Congenital heart defects: from a prenatal perspective

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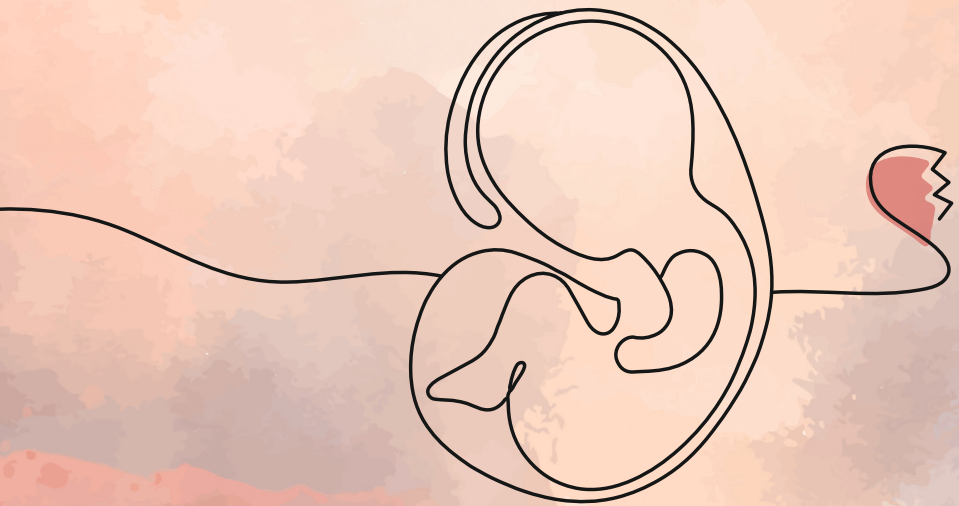

CHAPTER 5

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).

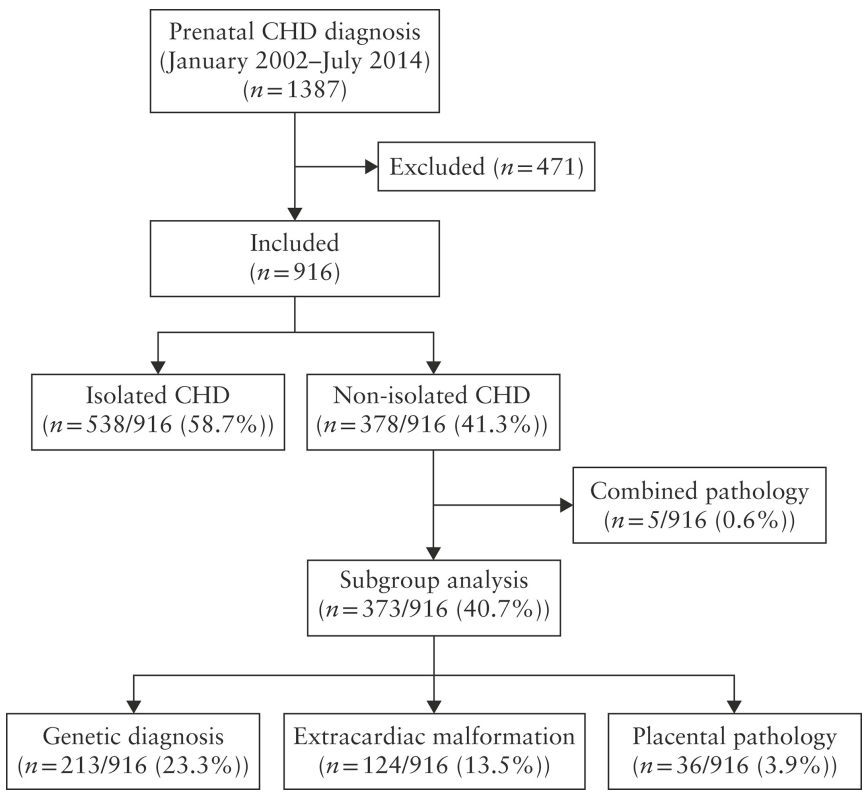

Figure 1. Flowchart summarizing case selection and inclusion from a cohort of fetuses with congenital heart defects.

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.

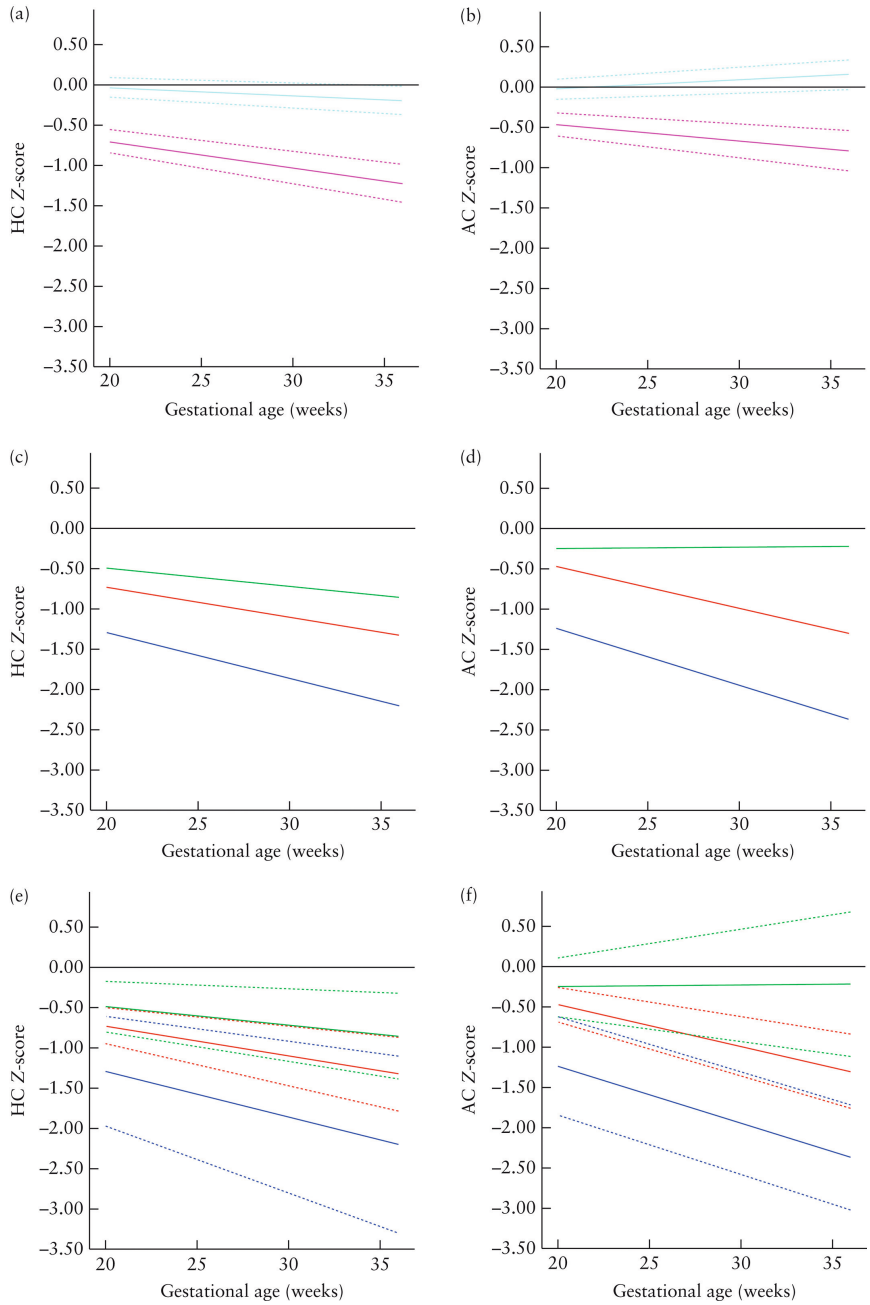

Figure 2. Head (a,c,e) and abdominal (b,d,f) growth trajectories of fetuses with congenital heart defect (CHD): (a,b) in all cases, according to whether CHD was isolated (cyan) or non-isolated (magenta); (c–f) in non-isolated CHD cases, subdivided according to additional pathology (red, genetic anomaly; 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**				