Imaging the prenatal brain in congenital heart defects

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

CHAPTER 7

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ABSTRACT

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

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

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

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

INTRODUCTION

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

Methods

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

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

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

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

Clustering of CHDs

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

Statistical analysis

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

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

Results

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

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

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

Sub group analysis

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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Tables

Table 1 *Baseline characteristics of included cases*

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

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

† Other major CHD include:

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

‡ Other minor CHD include:

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

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

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

Legend:

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

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

Flow: aortic arch blood flow.

O2: Oxygen saturation in the ascending aorta.

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

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

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

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

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

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

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

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

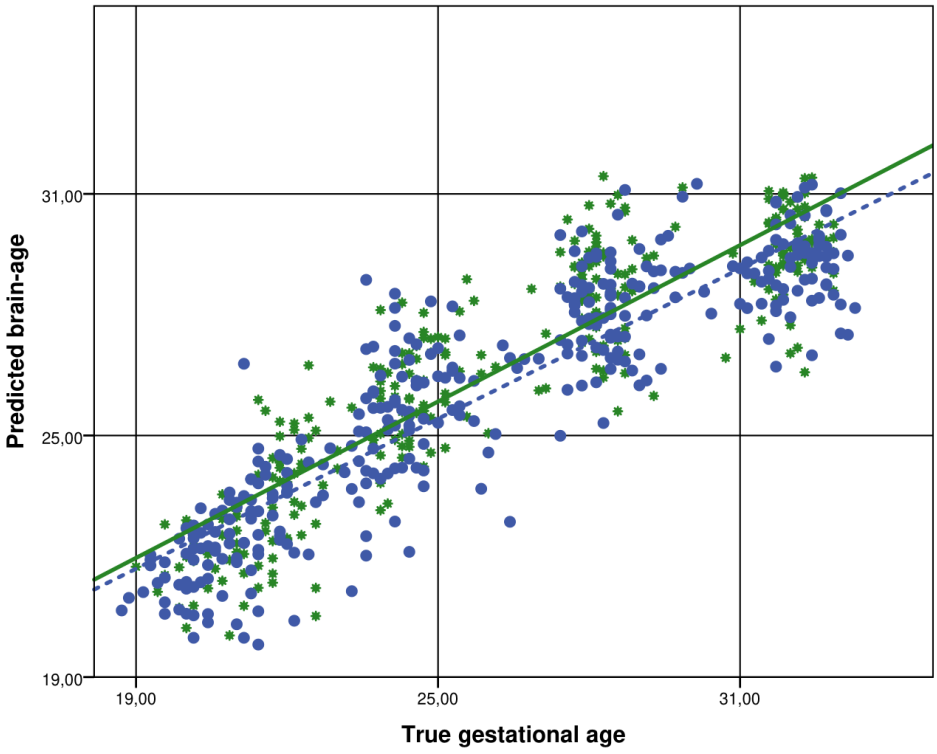

Figure 1 *Predicted brain age (y-axis) is plotted against the true gestational age (x-axis) in weeks. Linear lines show results of linear mixed model regression. Green star (✱) and continuous line (—) all controls. Blue dot (●) and - - - (dashed line) all cases.*

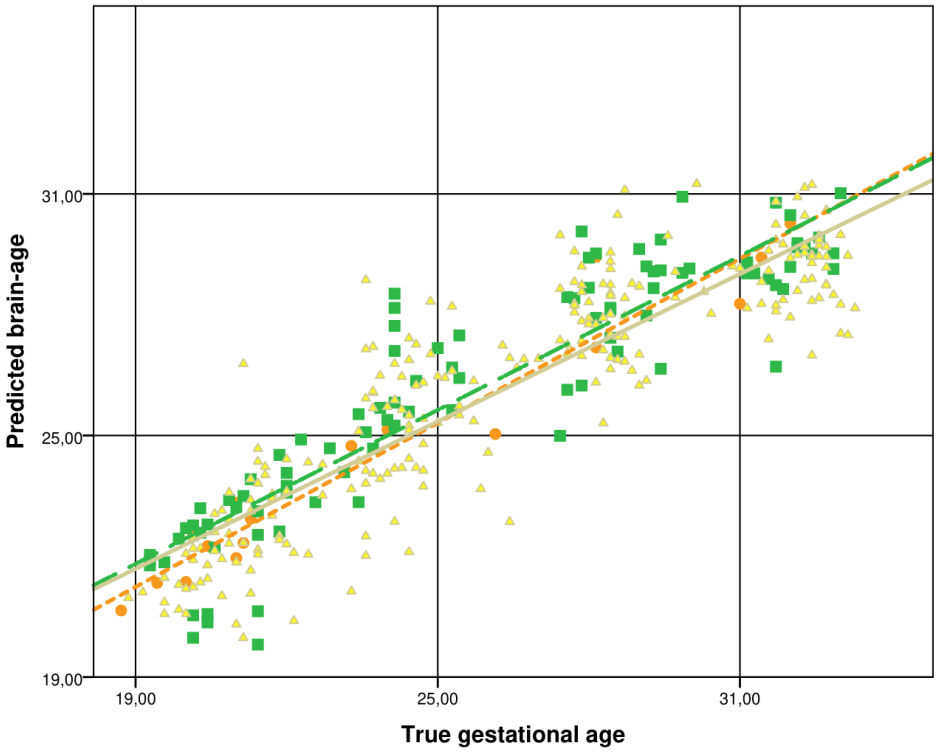

Figure 2 *Influence of aortic arch flow towards the fetal brain on brain-age in fetuses with isolated congenital heart defects. Linear lines show results of sub-analysis with linear mixed model regression. Green square (■) and interrupted line (– –) obstructed flow, orange dot (●) and dashed line (-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

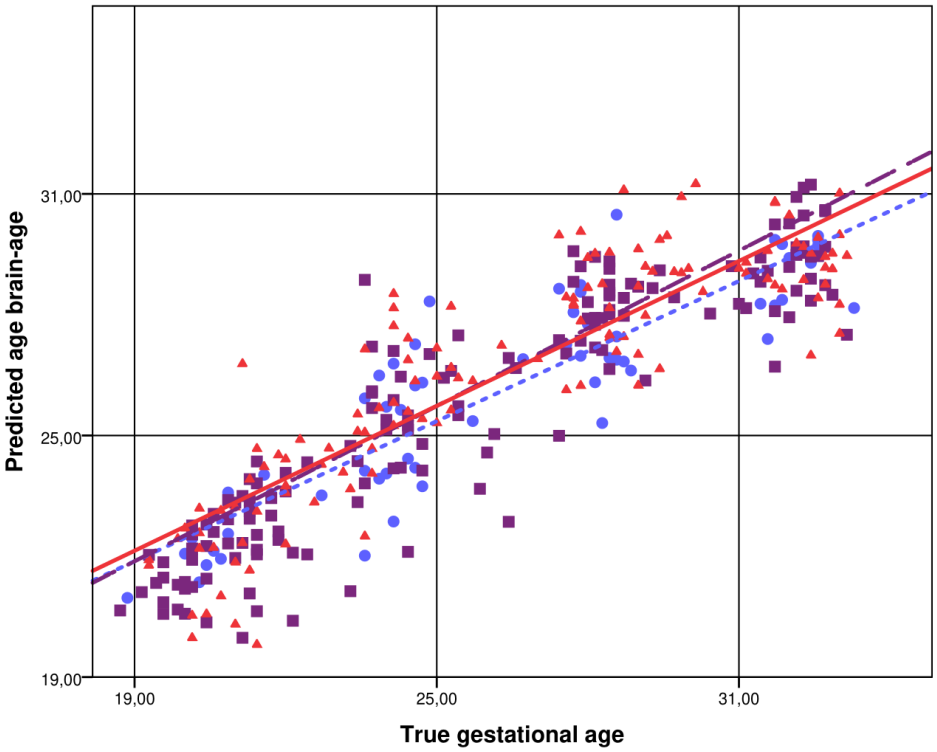

Figure 3 *Influence of ascending aorta oxygen saturation on brain-age in fetuses with isolated congenital heart defects. Linear lines show results of subanalysis with linear mixed model regression. Blue dot (●) and dashed line (- - -) low oxygen saturation, purple square (■) and interrupted line (— —)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

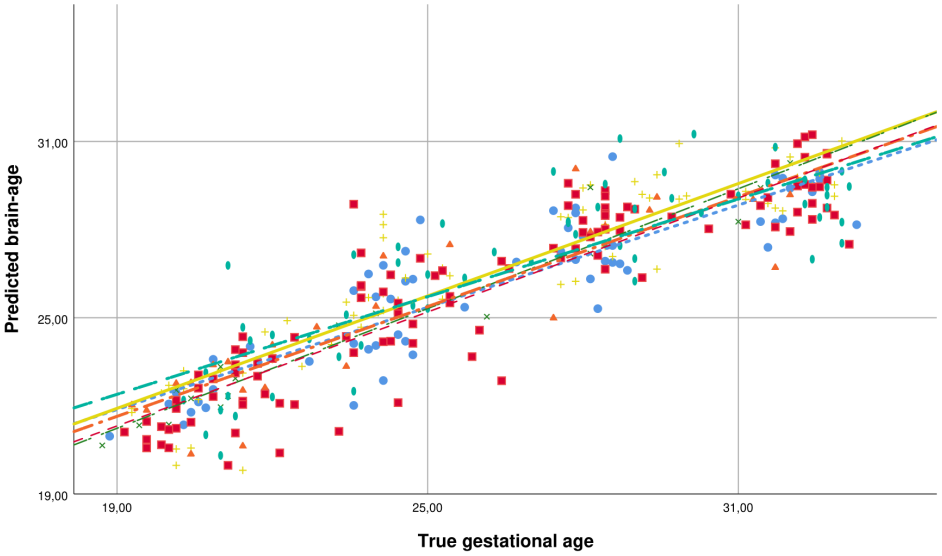

Figure 2 *Influence of CHD diagnoses on estimated brain-age. Linear lines show results of subanalysis with linear mixed model regression.*
Blue dot (●) and dashed line (- - -) normal flow + low O2: Transposition of the great arteries.
Yellow plus (+) and continuous line (——) obstructed flow + normal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

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

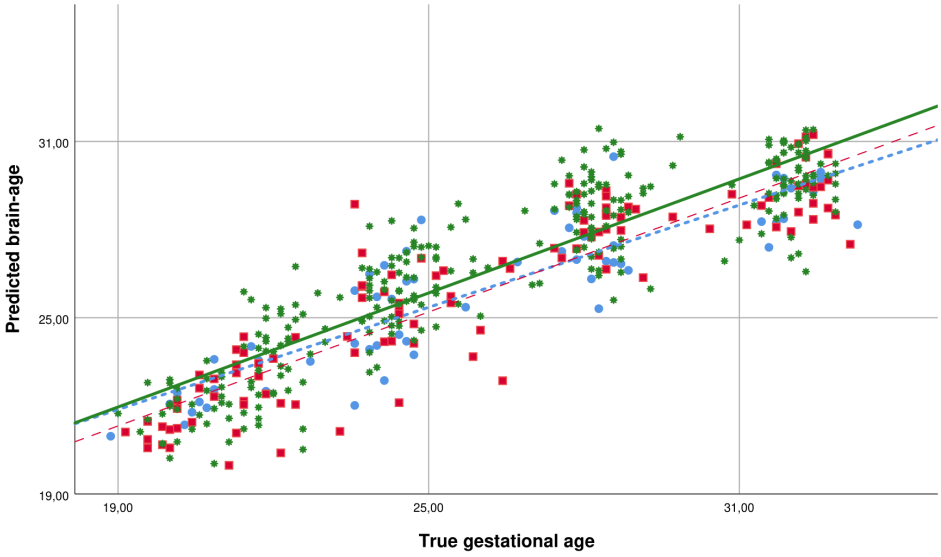

Figure 5 *Significant influence of transposition of the great arteries and Fallot/ Fallot-like congenital heart defects on prenatal brain-age as compared to controls. Linear lines show results of subanalysis with linear mixed model regression. Green star (✱) and continuous line (—) : all controls. Blue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