease”[tw] OR “genetic defect”[tw] OR “gene defect”[tw] OR “hereditary diseases”[tw] OR “genetic defects”[tw] OR “gene defects”[tw] OR “Syndrome”[Mesh] OR “syndrome”[tw] OR “Treatment Outcome”[tw] OR “Therapeutic Index”[tw] OR “Treatment Failure”[tw] OR “Outcome Assessment (Health Care)”[Mesh] OR “birth”[tw] OR “ births”[tw] OR “growth”[tw] OR “lifespan”[tw] OR “Comparative Study”[Publication Type] OR compar*[tw]) **AND** (“Fetus”[Mesh] OR “fetus”[tw] OR “fetuses”[tw] OR “foetus”[tw] OR “foetuses”[tw] OR “fetal”[tw] OR “foetal”[tw] OR “Prenatal Diagnosis”[Mesh] OR “prenatal diagnosis”[tw] OR Prenatal Diagnos*[tw] OR “Intrauterine Diagnosis”[tw] OR “Intra-uterine Diagnosis”[tw] OR Intrauterine Diagnos*[tw] OR Intra-uterine Diagnos*[tw] OR “Antenatal diagnosis”[tw] OR Antenatal Diagnos*[tw] OR “Prenatal Screening”[tw] OR Prenatal Screen*[tw] OR “Antenatal Screening”[tw] OR Antenatal Screen*[tw] OR “Ultrasonography, Prenatal”[Mesh] OR Prenatal Ultraso*[tw] OR Prenatal echogra*[tw] OR Antenatal Ultraso*[tw] OR “prenatal detection”[tw] OR “antenatal detection”[tw] OR antenatal*[tw] OR prenatal*[tw]) **AND** (“Truncus arteriosus”[Mesh] OR “Truncus arteriosus, persistent”[Mesh] OR “truncus arteriosus”[tw] OR “common arterial trunk”[tw] OR “persistent truncus arteriosus”[tw] OR “common truncus arteriosus”[tw] OR “common trunk”[tw] OR “persistent truncus”[tw] OR “truncus communis”[tw] OR truncus commun*[tw] OR “common trunc”[tw] OR “common truncal valve”[tw] OR “common truncus”[tw] OR “common trunks”[tw] OR “conotruncal anomaly”[tw] OR “cono truncal anomaly”[tw] OR “conotruncal anomalies”[tw] OR “cono truncal anomalies”[tw] OR “conotruncal malformation”[tw] OR “cono truncal malformation”[tw] OR “conotruncal malformations”[tw] OR “cono truncal malformations”[tw] OR “fetal congenital heart disease”[tw] OR “fetal congenital heart diseases”[tw] OR “foetal congenital heart disease”[tw] OR “foetal congenital heart diseases”[tw] OR ((“congenital heart disease”[ti] OR “congenital heart diseases”[ti]) AND (“fetus”[ti] OR “foetus”[ti])))) **NOT** (“Animals”[mesh] NOT “Humans”[mesh]) AND (english[la] OR dutch[la] OR “swedish”[la]))

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Appendix S2. Associated anomalies in survivors and non-survivors reported in the studies that described outcome & additional morbidity

	Survivors (TOP excl.)				Deceased, IUFD (TOP excl.)				Deceased, Neonatal death (Liveborns)			
	%	Isolated	Additional morbidity		%	Isolated	Additional morbidity		%	Isolated	Additional morbidity	
Allan *et al.*,1984	100% (1/1)	100% (1/1)	0% (0/1)		-	-	-	-	-	-	-	-
Tometzki *et al.*,1999	50% (1/2)	0% (0/2)	50% (1/2)	1 CHARGE syndrome	50% (1/2)	0% (0/2)	50% (1/2)	1 Trisomy 13	-	-	-	-
Duke *et al.*,2001	38% (5/13)	23% (3/13)	15% (2/13)	1 22q11.2 DS 1 Pierre Robin sequence, hemivertebrae, cleft palate	-	-	-	-	54% (7/13)	31% (4/13)	23% (3/13)	1 22q11.2 DS 1 MCA 1 Hydrocephaly
Volpe *et al.*,2003	53% (8/15)	47% (7/15)	7% (1/15)	1 22q11.2 DS, unilateral renal agenesis	13% (2/15)	7% (1/15)	7% (1/15)	1 Severe IUGR	-	-	-	-
Galindo *et al.*,2009	38% (3/8)	25% (2/8)	13% (1/8)	1 not specified	-	-	-	-	23% (3/8)	25% (2/8)	13% (1/8)	1 *not specified*
Lee *et al.*,2013	75% (6/8)	63% (5/8)	13% (1/8)	1 22q11.2 DS	-	-	-	-	25% (2/8)	13% (1/8)	13% (1/8)	1 congenital diaphragmatic hernia
Gómez *et al.*,2016	100% (1/1)	100% (1/1)	0% (0/1)		-	-	-	-	-	-	-	-
Morgan *et al.*,2013	-	-	-	-	3% (1/38)	0% (0/38)	3% (1/38)	1 Trisomy 13	-	-	-	-

Appendix S2. *(continued)*

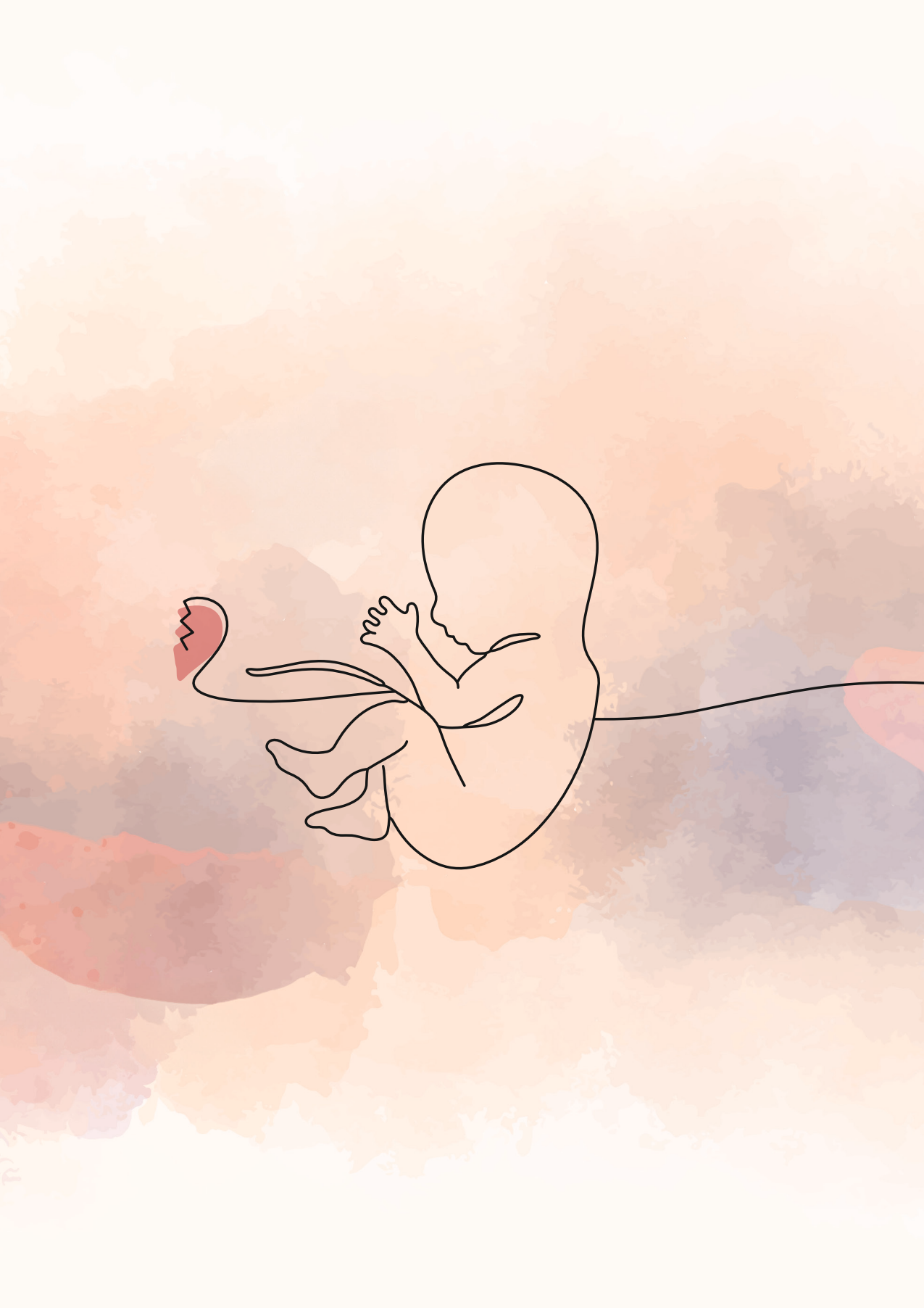
	Survivors (TOP excl.)				Deceased, IUFD (TOP excl.)				Deceased, Neonatal death (Liveborns)			
	%	**Isolated**	**Additional morbidity**		*%*	**Isolated**	**Additional morbidity**		*%*	**Isolated**	**Additional morbidity**	
Original data *This study*	60% (12/20)	35% (7/20)	25% (5/20)	3 22q11.2 DS 1 Adams-Oliver syndrome 1 Intestinal atresia	10% (2/20)	10% (2/20)	0% (0/20)	*2 Severe truncal valve regurgitation*	22% (4/18)	0% (0/18)	22% (4/18)	1 22q11.2 DS 1 CHARGE syndrome 1 Cri-du-Chat syndrome 1 IUGR
Total		**70% (26/37)**	**30% (11/37)**	**73% (8/11) genetic diagnosis 18% (2/11) ECM only 9% (1/11) *not specified***		**50% (3/6)**	**50% (3/6)**	**67% (2/3) genetic diagnosis 33% (1/3) ECM only**		**25% (8/32)**	**75% (14/32)**	**44% (4/9) genetic diagnosis 44% (4/9) ECM only 11% (1/9) *not specified***
Total (*Infant death not reported*)	**54% (37/68)**	**37% (25/68)**	**17% (11/68)**		**8% (6/75)**	**4% (3/75)**	**4% (3/75)**		**17% (16/92)**	**8% (7/92)**	**10% (9/92)**	

TOP termination of pregnancy, IUFD intra-uterine fetal death, 22q11.2 DS 22q11.2 deletion syndrome, MCA multiple congenital anomalies, IUGR intra-uterine growth restriction, ECM extracardiac malformation

Appendix S3. Quality assessment of included studies to assess risk of bias; QUIPs (Quality in Prognosis Studies) tool23

	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounders	Statistical Analysis & Reporting
Allan *et al.*,1984	Moderate	Low	Low	Moderate	Moderate	Low
Paladini *et al.*,1996	Low	Low	Low	Low	Low	Low
Hafner *et al.*,1998	Low	Low	Low	High	Low	Moderate
Tometzki *et al.*,1999	Moderate	Moderate	Low	Moderate	Moderate	Moderate
Duke *et al.*,2001	Low	Low	Low	Low	Low	Low
Volpe *et al.*,2003	Low	Low	Low	Low	Low	Low
Galindo *et al.*,2009	Low	Moderate	Low	Low	Low	Low
Swanson *et al.*,2009	Low	Low	Low	Low	Moderate	Low
Bourdial *et al.*, 2012	Low	Low	Moderate	Moderate	Moderate	High
Lee *et al.*,2013	Low	High	Low	Low	Low	Low
Gómez *et al.*,2016	Low	Low	Low	Low	Low	Low
Morgan *et al.*,2019	Low	Low	Low	Low	Moderate	Moderate
Traisrisilp *et al.*,2015	Low	High	Low	Low	Low	Low

Low: low risk of bias, Moderate: moderate risk of bias, High: high risk of bias.



CHAPTER 8

The aorto-left ventricular tunnel from a fetal perspective

Original case series and literature review

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ABSTRACT

Introduction

Aorto-left ventricular tunnel (ALVT) accounts for <0.1% of congenital heart defects. Evidence on the prognosis from a fetal perspective is limited. With this retrospective international case series, we provide information on the outcome of fetuses with ALVT.

Methods

All members of the Association for European Paediatric and Congenital Cardiology's (AEPC) Fetal Working Group and fetal medicine units worldwide were invited for participation. We observed antenatal parameters, neonatal outcome and postnatal follow-up. Additionally, a systematic search of the literature was performed.

Results

Twenty fetuses with ALVT were identified in 10 participating centers (2001-2019). Fetal echocardiographic characteristics of ALVT included an increased cardiac-thorax ratio (95%), left-ventricular end-diastolic diameter (90%) and a dysplastic aortic valve (90%). Extracardiac malformations were rare (5%). Eight fetuses died at a median gestational age (GA) of 21+6 weeks (range, 19-24): all showed signs of hydrops prior to 24 weeks or at autopsy. All others (60%, 12/2) were live-born (median GA 38+4, range 37-40), underwent surgery and were alive at last follow up (median 3.2 years, range 0.1-17). The literature reported 22 ALVT fetuses with similar outcome.

Conclusions

In the absence of fetal hydrops, ALVT carries a good prognosis. Fetuses who survive to 24 weeks without hydrops are likely to have a good outcome.

INTRODUCTION

Although the exact incidence of an aorto-left ventricular tunnel (ALVT) is unknown, it is estimated between 0.001% and 0.1% of all congenital heart defects.[1, 2] This defect is characterized by an abnormal connection between the ascending aorta and left ventricle that bypasses the aortic valve (AoV). This 'tunnel' provides a site for diastolic run-off from ascending aorta to the left ventricle, similar to significant aortic regurgitation, which may cause left ventricular (LV) dysfunction and congestive heart failure (CHF) as a result of the LV volume overload.[1]

Over the past 20 years, the introduction of an accessible and well-organized prenatal screening programs has considerably improved prenatal detection rates of CHDs in general from 23% to 59.5% [3, 4], with a pooled estimate of 45.1% for all isolated CHDs in unselected populations[5]. This development has likely improved the prenatal detection rate of ALVTs as well. Especially the most severely affected cases will be referred to fetal cardiology units, because of the enlarged left ventricle with an abnormal appearing left ventricular outflow tract. Due to the rarity of this heart defect, correctly diagnosing the cause of these ultrasonographic features remains a challenge, as well as estimating the risk of intrauterine decompensation following a correct diagnosis.

The few case reports on fetal ALVT focus on the characteristics of the diagnosis rather than outcomes beyond surgery.[6, 7] Conversely, postnatal studies focus on the prognosis of ALVT following a diagnosis in the neonatal period or in childhood.[8-11] As the age at diagnosis is related to the severity of heart defects in general, it can be expected that the prognosis of antenatal cases is worse, compared to ALVT cases detected in early infancy.[12] On the other hand, a prenatal diagnosis of ALVT might improve the prognosis, if cases benefit from timely intervention to mitigate deterioration after birth.[13]

Severe paravalvular regurgitation and signs of LV failure are critical conditions in utero and may potentially be important predictors of fetal death or neonatal death shortly after birth in ALVT cases.[12] However, the onset, severity and progression of congestive heart failure can vary significantly between patients.[1] To enable proper prenatal counselling for this condition, studies that describe the natural history and prenatal predictors at presentation in relation to outcome, are essential. With this study we aimed to gather up-to-date information on the outcome of a relatively large cohort of prenatally diagnosed ALVT cases worldwide. In addition, we systematically reviewed the literature for risk factors of intrauterine demise and neonatal outcome in fetuses with ALVT.

METHODS

We invited all members of the Association for European Paediatric and Congenital Cardiology's (AEPC) Fetal Cardiology Working Group and other fetal medicine units worldwide to participate in this retrospective multi-centre case series. Electronic databases or fetal registries were searched for patients with a prenatal diagnosis of ALVT. ALVT was defined as the presence of paravalvular aortic-left ventricular regurgitation that was visible on prenatal ultrasound and confirmation of the diagnosis on postnatal echocardiography amongst those that were liveborn. The Medical Ethics Committee of the Leiden University Medical Center approved this study.

Participating centres reviewed the patient charts and databases. Prenatal characteristics and course of disease after diagnosis were recorded using a pre-defined case record form (CRF). This CRF included the following antenatal parameters: gestational age at diagnosis, LV function (fractional shortening or ejection fraction) and cardiac biometry at diagnosis, Doppler flow velocimetry waveforms (umbilical artery (UA), middle cerebral artery (MCA), umbilical vein (UV) and ductus venosus (DV)), signs of fetal hydrops, cardiovascular profile score (CVPS), the presence of significant additional (extra-)cardiac malformations, the use of transplacental pharmacological treatment and pregnancy outcome. We supplemented an illustration indicating how the cardiothoracic ratio by circumference (CTR)[14, 15], left ventricular end-diastolic diameter (LVEDD)[16], aortic valve annulus[16], ascending aorta[16] and Doppler measurements[17-19] should be measured to minimize measurement error (Appendix 1). The left ventricular myocardial performance index (MPI), with a reported normal range from 0.35±0.03 to 0.40±0.05 with advancing gestation[20-22], was calculated as reported by Tei *et al.*[23]. The formula by Huhta *et al.*[24] was used to calculate the CVPS. The fetal heart was considered enlarged (cardiomegaly), if the CTR was greater than 0.5[15], by measurement or recorded as such at time of examination. The left ventricle, aortic valve annulus or ascending aorta was reported to be enlarged, if the specific measurement did not fall within the normal range (2 standard deviations above the mean for its gestational age).[16] Fetal LV function was defined abnormal, based on the normal range for left ventricular fractional shortening (LV-FS) or ejection fraction (LV-EF) for its respective gestational age.[25] Fetal hydrops was defined as the presence of at least 2 symptoms, including ascites, pleural effusion, pericardial effusion, skin edema or polyhydramnios.

In addition, we recorded interventions to prevent fetal demise (including premature delivery), the occurrence of fetal death, gestational age at birth, birth weight, Apgar scores at 1, 5 and 10 minutes and admission to the neonatal intensive care unit. We furthermore collected data on postnatal status: postnatal cardiac measurements,

age at (corrective) surgery, site of ALVT closure, presence of a residual shunt, surgical complications, postoperative cardiac function and hospital stay, morbidity, mortality and age at last follow-up visit.

The development of fetal hydrops was chosen as our primary outcome, because a considerable number of reported fetuses with ALVT demise before a viable age is reached[2, 26], whereas those that do make it to term generally have a good postoperative prognosis[6, 7, 27-33]. To evaluate potential prognostic factors, we assessed the association between the prenatal characteristics of ALVT at diagnosis and the risk of demise, as well as postnatal outcome for those that survived until birth. Although this is the first study that describes a relatively large series of fetuses with this rare diagnosis, these numbers remain small for extensive statistical analysis. Descriptive statistics, including the median ± range and proportions, were therefore used to report on prenatal characteristics, postnatal outcome and potential associations.

Literature review

We systematically searched the electronic PubMed, Embase, Web of Science, Cochrane Library and Emcare databases from database inception to December 2 2021, using search terms related to "aorto-left ventricular tunnel" and "prenatal diagnosis", to identify reported cases with a prenatal diagnosis of ALVT in the literature (Appendix 2). Research results are reported in agreement with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Screening for relevant articles by title and abstract was performed by one reviewer (A. van Nisselrooij). If relevant, full-text was retrieved and selected articles were cross-referenced (Figure 2). The following data were collected from the included articles: year of publication, first author, time of diagnosis, cardiac features at prenatal diagnosis, presence of fetal hydrops, gestational age at birth, (age at) surgery and follow-up.

RESULTS

Prenatal diagnosis

Fifty-six percent of the approached centres (10/18) were able to contribute cases to this study. We included 20 cases with a prenatal diagnosis of ALVT from 10 fetal cardiology units worldwide over a period of 20 years (2000-2019), that had not been previously reported in the literature. Prenatal diagnoses were made in the second trimester in 75% (15/20) of cases with a median gestational age at diagnosis of 21+2 [range: 14+4 - 38+1]. The diagnosis of ALVT had been confirmed on autopsy (88%, 6/7 fetal demised cases) or postnatal transthoracic echocardiography (all liveborn cases).

Characteristics of the patients with ALVT on fetal echocardiography involved an increased CTR in 95% (19/20), LV dilatation (90%, 18/20) and increased diameter of the ascending aorta (100%, 20/20) (Figure 1). The majority of cases presented with LV dysfunction (70%, 14/20) and a dysplastic aortic valve (90%, 18/20). Abnormal mitral valve (dysplasia/accessory tissue) was encountered in 10% (2/20). Significant extracardiac malformations were found in only one case, in which a 3p14.1 microdeletion was diagnosed (Table 1).

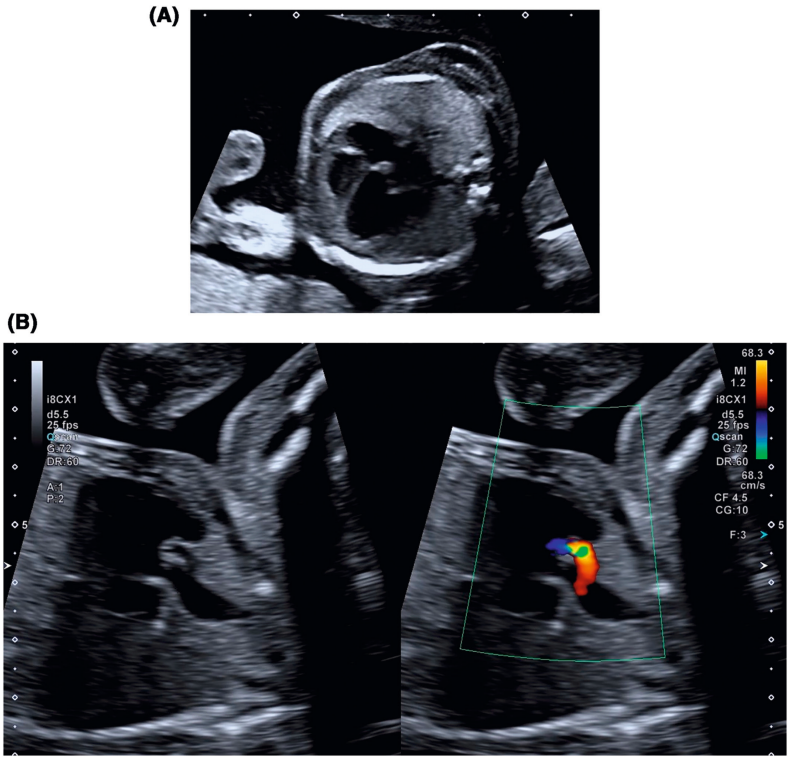

Figure 1.
(A) Four chamber view clearly showing cardiomegaly with left ventricular (LV) dilatation.
(B) LV outflow tract view in diastole. The flow through the tunnel that bypasses the aortic valve is clearly visible

Table 1. Prenatal characteristics at diagnosis

Number of cases		20	
GA at diagnosis	Median (weeks + days)	21 + 2	[14 + 4 - 38 + 1]
Diagnosis in second trimester		16	(80.0%)
Cardiac features at first presentation			
Cardiomegaly (CTR>0.5)[15]		19	(95.0%)
CTR		59%	[43 - 81%]
LVEDD[16]	enlarged	18	(90.0%)
LV aspect	hypertrophic	11	(55.0%)
LV function	dysfunction	14	(70.0%)
	fractional shortening	25.0%	[11 - 40%]

Table 1. *(continued)*

	ejection fraction	48.6%	[28 - 57%]
AoV annulus[16]	enlarged	11	(57.9%)
AoV aspect	dysplastic	18	(90.0%)
AoV function	insufficiency	7	(38.9%)
Asc. Aorta diameter[16]	enlarged	20	(100.0%)
ALVT	size (mm)	3.4	[1.5 - 8.0]
Other structural cardiac malformations		2	(10.5%)
	extracardiac	1	(5.3%)

Data are given as n (%) or median [range]. Data on the size and function of the aortic valve were missing in 1 and 2 cases, respectively.

The fetal heart, left ventricle, aortic valve annulus and ascending aorta were 'enlarged', if: (1) the specific measurement did not fall within the normal range with respect to the gestational age15, 16 or (2) the exact size had not been measured, but the size appeared evidently increased according to the cardiac expert involved in the treatment for that particular case.

Abbreviations: Asc, aorta ascending aorta; ALVT, aorto-left ventricular tunnel; AoV aortic valve; CTR, cardiothoracic ratio; GA, gestational age; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter.

Outcome

Forty percent (8/20) of cases showed signs of fetal hydrops, including skin edema, ascites and pleural or pericardial effusion at diagnosis or autopsy. The pregnancy was terminated on request of the parents before 24 weeks of gestation in 20% (4/20) of cases and spontaneous fetal death occurred in 3 cases (15%; 3/20), all of whom showed hydrops at time of diagnosis (<24 weeks GA). The remaining 65% (13/20) of cases resulted in a liveborn term neonate (median birth weight: 3420 gram; 92% ≥ 50th centile). The Apgar score at 5 minutes was ≥7 in all but one newborn (case 8), who eventually died (5%, 1/20). This case was diagnosed at 31 weeks of gestation, showing severe LV dysfunction and fetal hydrops, and quickly deteriorated after birth, requiring mechanical ventilation and inotropic support. Postnatal echocardiography, as well as MRI, were performed, displaying signs of a non-compaction cardiomyopathy with severe systolic dysfunction and mitral valve dysplasia. As extracorporeal membrane oxygenation (ECMO) was not yet available at the time in this facility (2010), it was decided not to intervene and the neonate died the 8th day of life due to progression of CHF.

All other liveborn neonates (60%, 12/20) underwent corrective surgery at a median age of 16 days [range: 1-44 days] and were discharged home after 22 days [median, range: 9-45 days]. In the majority of cases the tunnel was closed at the aortic site (75%, 9/12), whereas in 25% (3/12) of cases both sites were closed. The origin of the

left (LCA) and right coronary artery (RCA) were normal in 83% (10/12). In two cases (case 10 and 15) the RCA had to be separated from the tunnel. A residual paravalvar leak was described in three neonates (25%, 3/12) and surgery-related complications were encountered in one neonate (8%, 1/12). The latter involved a case that presented with clinical seizures requiring antiepileptic treatment the day after surgery, associated with hypoxic-ischemic brain injury, which was confirmed on MRI. Although the epileptic activity appeared transient, mild unilateral spastic cerebral palsy (Gross Motor Function Classification System (GMFCS), level 1) and delayed language development was reported on neurological follow-up (case 14). Altogether, 60% (12/20) of fetuses with a prenatal diagnosis of ALVT were alive at time of our analysis. The majority of patients are free of morbidity (58%; 7/12) at a median follow-up time of 3.2 years (range, 16 days - 17 years). In the remainding, aortic stenosis was present in three (25%, 3/12), CHF with reduced left and right ventricular function in one (8%, 1/12) and neurological symptoms in one case (8%, 1/12) (Table 2).

Table 2. Prenatal features in relation to outcome

Case description			Characteristics at presentation				Peripheral Dopplers					Outcome			
Nr	GA dx	ALVT (mm)	LVH	AoV size	LV function	Fetal hydrops	UV flow	DV a-wave†	UA EDF†	MCA EDF†	IUT	Pregnancy outcome		Surg-ery	Mort-ality
1	21+3	2.6	+	enlarged	dysfunction	+	–	–	–	–	no	TOP	21+6		+
2	18+2	3.0	+	enlarged	dysfunction	+	–	–	–	–	no	TOP	20+1		+
3	18+4	2.5	+	enlarged	normal	+	–	–	–	–	no	TOP	19+5		+
4	21+1	1.9	+	enlarged	dysfunction	+	normal	normal	normal	normal	no	TOP	21+5		+
5	23+0	3.3	-	normal	dysfunction	+	pulsatile	abnormal	abnormal	normal	no	FD	N.A.		+
6	20+3	4.5	-	normal	dysfunction	+	–	abnormal	abnormal	–	–	FD	24+0		+
7	20+0	8.0	-	enlarged	dysfunction	+ ‡	pulsatile	abnormal	abnormal	abnormal	yes	FD	23+0		+
8	31+3	6.0	+	enlarged	dysfunction	+	–	–	–	–	no	Livebirth	37+6	–	+
9	19+0	1.5	-	normal	normal	–	normal	–	normal	–	no	Livebirth	38+6	+	
10	29+1	–	+	–	dysfunction	–	–	–	–	–	–	Livebirth	38+2	+	
11	38+1	5.0	+	enlarged	dysfunction	–	–	–	–	–	no	Livebirth	38+0	+	
12	14+4	5.5	+	enlarged	normal	–	normal	normal	normal	normal	no	Livebirth	39+4	+	
13	23+0	2.8	-	normal	dysfunction	–	normal	normal	normal	normal	no	Livebirth	39+6	+	
14	20+4	3.5	-	enlarged	normal	–	normal	normal	normal	abnormal	–	Livebirth	37+0	+	
15	20+5	3.0	+	normal	normal	–	normal	normal	abnormal	abnormal	yes	Livebirth	38+0	+	
16	20+6	1.5	-	normal	normal	–	normal	normal	normal	normal	no	Livebirth	38+0	+	
17	22+3	4.0	+	enlarged	dysfunction	–	normal	normal	normal	abnormal	yes	Livebirth	40+0	+	
18	32+3	–	-	normal	dysfunction	–	–	–	–	–	–	Livebirth	38+2	+	
19	23+0	4.0	-	normal	dysfunction	–	pulsatile	normal	–	–	no	Livebirth	39+4	+	

Table 2. *(Continued)*

Case description			Characteristics at presentation				Peripheral Dopplers					Outcome			
Nr	**GA dx**	**ALVT (mm)**	**LVH**	**AoV size**	**LV function**	**Fetal hydrops**	**UV flow**	**DV a-wave†**	**UA EDF†**	**MCA EDF†**	**IUT**	**Pregnancy outcome**		**Surg-ery**	**Mort-ality**
20	30+4	4.2	+	enlarged	dysfunction	–	normal	normal	abnormal	abnormal	yes	Livebirth	40+0	+	
Risk of mortality															40%
	If normal		33.3%	25.0%	16.7%	7.7%	11.1%	11.1%	14.3%	40.0%	50.0%				
	If abnormal		45.5%	54.5%	50.0%	100.0%	66.7%	100.0%	60.0%	20.0%	25.0%				
	OR (95% CI)		1.7 (0.3 –10.3)	3.6 (0.5 – 26.4)	5.0 (0.5 – 54.5)	n.a.	16 (0.7 – 383.0)	n.a.	9.0 (0.6 – 143.9)	0.4 (0.02 – 6.3)	0.3 (0.03 – 4.2)				

Abbreviation: ALVT, aorto-left ventricular tunnel; AoV, aortic valve; CHF, congestive heart failure; CI, confidence interval; DV, ductus venosus; EDF, end-diastolic flow; FD, fetal death (spontaneous); GA, dx gestational age at diagnosis; IUT, intra-uterine (pharmacological) treatment; LV, left ventricular; LVH, left ventricular hypertrophy; MCA, middle cerebral artery; OR odds ratio, TOP, termination of pregnancy; UA, umbilical artery; UV, umbilical vein.
† abnormal, if end-diastolic flow was absent or reversed, ‡ fetal hydrops was present at autopsy.

Prognostic factors

If the ALVT had been diagnosed in the second trimester (75%, 15/20) and signs of hydrops did not occur before 24 weeks of gestation (8/15), the fetal condition remained stable during the course of pregnancy and all survived to surgery. However, all fetuses with signs of fetal hydrops before 24 weeks of gestation (7/20) resulted in elective or spontaneous termination of pregnancy. The presence of fetal hydrops appeared equally important in cases with a prenatal diagnosis in the third trimester, as all without signs of fetal hydrops survived (4/4), whereas the one presenting with hydrops at first evaluation died in the neonatal period (1/1).

Although LV-FS or -EF could only be obtained in 60% (12/20) of cases, the degree of LV dysfunction at presentation seemed related to the presence of fetal hydrops. We found a LV-FS ≤ 25% [range: 11 – 25%] or -EF ≤ 51% [range: 28 - 51%] at presentation in 87.5% of cases (7/8) that developed fetal hydrops during the course of pregnancy. Decreased LV-FS or EF, on the other hand, was never found amongst survivors (FS range: 27 - 40%; EF range: 40 – 57%). Other factors associated with an increased mortality risk included an enlarged aortic valve diameter (54.5% vs 25%, in those with normal aortic valve diameter) or left ventricular hypertrophy (LVH) at presentation (45.5% vs 33.3%, in absence of LVH).

Furthermore, evaluation of abnormal flow patterns on pulsed wave Doppler velocimetry can potentially aid to the differentiation between ALVT fetuses with a high mortality risk and those with a better chance of survival, based on the presence of absent or reversed end-diastolic flow in the UA (mortality: 60.0% vs 14.3%, if UA flow was normal), pulsatile flow in the UV (mortality: 66.7% vs 11.1%, if UV was not pulsatile) or a reverse a-wave in the ductus venosus (DV) (mortality: 100.0% vs 11.1%, if DV flow was normal).

Although the proportion of fetuses with aortic valve insufficiency tended to increase with advancing gestation (39% at presentation vs 82% shortly after birth), this was not associated with mortality. A correlation between mortality and abnormal flow in the middle cerebral artery (MCA) did not seem evident either. Unfortunately, we were unable to assess the potential predictive value of the MPI or CVPS, as these could not be calculated in 65% (13/20) and 60% (12/20) of the cases, respectively. Only a few fetuses received transplacental pharmacological treatment with digoxin alone or a combination of digoxin and metoprolol following a prenatal diagnosis of ALVT (20%, 4/20). Although there was no progression of CHF in treated fetuses, this impedes us to reliably assess this potential effect on the risk of mortality as well (Table 3).

Table 3. Postoperative outcome & follow-up of all surviving ALVT cases

	Postnatal course					General well-being			
Case	**Age at surgery†**	**LV dysfunction**	**Residual shunt**	**Complications**	**Discharge home (days)**	**Follow-up**		**Reinterventions**	**Morbidity**
9	42	no	–	NEC, residual AoS	16	Alive (17 yr.)	2	Surgical valve repair, AoV replacement (14 + 16.5 yr)	Alive and healthy
10	44	no	no	–	N.A.	Alive (10 yr.)	1	Commisurotomy AoV + PV (7 mo)	AoS (mild-abnormal)
11	10	no	–	–	45	Alive (13 yr.)	1	Bentall procedure (13 yr)	AoS (abnormal), AoI, AoAsc aneurysm
12	1	no	no	unknown	10	Alive (9 yr.)	0		AoS (mild)
13	1	yes	–	–	22	Alive (10 yr.)	0		CHF (LV+RV dysfunction)
14	5	yes	–	hypoxic-ischemic brain injury *po.*	21	Alive (4 yr.)	0		Spastic cerabral palsy (GMFCS I), AoI, residual aneurysm ALVT‡
15	25	yes	yes	–	34	Alive (2 yr.)	0		Alive and healthy
16	1	yes	no	SVT°	19	Alive (16 mo.)	0		Alive and healthy
17	29	no	yes	–	22	Alive (1.5 mo.)	0		Alive and healthy
18	4	–	yes	–	16	Alive (16 days)	0		Alive and healthy
19	25	yes	–	–	9	Alive (3.5 wks)	0		Alive and healthy
20	21	no	no	unknown	30	Alive (1.5 mo.)	0		Alive and healthy

Data are given as median or proportion of cases with complete information.
Abbreviations: ALVT, aorto-left ventricular tunnel; AoAsc, ascending aorta; AoS, aortic stenosis; AoV, aortic valve; CHF, congestive heart failure; GMFCS, I Gross Motor Function Classification System grade I; LV, left ventricular; mo, months; NEC, necrotizing enterocolitis; po, postoperative; PV, pulmonary valve; RV, right ventricular; SVT, supraventricular tachycardia; wks, weeks; yr, years.
† Age at surgery in days. ‡ Supraventricular tachycardia requiring medication

Literature review

The literature search yielded 42 articles. After screening of the title and abstract and reviewing of the full-text, 17 articles were found eligible for inclusion. These articles together reported on 22 fetuses prenatally diagnosed with an ALVT (1996-2021).[2, 6, 7, 12, 26-38] Patient characteristics and outcome of the reported fetuses are summarized in Table 4. Overall, 65% (15/23) were alive and thriving at the end of the follow-up period. Fetal hydrops was reported in 6/15 (33%) fetuses with a prenatal diagnosis in the second trimester, of which four resulted in fetal or postnatal demise (68%). On the other hand, fetal hydrops was never reported in those diagnosed in the third trimester, and only one case diagnosed in the third trimester demised (14%).

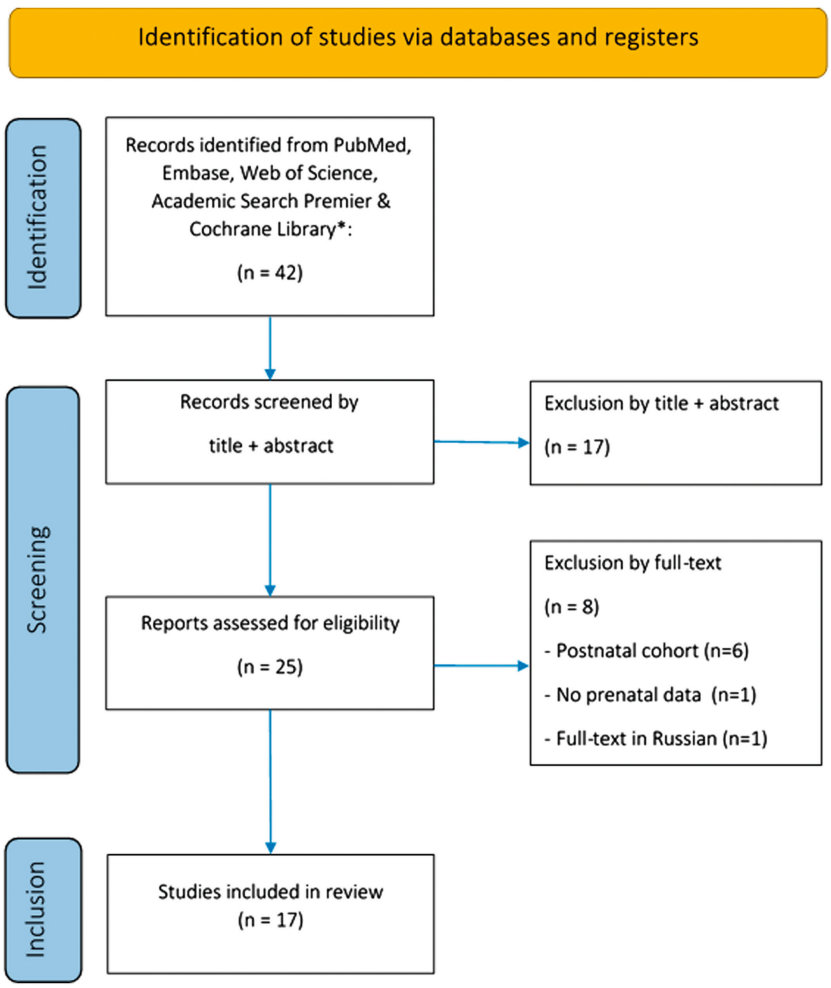

Figure 2. Identification of eligible studies in the literature, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

Table 4. Summary of fetuses diagnosed with ALVT reported in the literature (n=2238)

Prenatal diagnosis in second trimester								
Case	**Year**	**Author**	**Time of diagnosis**	**Cardiac features at prenatal diagnosis**	**Fetal hydrops**	**GA at birth**	**Surgery (age, days)**	**Follow-up**
1	1995	Cook[2]	22 GA	LV dilatation and hypertrophy, turbulent flow through ALVT	*unknown*	-		Termination of pregnancy
2	1996	Sousa-Uva [26]	22-24 GA	severe LV dysfunction and dilatation, dysplastic aortic cusps, paravalvular aortoventricular reflux	yes	-		Termination of pregnancy
3	1996	Sousa-Uva [26]	22-24 GA	severe LV dysfunction, myocardial hypertrophy, dysplastic regurgitant aortic cusps	yes	-		Spontaneous fetal demise (27 wks of gestation)
4	2013	Terry[12]	21 GA	Severe LV dilation/hypertrophy, poor contractility, reverse LV outflow adjacent to aortic valve. Endocardial fibrosis	yes	-	-	Spontaneous fetal demise (30 wks of gestation)
5	2007	Pascoli[34]	26 GA	LV dilatation and hypertrophy, enlarged aortic root, displastic AoV and aortic regurgitation	no	39	Yes (-)	Neonatal death (day 10), due to persistent ischemic failure of extremities
6	2011	Singh[6]	20 GA	-	yes	37	Yes (2)	Postnatal demise (5 wks), due to multisystem failure
7	2005	Biffanti[33]	22 GA	LV dilatation and dysfunction, ALVT	yes	35	Yes (2)	Alive and thriving at discharge, FU 9 weeks postoperatively

Table 4. *(Continued)*

Prenatal diagnosis in second trimester								
Case	**Year**	**Author**	**Time of diagnosis**	**Cardiac features at prenatal diagnosis**	**Fetal hydrops**	**GA at birth**	**Surgery (age, days)**	**Follow-up**
8	2007	Kenny[7]	26 GA	significant eccentric jet of aortic regurgitation wit LV dilatation, large ALVT	*unknown*	40	Yes (11)	Alive, FU on postoperative course unknown
9	2008	Henaine[29]	22 GA	enlarged LV, abnormal systolo-diastolic flow in the ascending aorta	*unknown*	term	Yes (6)	Alive and thriving, FU 2 years
10	2011	Singh[6]	22 GA	-	no	38	Yes (1)	Alive and thriving, FU unknown
11	2011	Singh[6]	20 GA	-	no	40	Yes (1)	Alive and thriving, FU unknown
12	2011	Singh[6]	23 GA	-	no	40	Yes (1)	Alive and thriving, FU unknown
13	2014	Jone[31]	25 GA	LV dilatation and dysfunction, dysplastic aortic cups, ascending aorta dilatation, ALVT	no	36	Yes (3)	Alive and thriving at 9 months FU
14	2016	Smith[35]	23 GA	left and right ventricular dilatation and dysfunction	no	term	Yes (3)	Alive and asymptomatic at 5 months FU
15	2020	Truong[37]	24 GA	LV dilatation, dysfunction, endocardial fibrosis and aortic regurgitation through a tunnel near the IVS	yes	term	yes (3)	Alive with normal LV function at 2 years FU

Table 4. *(Continued)*

Prenatal diagnosis beyond second trimester (or unknown)								
Case	**Year**	**Author**	**Time of diagnosis**	**Cardiac features at prenatal diagnosis**	**Fetal hydrops**	**GA at birth**	**Surgery (age)**	**Follow-up**
16	2017	Kosutic[36]	30 GA	LV and ascending aorta dilatation, separate ALVT	no	38	Yes (9 days)	Postnatal demise (8wks), due to peri-/postoperative complications
17	2000	Grab[28]	31 GA	LV and aortic root dilatation, large ALVT around the annulus	no	40	Yes (3 months)	Alive and thriving at 8 months FU
18	2005	Kolcz[30]	35 GA	enlargement/severe hypertrophy LV, aortic root dilatation, ALVT with paravalvular regurgitation	*unknown*	40	Yes (1 day)	Alive and thriving at 2 years FU
19	2015	Christmann[27]	35 GA	LV dilatation/hypertrophy, paravalvular regurgitation around the annulus	*unknown*	-	Yes (18 days)	Alive and asymptomatic at 5 year FU
20	2016	Nakamura[32]	31 GA	LV dilatation/dysfunction, dysplastic aortic valve, ALVT	no	37	Yes (1 hour)	Alive and thriving at 2 months FU
21	2021	Ito[38]	30 GA	LV dilatation, severe regurgitation through ALVT	*unknown*	36	yes (1)	Alive, trivial aortic regurgitation at 1 month after surgery
22	2016	Smith[35]	unknown	-	*unknown (CHF reported)*		Yes (1 day)	Alive and thriving, mild cardiomyopathie at 12 years FU

Abbreviations: FU, follow-up; IVS, intraventricular septum; LVOT, left ventricular outflow tract.

DISCUSSION

This is the first study that reports on the fetal course of ALVT in relation to outcome in a contemporary cohort of prenatally diagnosed cases worldwide. Despite the significant risk of fetal or neonatal demise (40% of cases in our study), we found that in the absence of symptoms of fetal hydrops, all patients survived with good clinical outcome at a median age of 3.2 years (range, 1 month to 18 years).

This series shows that the key features leading to the fetal diagnosis of ALVT are cardiomegaly, LV dilatation, dilatation of the ascending aorta and paravalvular aorto-ventricular regurgitation. The retrograde flow that passes to the left ventricle beside the aortic valve, is the cornerstone of the diagnosis. With modern ultrasound systems the tunnel itself may even be visible (Figure 2). Majority of cases show an abnormal LV function at presentation, defined as an impaired LV-FS or -EF.[25] Impaired LV contractility may eventually lead to low cardiac output, elevated venous pressure, fetal hydrops and fetal or neonatal demise. ALVT usually presents as an isolated defect, though aortic valve abnormalities including bicuspid aortic valve may also be present.

Not surprisingly, fetal hydrops appears to be associated the most with adverse outcome. Prognosis of fetuses with symptoms of hydrops (including ascites, pleural effusion or skin edema) was very poor, as all cases died before a viable age was reached or surgery could be performed. However, if fetuses did not develop hydrops, all successfully received corrective surgery and the majority are free of morbidity at 3.2 years of age. Presence or absence of hydrops was described in 15/22 ALVT cases reported in the literature.[6, 12, 26, 28, 32, 33, 36, 37] Six cases had signs of hydrops with similar outcome compared to this series, as the majority resulted in fetal or postnatal demise (68%).[6, 26, 33] Similar to our experience, the literature review showed that in the absence of hydrops, fetal or neonatal mortality occurred in only 14% of cases.[6, 28, 31, 32, 34-36] Our finding that stable non-hydropic fetuses with ALVT, despite the impressive cardiomegaly and impaired contractility, generally survive to term with good clinical outcome, is essential for prenatal counselling; it is important that the parents are aware that the prognosis in those who survive without hydrops to near term is better than prognosis if there is evolving hydrops in mid-gestation before they make decisions regarding the pregnancy.[8]

We encountered a strong relationship between abnormal peripheral Doppler measurements and perinatal death in fetuses with ALVT. In this study 100% of cases with a reverse a-wave in the DV died, compared to 13% if the a-wave remained positive. The risk of mortality was also considerably higher among fetuses with pulsatile flow

in the UV and absent or reversed end-diastolic flow in the UA. As abnormal venous Dopplers are a result of congestive heart failure[24], these parameters reflect on the condition of the fetus with development of fetal hydrops as an end-stage and do not influence the prognosis independently. This is in line with a previous study by Gudmundsson *et al.* stating that the presence of umbilical venous pulsations is the most useful predictor of perinatal death in cases with fetal hydrops.[39]However, it should be stressed that abnormal flow in the umbilical artery may be incorrectly interpreted as a sign of placental disfunction, rather than a result of the presence and size an ALVT. Finally, an enlarged aortic valve diameter at presentation also seemed associated with the development of fetal hydrops and adverse outcome.

Safe and effective treatment options for ALVT patients in fetal life have not been reported to date. Although some studies suggest that the use of intrauterine digoxin and beta-blocker therapy may improve CVPS with little risk to do potential harm, case numbers in our study were limited and current evidence is too scarce to suggest IUT as a fetal therapy in cases with fetal ALVT.

The majority of liveborn neonates (92%) received corrective surgery without significant postoperative complications. Neonatal death occurred in the only liveborn that had presented with hydrops in the third trimester and quickly deteriorated after birth. Although we did not observe postoperative deaths in this study, postoperative mortality has been reported in three cases in the literature.[6, 34, 36] All demised due to rapid progressive deterioration perioperatively and/or postoperative complications. The age at surgery varied considerably amongst our original cases from a few hours after birth to 6 weeks of age. This is consistent with the literature, describing neonates that require surgery within the first days of life[6, 30-32, 34, 35, 37], as well as those in which surgery could be delayed up to 3 months or even 1 year of age[6, 28]. This reflects the heterogeneity in clinical conditions in ALVT patients, as well as changing attitudes regarding and expertise with neonatal surgical interventions in general. Altogether, our results stress that close monitoring of these neonates is warranted, given the risk of rapid and sudden deterioration despite aggressive anti-congestive therapy and the unpredictability of postoperative recovery.

Overall survival in fetuses with ALVT was 60% with good clinical outcome in the survivors. Fifty-eight percent of these cases did not show signs of additional morbidity with a normal cardiac function at a median age of 3.2 years. The majority of cases remained asymptomatic until the end of primary school, after which cardiac signs, such as a mild to moderate aortic stenosis, became more apparent and surgical intervention involving replacement of the aortic valve was necessary. In this study three cases

needed aortic valve replacement at a median age of 14 years and 3/4 patients over age 10 has had additional surgery at the time of this writing.

Despite the international character of this study, with the aim to include a maximal number of available fetuses with this extremely rare heart defect, we were still underpowered to perform extensive statistical analyses. The retrospective character and broad time-period from which cases were retrieved, therewith precluded us to evaluate all potentially prognostic factors for adverse outcome, amongst which the presence of pathogenic variants after genetic testing. As a third, the considerable changes in the prenatal detection of CHDs in general, as well as care for these patients over the past decades may have resulted in the description of a selected population and the results may not be applicable to cases in the current period. To develop an accurate prediction model that aids prenatal counseling by truly discriminating those at risk of fetal or neonatal demise from those with a generally good clinical outcome, a global registry is necessary to obtain a larger study population with time.

In conclusion, evaluation of fetal hydrops and concomitant fetal Doppler anomalies before 24 weeks of gestation seem particularly useful to indicate cases of ALVT at risk for perinatal death. In the absence of signs of fetal hydrops, all cases reached term and were liveborn. Although close monitoring after birth is warranted, the vast majority will make it to surgery and survive with good clinical outcome. To improve our understanding of this disorder and the variability in clinical presentations, autopsy and genetic studies are necessary.

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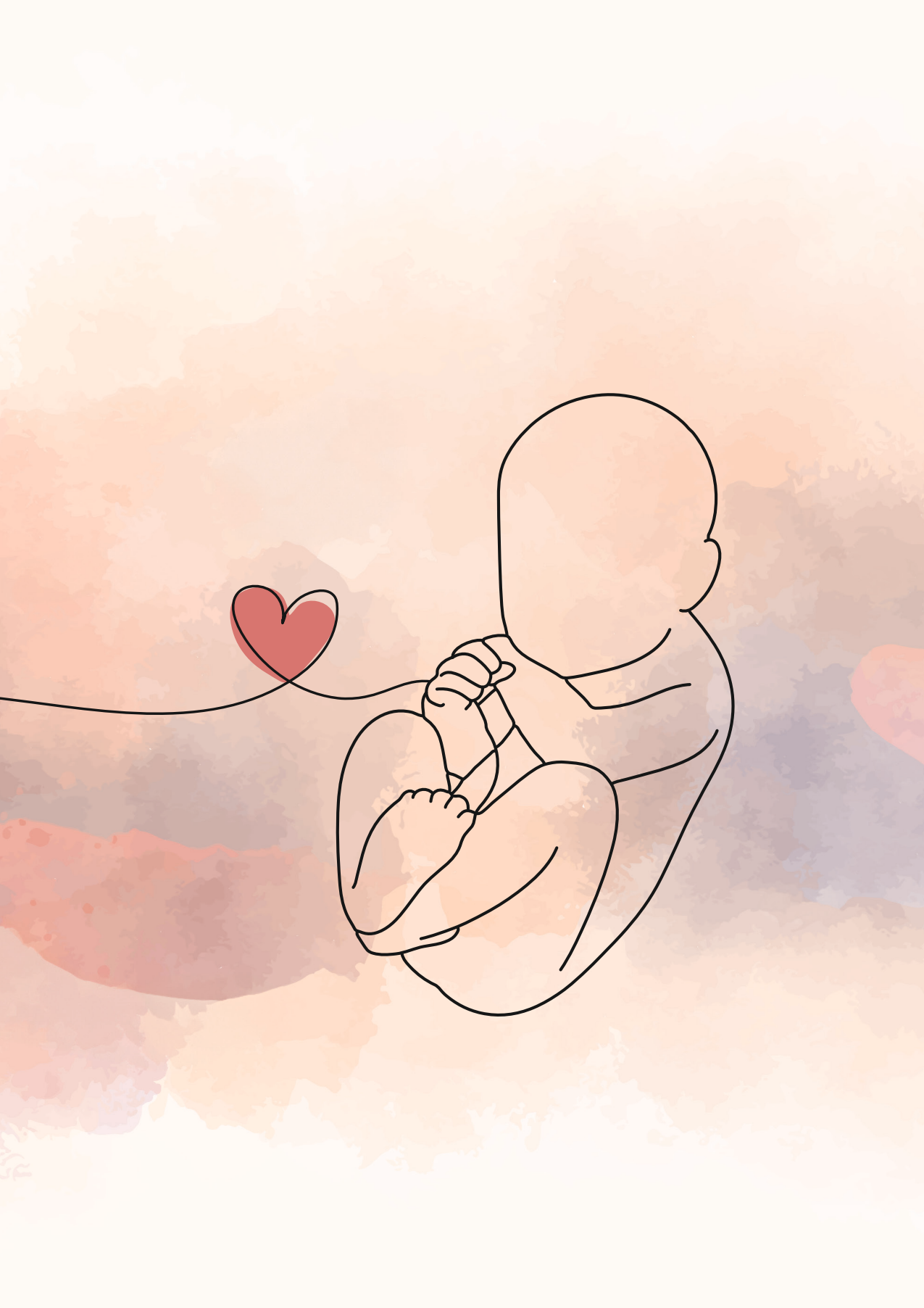

CHAPTER 9

General discussion

Recent innovations in ultrasound technology and the increase in expertise have caused a rapid elevation in prenatal detection rates for congenital heart defects. Prenatal detection is nowadays possible at an early gestational age. These developments, however, also raised challenges for prenatal counseling. This thesis focuses on several aspects of screening for congenital heart defects (CHD) in the fetus and on the establishment of the prognosis following prenatal CHD diagnosis. To improve the performance of screening, chapter 2 in this thesis displays potential (adjustable) risk factors for a missed prenatal diagnosis. Other studies in this thesis elaborate on the prognosis of CHD from a fetal perspective, as we have shown that this may vary significantly from the prognosis following a postnatal diagnosis or the prognosis after an intervention. This chapter will elaborate on several aspects in the care for fetuses with CHD. This can roughly be divided into three main themes: the prognosis of a CHD at mid-gestation, the performance of prenatal screening and ethical considerations regarding screening for CHD.

PROGNOSIS OF A CONGENITAL HEART DEFECT AT MID-GESTATION

Prenatal detection of cardiac anomalies has - with the development of structural screening programs - significantly increased over time. The prognosis of early diagnosed congenital heart defects, particularly in the fetal period, may, however, be challenging to determine. This prognosis is mainly based on the reported outcome in cohort studies. The majority of these cohort studies, however, concern a specific cardiac defect, following cardiac surgery or a cardiac intervention. The postoperative or post-intervention prognosis differs from the prognosis at mid-gestation for two reasons. First of all, the time-interval from mid-gestation to postnatal intervention is considerable and significant changes or complications may have occurred. Secondly, the prognosis may differ due to the fact that prenatally detected and undetected cases may not be similar at baseline. Variation in these study populations occurs because some cases result in spontaneous fetal demise, in some cases parents may opt for pregnancy termination and some newborns die after birth before surgery or cardiac interventions have been performed. Thus, fetuses diagnosed with a CHD at mid-gestation in general comprise a significantly different population with a more severe phenotype compared to those diagnosed and followed up after birth. Therefore, the studies in this thesis specifically focus on the outcome of fetal congenital heart defects to gather more knowledge on the prognosis at this time of diagnosis to improve prenatal counseling.

One of the major determinants for prognosis is the presence of a genetic syndrome in CHD cases. It is generally known that congenital heart defects are strongly associated with genetic diagnoses, which increase the risk of fetal demise or postnatal complications. Fetal studies that report on the incidence of genetic diagnoses, beyond

screening for aneuploidy or 22q11.2 deletion syndrome, are scarce. In Chapter 3 we studied the incidence of genetic anomalies in the largest cohort of euploid fetuses with CHD so far and assessed the impact on several outcome parameters. This study showed that 16% of isolated and non-isolated euploid CHD fetuses were diagnosed with a genetic diagnosis following microarray analysis or exome sequencing. In this cohort, the presence of these additional genetic diagnoses increased the risk of fetal growth restriction, premature birth, mortality and developmental delay. Based on the results from this large unselected cohort of CHD fetuses, the presence of genetic diagnoses does not only occur more often in fetuses diagnosed at mid-gestation compared to children with CHDs, but it had significantly impact on the prognosis as well. It is therefore likely that the increased incidence of cases with genetic anomalies is an important contributor to the differences in prognosis between fetal and postnatal cohorts. Our study shows that knowledge on the presence or absence of genetic anomalies at time of mid-gestational diagnosis is essential for prenatal counseling to estimate the prognosis for the child. The estimation of prognosis in these children does not only involve the presence of neurodevelopmental delay, but the chance of survival as well. Further research to determine the incidence of specific genetic variants for each congenital heart defect separately from a fetal perspective is necessary to enable patient-specific counseling on the indication for additional genetic testing.

A very important prognostic factor for parents is (the risk of) neurodevelopmental delay, which has a major impact on the quality of life. Neurodevelopmental delay occurs in at least one third of children with complex CHD. Existing literature indicates that this delay may start in utero. In Chapter 5, we studied head growth as a marker for neurodevelopment in CHD fetuses. Our findings reveal that not only head growth, but overall growth is impaired in fetuses with CHD. Although fetal growth measurements often fall within normal limits, they are significantly smaller compared to healthy fetuses. This indicates that CHD fetuses generally do not achieve their growth potential. Intrauterine growth restriction appeared particularly caused by impaired placentation and to a lesser extent by genetic syndromes. The risk of placenta-related pathology is increased in fetuses with congenital heart defects. Consequently, there is an increased incidence of preeclampsia, lower birthweight and a higher rate of small for gestational age neonates. [1, 2] While these measurements could not directly be related to neurodevelopmental outcome in our studies, previous literature shows that (early) fetal growth restriction is associated with neurodevelopmental delay.[3] Therefore, impaired placentation and subsequently impaired fetal growth may have a significant influence on delayed neurodevelopment in CHD fetuses. Future research on brain development in CHD fetuses and infants should be related to overall fetal growth and additional pathology.

The significant difference in prognosis between prenatally detected and postnatally detected cases was also demonstrated in specific cardiac diagnoses. In Chapter 7 we report on the first relatively large cohort study that evaluates prognosis of a common arterial trunk from a fetal perspective. A systematic review was performed to include additional cases. Our results show that the risk of mortality in fetuses diagnosed with CAT is significant. In a considerable number of pregnancies with fetal common arterial trunk, the parents decided to terminate the pregnancy, mainly in cases with concomitant comorbidity or significant genetic syndromes. Despite this, still 40% of continuing pregnancies with an intention-to-treat resulted in fetal or postnatal demise and only 30% of survivors did not show signs of additional morbidity or developmental delay at the age of six. Postnatal cohort studies, on the other hand, generally tend to depict a more optimistic prognosis, with 1-year survival rates between 80 and 90%. This is comparable to the postoperative survival rate of 87% in our cohort, if we solely include cases that underwent surgery. This difference can be explained by the fact that demise mainly occurred in utero or shortly after birth, due to truncal valve incompetence or other complications as a result of a genetic syndrome, in particular when delivered prematurely. These results emphasize that characteristics at diagnosis (genetic diagnoses, extra-cardiac comorbidity etc.) and associated outcome parameters (complications due to risk of premature birth or genetic syndrome, mortality) are strongly correlated to - and significantly differ with - the time of diagnosis.

Chapter 8 of this thesis focuses on the prognosis of an aorto-left ventricular tunnel (ALVT), an extremely rare congenital heart defect. We initiated a retrospective multicenter study on this particular defect, due to the diagnosis of ALVT in a fetus of 20 weeks' GA in our own center. The dilatation and poor contractility of the left ventricle was impressive, but prenatal counseling on the prognosis was hampered by the scarce literature on this diagnosis in fetuses. The internationally collected fetal cases were combined with a systematic review of the literature, which resulted in a total of 42 fetuses with ALVT. This study shows that 40% of fetuses with ALVT results in fetal or neonatal demise before a viable age was reached or surgery could be performed. Not surprisingly, symptoms of fetal hydrops (such as ascites, pleural effusion or skin edema) were present in all cases resulting in fetal demise. On the other hand, the fetuses that did not develop hydrops before 24 weeks' GA, all made it to term, received corrective surgery and were alive without additional morbidity at the age of 3. Similar to our findings, a review of cases in the literature showed that in the absence of hydrops, fetal or neonatal mortality rarely occurred. These findings are extremely important for prenatal counseling, as it indicates that stable non-hydropic fetuses with ALVT generally survive to term with good clinical outcome, despite the impressive cardiomegaly and

impaired contractility. Counseling should therefore clearly address that the prognosis at mid-gestation highly depends on the presence of hydrops at that time.

Lastly, a prenatal diagnosis does not always equal the diagnosis after birth. First of all, errors in the cardiac diagnosis can explain some of these differences, for several reasons. Secondly, some CHDs simply do not become apparent until the third trimester or even develop in the postnatal period. In Chapter 6 we describe the outcome of cardiac ventricular size disproportion at mid-gestation. It is well known that this feature is a marker in fetal life for the development of aortic coarctation after closure of the arterial duct in the first days of life. Although prenatally counseled to be at risk for the development of aortic coarctation, only 40% truly develop an aortic coarctation. Prenatal counseling addresses the risk of coarctation, as well as the considerable risk of a false-positive finding. However, the clinical course of this group had never been studied before. In Chapter 6 we specifically focused on these false-positive cases. Amongst those that did not develop aortic coarctation, a large proportion of these neonates showed prenatally undetected congenital defects (46%) and/or pulmonary or transition problems (35%) during postnatal follow-up. Only 45% of these cases did not need any medical service in the neonatal period, nor did they require medication, postnatal intervention or surgery at all.

It is well-known that cases at risk for aortic coarctation are one of the most difficult to identify in the fetus. Due to the low detection rate for aortic coarctation, together with the high number of false positives, the majority of studies have primarily focused on enhancing detection rates and differentiating coarctation cases from those that appear 'normal' after birth. The results from our study have shown that literature should not solely focus on the 'true' aortic coarctation cases, as those that do not develop aortic coarctation are still at significant risk of transitional problems after birth, that may require intensive care or present with other congenital defects. Close observation should therefore be warranted, regardless of the initial postnatal diagnosis, and equally represented in the literature to improve counseling on prognosis of true- and false-positive cases.

These results emphasize that referencing postnatal studies for prenatal counseling should be avoided or done with caution, as it might present a falsely optimistic perspective and result in a significant overestimation of the prognosis at mid-gestation. Due to rapid innovations in the care for and outcome of fetus and neonates with CHD, additional research should not merely elaborate on (postoperative) survival, but gather evidence on long-term outcome and wellbeing in general as well.

In conclusion, fetuses with a CHD at mid-gestation comprise a significantly different population compared to those diagnosed or studied after birth. Postnatal cohorts represent only a selected proportion of all fetuses diagnosed with CHD at mid-gestation. These selected cases generally have a better chance of survival and prognosis after birth, due to a lower incidence of genetic diagnoses or associated risks, such as premature birth and comorbidities. Although evidence on the prognosis of CHD from a fetal perspective is essential, the availability is still limited. To enhance prenatal counseling, further research from a fetal perspective is necessary to provide a more accurate and patient specific estimation of prognosis and quality of life, including long-term outcome in general. Until then, counsellors and scientists should be aware of this significant selection bias and statements regarding the expected prognosis should be made with caution.

IMPROVEMENT OF PRENATAL SCREENING FOR CHD

Prenatal detection of CHD remains a hot topic in current literature, with the aim to optimize timely treatment of these significant defects. Despite multiple interventions to improve detection rates, including a standardized approach to assess the cardiac anatomy and the addition of the three-vessel trachea view, still a considerable number of cases is missed antenatally. Prenatal detection rates for congenital heart disease range from 30-60% in developed countries, but have generally increased over time. The detection rate of critical or significant heart defects is much higher, with a detection rate for single ventricular anomalies of almost 100% in countries with well-organized screening programs nowadays. If additional extracardiac malformations are present, the likelihood of antenatal detection of CHD also increases. The use of a systematic approach with mainly transversal planes, including the four-chamber view, left and right ventricular outflow tracts, three-vessel view and three-vessel-trachea view, has become standard practice to structurally assess the fetal heart and maximizes the cost-effectiveness of prenatal screening.[4, 5] However, isolated heart defects that present with a normal four-chamber view, such as transposition of the great arteries or aortic coarctation, remain easily missed.[6, 7] In this section, we will further discuss risk factors for an antenatal miss and elaborate on potential strategies to improve prenatal detection rates in the future.

Current literature reports numerous studies that aim to develop strategies to improve prenatal detection rates. Little is known about factors that contribute to or cause antenatal miss in these cases. In the PRECOR registry, all fetus or children with a diagnosis of a severe congenital heart defect have been registered since 2002. We identified all undetected and detected cases in PRECOR to study impeding factors

and potential causes for an antenatal miss by gathering their original ultrasound scans (Chapter 2). The results of this study have shown that the quality of cardiac planes obtained during the standard anomaly scan was significantly better in detected compared to undetected cases and increased with the number of ultrasound scans performed by a sonographer per year. While assessing potential causes of a missed prenatal diagnosis, we observed that in half of the undetected cases the cardiac planes had not technically been obtained adequately, resulting in images of insufficient quality to visualize the cardiac defect. In 30% of undetected cases, however, the images were of sufficient quality to visualize the defect, but the CHD had not been recognized by the sonographer. It was thought that a missed prenatal diagnosis was inevitable in the remaining 20%, as the quality was sufficient, but the cardiac anatomy appeared normal at mid-gestation. This information is essential to identify and address determinants for a prenatal diagnosis to further improve antenatal detection of CHD.

The main cause for an antenatal miss appeared a lack of competence to obtain technically correct cardiac images in abnormal cases, although sonographers were capable to produce these images in normal cases. Previous literature has shown that education and specific hands-on training programs to acquire satisfactory views has a positive impact on the quality of the structural anomaly scan. These studies, however, were able to improve detection rates for CHD up to 60%, which equals the current detection in an increasing number of Western countries, including the Netherlands. It is therefore questionable whether increasing the amount of training could further improve current detection rates in these countries. The international guidelines available to standardize quality, obligatory training programs to become and/or stay qualified as a sonographer and routinely performed monitoring audits are all focused to improve and guarantee adequate quality of screening in a normal setting.[8-11] However, it seems that we do not train our sonographers how to adapt to the abnormal setting and how to enable them to acquire competent views in those cases as well. There could be various psychological reasons for sonographers to perform well in normal cases, but not show similar capability in abnormal cases. This could be attributed to specific personality traits, such as tolerating suboptimal images occasionally, or due to limited experience, causing sonographers to rather question their own abilities rather than the fetus's anatomy.

In Chapter 2, we also found that the quality of cardiac scans in abnormal cases increased with the volume of ultrasound scans performed by the sonographer and the yearly volume of scans in the screening center. This correlation is a probably a result of multiple factors. First of all, sonographers that perform more ultrasound scans will likely work in screening centers with high volumes, which may increase their

exposure to abnormal cases when discussing or evaluating images of patients from fellow colleagues. Secondly, as the number of ultrasound scans performed on a regular base increase, the more acquainted the sonographer will be with the normal cardiac anatomy and more likely to differentiate abnormal from normal. This acquaintance might also assure that the sonographer will question the cardiac anatomy rather than their personal technical competence.

The results from this audit enable the development of strategies to increase prenatal detection of CHDs by improving the quality of cardiac scanning, as well as recognition of abnormalities. First of all, centralization of care and thus an increase in the sonographer's annual volumes might improve the quality of cardiac scans. Due to significant implications of this intervention for screening centers, sonographers and the accessibility to health care in general, a critical assessment considering various aspects should be performed. This includes the evaluation of the feasibility and effectiveness of this intervention compared to other strategies to strengthen the performance of sonographers, as well as the perspectives of sonographers.

The incorporation of training in abnormal cases to obtain adaptational skills, rather than the evaluation of the performance of the sonographer in the normal setting could also be of additional value. Although it would be desirable to include scanning of real CHD fetuses in the sonographer's training programs, for example in a tertiary referral center, this is not feasible due to the high number of sonographers versus the emotional burden for these pregnant women. Ultrasound simulators to train both normal and pathological findings are potentially an ideal tool to teach, to improve and monitor physicians' ultrasound skills in detecting fetal anomalies. In fact, the use of ultrasound simulators was already reported 20 years ago to train fetal nuchal translucency thickness and crown-rump length measurements.[12]

The recent editioral by Yagel and Moon-Grady[4], suggests more frequent or extensive auditing of individual operators and ultrasound departments in general to enhance screening performance. In the Netherlands the sonographer's performance is already monitored every two years to remain qualified. As our data show that sonographers primarily experience difficulty in adapting to abnormal anatomy, intensifying monitoring will be of limited additional value. Therefore, time and resources should be invested in interventions with a potentially higher yield.

Strategies to enhance prenatal detection should also incorporate measures to increase exposure to abnormal anatomy and improve recognition of cardiac defects. Currently available educational platforms, such as 'Fetal Heart Academy', include fetal ultrasound

images of cardiac defects to subject sonographers to abnormal scans. It has never been studied whether the use of these platforms on a frequent basis could increase knowledge and therewith recognition of fetal heart defects. The development of a platform that enables sonographers to safely and anonymously share difficult cases with fellow sonographers or experts could be a promising tool. Operators should acquire videoclips in addition to 'still images' to not only improve scan quality, but optimize consultation by a third party in these difficult cases as well. The use of such platforms would not only increase exposure to abnormal anatomy amongst sonographers, but could lower the threshold to consult colleagues as well. This potentially results in less but more appropriate referrals to tertiary care centers, and therewith even reduce the costs of screening for CHD in general.

Heart defects that had not been recognized antenatally often involved those with a normal four-chamber view that could only be recognized in the outflow tract views. Utilizing innovative approaches such as 'automatic image recognition' through Artificial Intelligence (AI) can significantly enhance defect recognition, which may significantly improve prenatal detection of CHDs. Colleagues from the University of San Francisco already showed promising preliminary results on the use of 'automatic image recognition' in original acquired images of CHD cases and normal controls. Its applicability in prenatal screening for CHD requires further study to ensure both high sensitivity for patient detection and specificity to minimize unnecessary referrals. If future research shows that CHD detection by AI is comparable to our current golden standard, the fetal cardiology experts, this tool would be a major step forward to further improve prenatal detection rates in both Western and non-Western countries. It is however crucial to acknowledge that variations in image quality due to differences in expertise and settings can impact the overall yield and diagnostic value.

Altogether, these results suggest several potencies to improve prenatal detection for CHD.

Quality of prenatal screening can potentially be improved by enforcing a minimal annual volume or centralization of care. Inadequate adaptational skills or insufficient recognition of CHDs impedes prenatal detection in the majority of undetected cases. Interventions to potentially enhance the sonographer's performance include the use of interprofessional platforms for discussion, ultrasound training simulators, the use of automatic imaging recognition or even psychological training to cope with triggered insecurities. Future research is required to evaluate the most potent, feasible and cost-effective approach to accomplish this.

ETHICAL CONSIDERATIONS IN SCREENING FOR CHD

Screening programs intend to identify individuals in the pre-symptomatic phase of disease amongst those that appear well and did not seek medical attention yet.[13, 14] These programs aim to minimize consequences of disease through early identification and optimal treatment. The organization of a screening program, however, requires sufficient financial resources, equipment, qualified personnel and monitoring.

In 1968 the World Health Organization published a report by Wilson and Jungner describing 10 principles of screening.[15] This was later transformed into a set of 3 domains including the 12 modified criteria that should be considered before initiating a screening program.[16, 17] Following these criteria, it is impeccable that congenital heart defects meet the requirements in which screening should be concerned. First of all, CHDs have a major impact on a child's health and are known to be the most common cause of infant death and mortality from all birth defects.[18] The target population is well-defined and generally includes all first or second trimester fetuses, because the vast majority of CHD cases occur in low-risk populations. Interestingly, the main purpose of screening for congenital anomalies - as described by the 'National Institute for Public Health and the Environment' (RIVM) – merely mentions the opportunity for parents to make reproductive choices.

The second domain includes criteria for the test' performance and possibilities for intervention. Regarding the performance, screening for CHD should be accurate and acceptable. Currently, prenatal detection rates of 60-80% have been reported in a low-risk setting, after a significant increase over the past 20 years.[7, 19] Theoretically, most cardiac lesions can be detected in the fetus – with the exception of some (minor) lesions such as secundum atrial septal defect and patent ductus arteriosus. In reality, approximately 30% remains undetected before birth. While there is potential to enhance the sensitivity and specificity of screening for CHD, prenatal detection rates have significantly improved over time due to the growing expertise and increase in evidence to sustain this progress. Another aspect of screening involves the insurance that the screening test' results are clearly interpretable. The majority of studies on the prognosis of CHD however include postnatal studies, whereas evidence from a fetal perspective is limited. This impedes counselors to estimate what the clinical value of their findings are in the fetus. Therefore, evidence on the prognosis after birth is available, but suboptimal at time of a diagnosis. With regards to the opportunity for intervention, prenatal screening does allow for it, but only in selection of CHD: fetus with valvular anomalies, in whom intrauterine treatment might be favorable. Invasive

interventions in utero are, however, only performed in few fetal surgical care centers and the treatment is still considered experimental.[20, 21]

The third domain assesses the potential benefit and harm of screening. A prenatal diagnosis of CHD has evident advantages, as it provides parents prognostic information before birth, enables them to make decisions concerning their (future) family and ensures optimal perinatal care management. Unpublished data from our cohort of nearly 4000 CHD fetuses (2002-2016), indicate that despite improved prenatal detection and genetic testing for fetal CHD, the proportion of elective pregnancy terminations did not increase. Thus, prenatal detection does not seem to lower the termination threshold but enables early identification of a larger, likely less severe, portion of congenital heart defects. Timely diagnosis of fetal CHD empowers informed decisions, reducing late-term terminations and its related complications.

Referencing a comprehensive meta-analysis, prenatal diagnosis has been shown to decrease the risk of cardiovascular compromise before surgery for critical cardiac lesions.[22] Additionally, in a neonatal cohort with duct-dependent critical CHD, a notable reduction in postnatal mortality and cardiac arrest risk prior to surgery was reported, along with a decrease in preoperative ventilation and vasoactive medication.[23] For a specific subset of patients with valvular anomalies, intrauterine treatments such as aortic or pulmonary balloon valvuloplasty, or atrial needle septoplasty, may potentially enhance prognosis.[24-26] However, it is essential to emphasize that critical diagnoses significantly benefit from prenatal diagnosis, but only represent a minority (comprising less than 25% of all CHDs). Evidence supporting the favorable impact of timely diagnosis on outcomes in other congenital heart defects is scarce and often inconsistent.

The potential harm of prenatal screening has hardly been studied. A prenatal diagnosis may cause increased parental stress or anxiety levels, which can (directly or indirectly) affect the fetal health. In some cases, a prenatal diagnosis cannot be made with certainty or the prognosis is unknown. In these situations, prenatal screening induces more insecurity about the fetus' prognosis, even potentially unnecessary. On the opposite, if the heart appears structurally normal on the first or second trimester scan, this does not guarantee a structurally normal heart at birth. One of the advantages of a prenatal diagnosis is the ability for adequate perinatal management. This is mainly essential in a fetus with a critical CHD, at risk for rapid cardiac deterioration and postnatal complications after birth. The impact of postnatal complications due to delayed diagnosis and inadequate care seems relatively low from a national perspective with current detection rates. Univentricular heart defects or lesions that present with an extremely abnormal four-chamber view are detected in nearly all fetuses. Some

critical congenital heart defects, among which transposition of the aorta, aortic stenosis and aortic coarctation, are however not uncommon to be missed.

Based on my personal experience and discussions with nearly 100 mothers of both prenatally detected and undetected CHD patients, I believe that the opportunity to prepare for a child with a significant anomaly such as CHD before birth, is highly valuable to (future) parents and outweighs the potential harm. Although psychological factors of an antenatal miss or prenatal diagnosis were not officially studied, many parents motivated the reason for their consent to participate spontaneously during follow-up (concerning the study 'Why are congenital heart defects being missed?', Chapter 2). Frequently, mothers of undetected cases mentioned to aspire the prevention of a missed diagnoses in similar cases in the future. This would potentially prevent cardiac deterioration in other children and timely prepare parents for having a child with CHD. Only a small proportion of mothers reported to be grateful that the diagnosis remained undetected before birth, as they enjoyed a carefree pregnancy and did not experience any negative effect of a delayed diagnosis for their child. It would be interesting to further assess the psychological effects of a missed diagnosis and false parental reassurance following prenatal screening. Especially for undetected critical CHD cases, as this has – to my knowledge – never been thoroughly studied so far.

In the Netherlands, the 'National Institute for Public Health and the Environment' (RIVM) is responsible for the management and provision of information regarding population screening programs. It also serves as an advisory body, providing information to policymakers in the country. Their formal report on prenatal screening (named 'Prenatal screening', published 2016), intended for the Dutch Health Council, solely describes prenatal screening as a measure to provide 'reproductive choices'. This emphasizes only a limited aspect of the motivation to screen for congenital anomalies. Given their mandate to consolidate knowledge, facilitate innovation in population screening when deemed necessary, and provide recommendations to policymakers, it is important that such institutes consider all aspects of screening, as advocated by the WHO in 1968.

In conclusion, fetuses with a prenatal diagnosis of a critical (duct-dependent) CHD benefit from a timely diagnosis and optimal perinatal care management. Yet, scientifically proving this for all structural cardiac anomalies remains challenging. This can be ascribed to the low prevalence of specific heart defects and the considerably lower detection rates, especially for non-critical CHDs, up to a decade ago. Based on numerous parental declarations, the opportunity to prepare for a child with a CHD before birth is highly valuable and outweighs the potential harm in my opinion. With increasing detection rates and subsequent options for genetic testing, potentially

negative effects of prenatal screening should be critically evaluated as well. Future research is needed in order to organize health care according to evidence-based best practices, and with respect to the benefits of timely diagnosis and the potential harm of screening.

FUTURE PERSPECTIVES

It is imperative to acquire evidence concerning the entire developmental journey and outcome, beginning as early as the fetal stage, to optimize care for children with CHD. Due to the rarity of cardiac defects and rapid innovations in this field, financial support is essential to develop and sustain a large national CHD registry. This registry would allow future studies to collection essential data and monitor the national screening performance.

First of all, optimal fetal and neonatal care requires early identification of cases, preferably in the fetus. Future research should evaluate the effectiveness of new strategies to improve prenatal detection for CHD, such as training in abnormal cases or psychological interventions to optimize referrals to specialized fetal echo facilities. Additionally, the use of artificial intelligence should be studied, as this may aid sonographers in the recognition of fetal cardiac abnormalities, both in Western and non-Western areas worldwide.

Secondly, counseling following a prenatal diagnosis demands sufficient evidence from a fetal perspective to enhance fetal and perinatal care management. Future studies should therefore always relate evidence regarding prognosis to the time of diagnosis. Lastly, with the rapidly evolving techniques and possibilities for genetic testing, parents should already be counseled on the impact of (additional) genetic diagnoses in an early stage and beyond the scope of pregnancy termination. Besides that, future research should aim to find a balance between optimizing fast and complete genetic testing for all variants, limiting the potentially negative consequences and potentially develop functional tests to aid the translation of these genetic results to their phenotypical consequences.

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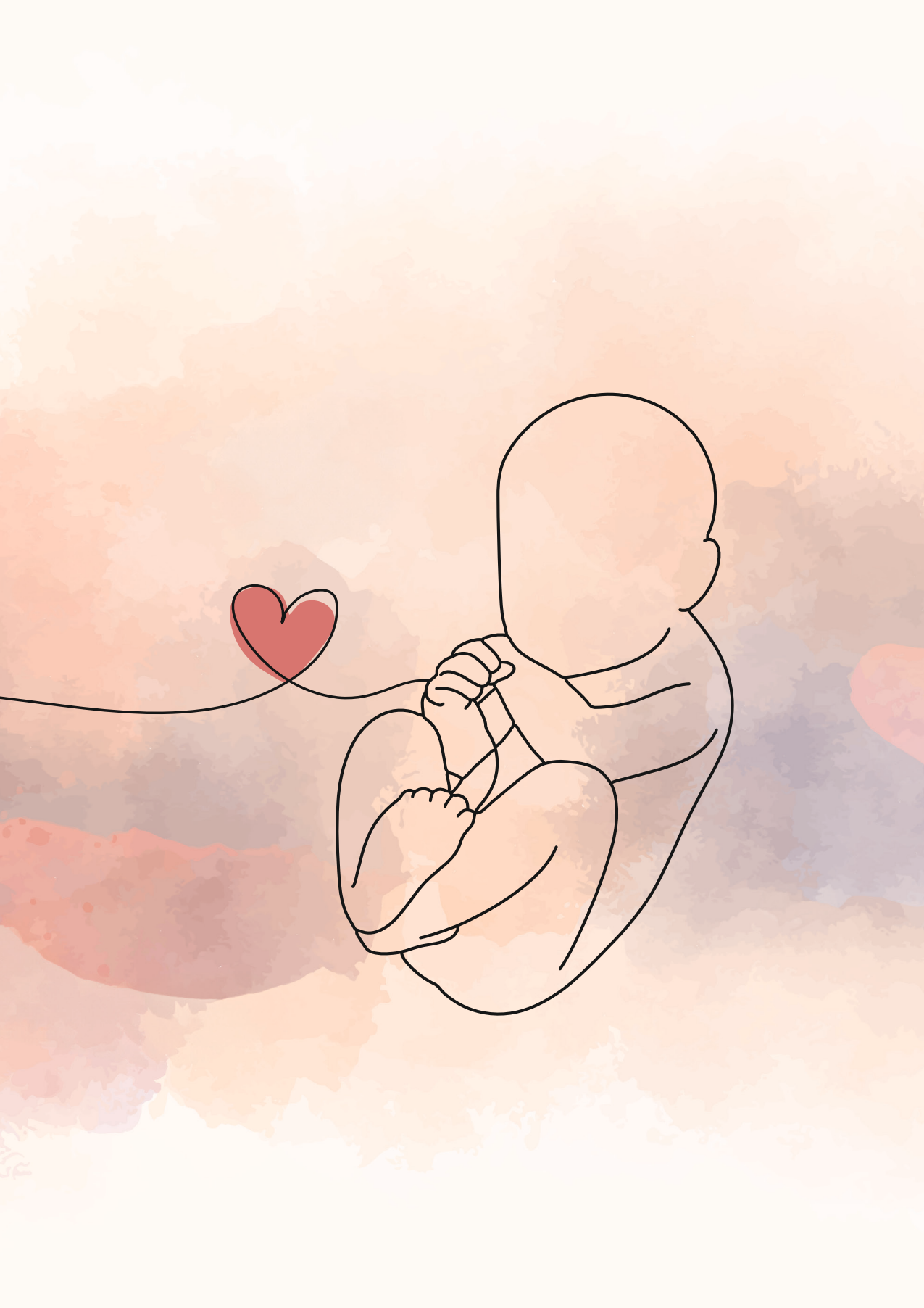

APPENDICES

SUMMARY

This thesis discusses the prenatal detection and outcome of congenital heart defects in the fetus.

This thesis is divided into two parts. *The first part focuses on determinants for a prenatal diagnosis in fetus with a structural congenital heart defect, whereas the second part elaborates on the prognosis of congenital heart defects in the fetus to improve prenatal counseling and postnatal care management.*

Prenatal detection

In **chapter 2** we present a case-control study that assessed the quality of fetal heart images and circumstantial factors in fetuses with prenatally undetected and detected congenital heart defects. Cardiac images were scored for technical correctness by two fetal echocardiography experts, blinded to prenatal detection and the specific cardiac diagnosis. Our results show that circumstantial factors, such as BMI or fetal position, hardly differed between prenatally undetected and detected cases. The group of undetected cases however, scored significantly lower than the detected group on the quality of all cardiac images obtained. A lack of adaptational skills when performing the cardiac scan appeared the main cause for a missed prenatal diagnosis (49%), followed by the inability to recognize the heart defect (31% of undetected cases). Prenatal detection appeared not feasible in 20% of undetected cases, as the heart defect was not visible at mid-gestation on adequate cardiac scans. Furthermore, the performance of a high volume of cardiac scans, especially in large ultrasound centers, significantly contributes to prenatal detection.

Outcome

This second part aims to provide evidence on the prognosis of fetal congenital heart defects, as prenatal counselling can be particularly challenging for diagnoses with limited evidence on outcome from a prenatal perspective.

In **chapter 3** we report on the prevalence of genetic diagnoses in fetus with severe congenital heart defects, either isolated or non-isolated, as well as the impact of these diagnoses on mortality and morbidity. After exclusion of aneuploidy cases, a genetic diagnosis was found in 16% of cases, comprising copy number variants in 10% and abnormalities in specific genes in 6% of cases. Therefore, exome sequencing should be considered in all fetuses with a normal chromosome microarray analysis, especially if extra-cardiac malformations are present.

In **chapter 4** we present our response to a Letter to the editor. With the use of the acquired data from chapter 3, we provide additional information on genetic syndromes, particularly those not detectable with chromosome micro-array analyses. The incidence of CNVs and sequence variants is reported for each CHD diagnosis, and sequentially for isolated and non-isolated cases separately. With regards to specific CHD diagnoses, exome sequencing had a particularly high yield amongst those with conotruncal anomalies, left isomerism and rhabdomyomas.

Chapter 5 involves a retrospective cohort study that assessed the impact of extracardiac pathology on fetal head growth and growth in general, as a marker for fetal brain development. Patients were allocated to the isolated group or assigned to one of the three non-isolated groups (genetic syndromes, extra-cardiac malformations or placental pathology). A mixed-linear regression model was used to study fetal growth over time. At mid-gestation, head circumference was significantly smaller in non-isolated compared to isolated cases, which decreased significantly further with advancing gestation. Placental pathology and genetic syndromes seem to be important attributors for restricted (head) growth. This effect appears irrespective of altered hemodynamics caused by the CHD.

In **chapter 6** we describe a case-control study to evaluate postnatal outcome of fetus with an isolated ventricular size disproportion, particularly those that do not develop an aortic coarctation. In this cohort, 60% of fetus did not develop an aortic coarctation. These cases showed prenatally undetected congenital defects and pulmonary or transition problems. Proper monitoring is therefore warranted and prenatal counseling should consider to incorporate the risks for additional morbidity and neonatal complications in these cases.

Chapter 7 reports our original cohort study to assess the mortality and postnatal morbidity in fetus with a common arterial trunk. Additionally, we performed a systematic review of the literature for additional cases. This study found that parents opted for elective pregnancy termination in almost half of the cases. Amongst ongoing pregnancies, 40% resulted in fetal or neonatal demise. Significant morbidity, such as 22q11 deletion syndrome, Adams-Oliver syndrome, intestinal atresia and neurodevelopmental delay, was found in 40% of survivors.

In **chapter 8**, we present an international case series of fetuses diagnosed with an aorto-left ventricular tunnel (AVLT) to provide evidence on the outcome of this rare diagnosis, especially before birth. We evaluated antenatal parameters, neonatal outcome, postnatal follow-up in these cases and systematically reviewed the literature

for similar cases. Echocardiographic characteristics of AVLT included an increased cardiac-thorax ratio (95%), increased left ventricular end-diastolic diameter (90%) and a dysplastic aortic valve (90%). Extracardiac malformations were rare (5%). All cases that resulted in fetal demise, showed signs of hydrops prior to 24 weeks of gestation or at autopsy. In the absence of fetal hydrops, however, ALVT generally carries a good prognosis.

NEDERLANDSE SAMENVATTING

Aangeboren hartafwijkingen (CHD) zijn de meest voorkomende aangeboren afwijking met een incidentie van 6-10 per 1000 pasgeborenen. In een kwart van de patiënten betreft dit ernstige hartafwijkingen, die een operatie of ingreep behoeven binnen het eerste levensjaar. De meerderheid van de neonaten met een ernstige CHD is symptomatisch na de geboorte en wordt hierdoor snel geïdentificeerd. Echter, tijdens routineonderzoek na de geboorte worden in 30% van de neonaten met een ernstige CHD geen afwijkingen waargenomen en worden zij ontslagen uit het ziekenhuis zonder diagnose. In de literatuur wordt een mortaliteitsrisico beschreven tot 30% bij neonaten die onopgemerkt blijven tijdens de ziekenhuisopname. Een langere tijd tot diagnose verhoogt het risico op zowel mortaliteit als morbiditeit in deze gevallen. De timing van klinische presentatie kan aanzienlijk verschillen tussen ernstige hartafwijkingen. Vooral hartafwijkingen die niet afhankelijk zijn van een open ductus arteriosus zullen asymptomatisch blijven na de eerste 72 uur. Cyanose wordt niet vaak klinisch herkend bij milde desaturatie (>80% saturatie), bloedarmoede of donker gepigmenteerde zuigelingen. Tijdige herkenning van CHD is derhalve noodzakelijk om potentieel levensbedreigende situaties of blijvende schade te voorkomen.

Antenatale screening voor CHD

Een prenatale diagnose maakt een geplande bevalling in een tertiaire zorginstelling met optimale behandelingsopties mogelijk. Daarnaast biedt het de mogelijkheid aanvullend genetisch onderzoek te doen om de prenatale counseling te optimaliseren en ouders voor te bereiden. Voorts biedt een prenatale diagnose reproductieve autonomie, en kan het leiden tot het besluit van ouders om de zwangerschap te beëindigen.

Tegenwoordig heeft de meerderheid van de westerse landen een screeningsprogramma om congenitale (hart-)afwijkingen in het eerste of tweede trimester van de zwangerschap te detecteren. Het structureel echografisch onderzoek (SEO), voor alle zwangeren tussen 18-21 weken zwangerschap, werd in Nederland geïntroduceerd als onderdeel van het bevolkingsonderzoek in 2007. Tijdens dit onderzoek worden alle cardiale doorsnedes systematisch beoordeeld om foetus te identificeren met een (risico op) aangeboren afwijking(en) in een laag-risico populatie en prenatale detectie te optimaliseren. Ondanks gestandaardiseerde richtlijnen en protocollen blijven structurele cardiale afwijkingen de meest gemiste aangeboren afwijkingen. Screeningprogramma's in laag-risico populaties rapporteren detectiepercentages tussen de 30–75% in westerse landen. Daarentegen worden detectiepercentages tot 80-90% beschreven, wanneer vanuit één of enkele centra wordt gerapporteerd. Dit detectie percentage is haalbaar, indien overal in de regio deze omstandigheden

gecreëerd zouden kunnen worden. Het identificeren van aanpasbare factoren die prenatale detectie belemmeren, is derhalve noodzakelijk om de opbrengst van regionale en nationale screeningsprogramma's toe te laten nemen.

Prenatale counseling

Een belangrijk voordeel van een prenatale diagnose is de mogelijkheid om ouders al voor de geboorte te informeren en voor te bereiden. De kwaliteit van prenatale counseling is afhankelijk van de zekerheid van een diagnose en wetenschappelijk bewijs over de te verwachte prognose. Deze kennis is belangrijk voor het optimaliseren van postnatale zorg, en cruciaal voor geïnformeerde besluitvorming in de gevallen waarbij ouders overwegen de zwangerschap te beëindigen.

Beschikbare literatuur richt zich vaak op de prognose van neonaten met een bepaalde hartafwijking na een (succesvolle) chirurgische correctie of ingreep. Foetussen gediagnosticeerd met een aangeboren hartafwijking vormen een significant andere populatie, aangezien degenen die voor de geboorte of in de eerste dagen van hun leven overlijden en om die reden niet aan een operatie toe komen, niet worden geïncludeerd. Aangeboren hartafwijkingen zijn tevens geassocieerd met de aanwezigheid van comorbiditeiten, waaronder genetische aandoeningen, additionele extra-cardiale afwijkingen of neurologische ontwikkelingsachterstand. Literatuur naar de incidentie van genetische diagnoses onder *foetussen* met een CHD is echter beperkt en relatief verouderd. Recentere technieken als micro-array onderzoek en exoomsequencing zijn in staat ook kleinere duplicaties, deleties en genetische mutaties diagnosticeren. Beschikbare literatuur beschrijft tot op heden vooral de incidentie van numerieke chromosoomafwijkingen, waaronder trisomieën, triploïdie of specifieke diagnostiek naar het 22q11.2-deletiesyndroom. Om accurate en patiënt-specifieke counseling ten tijde van een prenatale diagnose mogelijk te maken, inclusief het risico op comorbiditeiten, zijn studies naar de prognose van CHD vanuit een foetaal perspectief essentieel, waarbij de kans op genetische oorzaken meegenomen dient te worden.

Dit proefschrift beschrijft factoren, die van invloed zijn op het vermogen om aangeboren hartafwijkingen voor de geboorte op te sporen. Daarnaast wordt de prognose van (verschillende) aangeboren hartafwijkingen vanuit een foetaal perspectief beschreven om prenatale counseling en postnatale zorg te verbeteren.

Prenatale detectie

Deel I van dit proefschrift richt zich op het evalueren van factoren en identificeren van oorzaken voor het niet detecteren van aangeboren hartafwijkingen ten tijde van de SEO (ook wel '20 weken echo' genoemd). In **hoofdstuk 2** beschrijven we een case-

control studie waarbij patiënt-specifieke karakteristieken en omstandigheden, de ervaring van de echoscopist en kwaliteit van de beelden van de '20-weken echo' wordt vergeleken tussen prenataal gedetecteerde en niet-gedetecteerde foetussen met een aangeboren hartafwijking. Twee experts op het gebied van foetale echocardiografie scoorden de technische kwaliteit van de cardiale doorsnedes, geblindeerd voor de diagnose en wetenschap of de diagnose voor de geboorte was gesteld. De resultaten van deze studie tonen aan dat omstandigheden, zoals maternaal BMI of foetale positie, nauwelijks verschilden tussen prenataal gedetecteerde en niet-gedetecteerde foetus. De groep zonder prenatale diagnose scoorde echter significant lager dan de gedetecteerde groep op de kwaliteit van alle verkregen cardiale afbeeldingen. Een gebrek aan aanpassingsvermogen, bij het uitvoeren van een cardiale scan in gevallen met afwijkende anatomie, leek de belangrijkste oorzaak voor een gemiste prenatale diagnose (49%), gevolgd door een onvermogen om de hartafwijking te herkennen (31% van de onopgemerkte foetus). Prenatale detectie bleek niet haalbaar bij 20% van de niet-gedetecteerde foetus, omdat de hartafwijking niet zichtbaar was ten tijde van de SEO ondanks *adequate* cardiale scans. Bovendien lijkt de ervaring van de echoscopist en het centrum ook bijdragend aan een betere kwaliteit van de cardiale echo en daarmee de prenatale detectie van CHD.

Prognose

Het tweede deel van dit proefschrift richt zich op de prognose van verschillende aangeboren hartafwijkingen vanuit een foetaal perspectief. In **hoofdstuk 3** onderzochten we in een cohort studie de incidentie van genetische diagnoses onder aangeboren hartafwijkingen, zowel geïsoleerd als niet-geïsoleerd, en evalueerden we de impact van deze diagnoses op mortaliteit en morbiditeit. Na exclusie van casus met een aneuploïdie, werd een andere genetische diagnose gevonden in 16%. Dit betrof een copynumbervariatie (CNV) in 10% en een genetische mutatie of variatie in 6% van euploïde foetus. Genetische diagnoses bleken sterk geassocieerd met de aanwezigheid van additionele structurele afwijkingen, dysmorfieën en kwamen het vaakst voor bij foetus met een interruptie van de aortaboog, pulmonalis atresie en een ventrikel- of atrioventriculair septumdefect. De aanwezigheid van een genetische diagnose bij CHD foetus bleek het risico op mortaliteit te vergroten, alsmede de kans op morbiditeit en ontwikkelingsachterstand postnataal. Gezien de incidentie en impact van genetische variaties, niet detecteerbaar met conventionele technieken, concluderen wij dat exoom sequencing aangeboden dient te worden bij foetussen met een normale micro-array analyse, en zeker bij patiënten met een niet-geïsoleerde CHD.

Naar aanleiding van een 'Letter-to-the-Editor', verstrekken we in **hoofdstuk 4** in het bijzonder het percentage genetische syndromen, die *niet* detecteerbaar zijn met

chromosoom micro-array analyse, onder congenitale hartafwijkingen afzonderlijk en uitgesplitst naar geïsoleerde en niet-geïsoleerde casus. Hieruit bleek dat exoom sequencing een bijzonder hoog rendement heeft bij foetus met conotruncale afwijkingen, links-isomerisme en rhabdomyomen.

Neurologische ontwikkelingsachterstand komt vaak voor bij kinderen met aangeboren hartafwijkingen. In **hoofdstuk 5** rapporteren we een retrospectieve cohortstudie die de impact van extra-cardiale pathologie op de hoofdomtrek van de foetus, als marker voor hersenontwikkeling, evalueert in relatie tot de foetale groei in het algemeen. Foetus met een CHD werden verdeeld in een geïsoleerde en niet-geïsoleerde groep. De niet-geïsoleerde groep werd verder onderverdeeld in 3 groepen: genetische syndromen (1), extra-cardiale (2) of placentaire afwijkingen (3). Een mixed-lineaire regressie analyse werd gebruikt om de foetale groei in de loop van de tijd te bestuderen. De foetale hoofdomtrek van niet-geïsoleerde CHD foetus was significant kleiner ten opzichte van geïsoleerde foetus bij 20-weken zwangerschap, wat significant verder afnam met het vorderen van de zwangerschap. Placenta-gerelateerde afwijkingen en genetische syndromen lijken een belangrijke verklaring voor de beperkte (hoofd)groei onder foetus met een CHD. Dit effect lijkt onafhankelijk van de impact van de CHD op flow of oxygenatie naar het foetale brein.

De laatste hoofdstukken van dit proefschrift richten zich op prenatale diagnoses die moeilijk antenataal te counselen zijn door het ontbreken van informatie over de prognose vanuit een prenataal perspectief. **Hoofdstuk 6** beschrijft een case-control studie naar de postnatale uitkomsten van foetus met een geïsoleerde ventriculaire disproportie als antenatale marker voor de ontwikkeling van een coarctatio aortae. In dit cohort ontwikkelden 60% van deze foetus postnataal echter géén coarctatio van de aorta, maar presenteerden zich met andere (prenataal niet-gedetecteerde) structurele afwijkingen of pulmonale of transitie-problemen. Postnatale monitoring is derhalve aangewezen en prenatale counseling dient zowel het risico's van een coactatio aortae alsmede andere afwijkingen en neonatale complicaties te bespreken.

Hoofdstuk 7 beschrijft een cohortstudie en systematisch literatuuronderzoek naar de neonatale uitkomsten en het risico op genetische diagnoses, extra-cardiale afwijkingen en ontwikkelingsachterstand onder foetus met truncus arteriosus (CAT). Ouders kozen in bijna de helft van de gevallen voor beëindiging van de zwangerschap. Na exclusie van deze casus, betrof het risico op intra-uterien overlijden of neonatale sterfte 40% onder doorgaande zwangerschappen. Verder was er sprake van significante morbiditeit bij helft van de overlevenden, waaronder het 22q11-deletiesyndroom, het Adams-Oliver-syndroom, intestinale atresie en neurologische ontwikkelingsachterstand. In de andere

helft van de overlevenden (of 30% van doorgaande zwangerschappen) kwam de CAT geïsoleerd voor en werd een normale ontwikkeling gezien.

In **hoofdstuk 8** presenteren we een internationale case serie van foetussen gediagnosticeerd met een aorto-linkerventriculaire tunnel (AVLT) om informatie te vergaren over de uitkomst van deze zeldzame hartafwijking vanaf de antenatale diagnose. Echocardiografische kenmerken van een AVLT betreffen een vergrote cardiothoracale ratio (95%), linkerventrikel eind-diastolische diameter (90%) en een dysplastische aortaklep (90%). Extra-cardiale afwijkingen komen zelden voor (5%). Alle casus die resulteerden in foetale sterfte, vertoonden tekenen van hydrops vóór 24 weken zwangerschap of ten tijde van autopsie. In afwezigheid van foetale hydrops lijkt een ALVT echter een goede prognose te hebben.

Conclusie en toekomstperspectief

Kennis over de volledige ontwikkeling, beginnend in de foetus, is essentieel om de zorg voor kinderen met een CHD te kunnen verbeteren. Vanwege de zeldzaamheid van individuele cardiale afwijkingen en de snelle innovaties op gebied van diagnostiek en behandeling is financiële ondersteuning essentieel om een groot nationaal CHD-register te ontwikkelen en te behouden. Zo'n register levert over een paar jaar essentiële gegevens om de prestaties van de nationale screening te monitoren en betrouwbaar over de uitkomsten te kunnen rapporteren en counselen.

Vroege identificatie van casus kan optimale zorg voor foetus en pasgeborenen met een CHD bevorderen. Toekomstig onderzoek moet zich richten op de effectiviteit van nieuwe strategieën om prenatale detectie van CHD te verbeteren, waaronder training in abnormale casus of psychologische interventies om verwijzingen naar gespecialiseerde centra te optimaliseren. Evaluatie van de toepasselijkheid van kunstmatige intelligentie is wenselijk, aangezien dit echoscopisten kan helpen bij het herkennen van foetale cardiale afwijkingen, zowel in westerse als niet-westerse gebieden wereldwijd.

Prenatale counseling voor CHD vereist voldoende kennis vanuit een foetaal perspectief, aangezien de prognose van foetus met een CHD significant kan verschillen van de prognose van pasgeboren of post-chirurgische neonaten. Toekomstige studies dienen de prognose van een (cardiale) diagnose derhalve altijd te relateren aan het tijdstip van diagnose. Indien beschikbare literatuur wordt geëvalueerd, dienen counselors zich hiervan bewust te zijn.

Tot slot dienen ouders al in een vroeg stadium voorgelicht te worden over de impact van (aanvullende) genetische diagnoses bij foetus met een CHD, gezien de snel evoluerende

technieken en mogelijkheden voor prenataal genetisch onderzoek. Toekomstig onderzoek is gewenst om een balans na te streven tussen het optimaliseren van snelle en complete genetische tests voor alle varianten, het beperken van de potentieel negatieve gevolgen en mogelijkerwijs het ontwikkelen van functionele testen om de vertaling van genetische resultaten naar hun bijbehorende fenotype te kunnen maken.

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ABOUT THE AUTHOR

Amber van Nisselrooij werd geboren op 6 april 1994 in het Maxima Medisch Centrum te Veldhoven. Tijdens haar jeugd woonde ze met haar ouders en jongere broer Tijmen in Dommelen (Noord-Brabant).

In 2012 behaalde zij haar gymnasiumdiploma aan Scholengemeenschap Were Di in Valkenswaard, waarna ze startte met de studie Geneeskunde aan de Universiteit Leiden. In 2015 behaalde ze haar bachelorsdiploma Geneeskunde en rondde ze het Honours College-traject af aan de Universiteit Leiden. Dit traject richtte zich in het bijzonder op klinische epidemiologie. Vanuit haar interesse in kindercardiologie deed ze onderzoek onder begeleiding van prof. dr. M.C. Haak (aandachtsgebied: foetale cardiologie) en prof. dr. N.A. Blom (kindercardioloog) in het Leids Universitair Medisch Centrum (LUMC).

In 2016 begon ze - op haar verzoek - de master met een verlengde wetenschapsstage en presenteerde ze haar onderzoek op het congres van de International Society of Ultrasound in Obstetrics and Gynecology in Montréal. Het enthousiasme voor onderzoek naar aangeboren hartafwijkingen was geboren. In 2017 kreeg ze een beurs vanuit Stichting Hartekind om fulltime onderzoek te doen, waarvoor de master Geneeskunde werd gepauzeerd. In 2018 verkreeg Amber een tweejarige aanstelling als promovendus (MD-PhD) aan de Universiteit Leiden om het promotietraject voort te zetten, waaruit dit proefschrift is voortgekomen.

In 2020 heeft ze de master Geneeskunde hervat, waarna ze in 2022 cum laude het artsexamen heeft behaald. Vanaf maart 2023 was Amber werkzaam als arts-assistent Kindergeneeskunde in het Juliana Kinderziekenhuis in Den Haag. Inmiddels werkt ze met veel plezier op de Intensive Care Kinderen van het LUMC.

Amber heeft een bijzondere interesse ontwikkeld voor zorg in lage-inkomenslanden naar aanleiding van klinische stages in het Provincial General Hospital in Nakuru (Kenia) en het Kilimanjaro Christian Medical Centre in Moshi (Tanzania). Vanaf juni 2023 is Amber lid van de Young Leadership board van 'Save a Child's Heart Nederland' om de zorg voor kinderen met hartafwijkingen wereldwijd te verbeteren. Daarnaast hoopt ze in de toekomst niet alleen in Nederland werkzaam te zijn, maar tevens de zorg in niet-Westerse landen te ondersteunen.

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