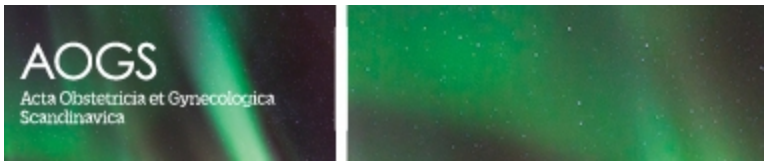


Cortical development in fetuses with congenital heart defects using an automated brain-age prediction algorithm

Journal:	*Acta Obstetricia et Gynecologica Scandinavica*
Manuscript ID	AOGS-19-0358.R1
Wiley - Manuscript type:	Original Research Article
Date Submitted by the Author:	14-Jun-2019
Complete List of Authors:	Everwijn, Sheila; Leiden University Medical Center, Obstetrics Namburete, Ana; University of Oxford, Institute of biomedical engineering van Geloven, Nan; Leiden Universitair Medisch Centrum Jansen, Fenna; Leiden University Medical Center, Obstetrics and fetal therapy Papageorghiou, Aris ; John Radcliffe Hospital, Obstetrics and Gynecology Noble, Alison; University of Oxford Teunissen, Aalbertine; Leiden Universitair Medisch Centrum, Obstetrics Rozendaal, Lieke; Leiden University Medical Center, Pediatric Cardiology Blom, Nico; Leids Universitair Medisch Centrum, Pediatric Cardiology van Lith, Jan; Leiden University Medical Center, Obstetrics and Fetal therapy Haak, Monique; Leiden University Medical Center, Obstetrics and fetal therapy
Keywords:	Prenatal diagnosis, Ultrasound

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

Sheila M. P. Everwijn[1]; Ana I. L. Namburete[2]; Nan van Geloven[3], Fenna A. R. Jansen[1], Aris T. Papageorghiou[4], Alison J. Noble[2] , Aalbertine K. K. Teunissen[1], Lieke Rozendaal[5], Nico A. Blom[5], Jan M. M. van Lith[1], Monique C. Haak[1]

1 Department of Obstetrics and Prenatal Diagnosis, Leiden University Medical Center, Leiden, Netherlands

2 Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Oxford, UK

3 Department of Biomedical Data Sciences, Section Medical Statistics, Leiden University Medical Center, Leiden, Netherlands

4 Nuffield Department of Obstetrics and Gynecology, John Radcliffe Hospital, University of Oxford, UK

5 Department of Pediatric Cardiology, Leiden University Medical Center, Leiden, Netherlands

Corresponding Author:

Sheila Maria Pieternella Everwijn

Leiden University Medical Center, Department of Obstetrics – prenatal diagnosis and fetal therapy K6-P, Postbus 9600, 2300 RC Leiden , The Netherlands

E-mail: s.m.p.everwijn@lumc.nl

Conflict of interest

None

Funding

The authors received no specific funding for the completion of this work. ATP is supported by the Oxford Partnership Comprehensive Biomedical Research Centre with funding from the NIHR Biomedical Research funding scheme.

ABSTRACT

Introduction: Congenital heart defects are associated with neurodevelopmental delay. It is hypothesized that fetuses affected by congenital heart defect have altered cerebral oxygen perfusion and are therefore prone to delay in cortical maturation. The aim of this study was to determine the difference in fetal brain age between consecutive congenital heart defect cases and controls in the second and third trimester using ultrasound. **Material and methods:** Since 2014, we have included 90 isolated severe congenital heart defect cases in the Heart And Neurodevelopment(HAND)-study. Every four weeks, detailed neurosonography was performed in these fetuses, including the recording of a 3D volume of the fetal brain, from 20 weeks onward. 75 healthy fetuses underwent the same protocol to serve as a control group. The volumes were analyzed by automated age prediction software which determines gestational age by the assessment of cortical maturation. **Results**: In total 477 volumes were analyzed using the age prediction software (199 volumes of 90 congenital heart defect cases; 278 volumes of 75 controls). 16 (3.2%) volume recordings were excluded because of imaging quality. The age distribution was 19-33 weeks. Mixed model analysis showed that the age predicted by brain maturation was 3.0 days delayed compared to the control group ($p = 0.002$). **Conclusions**: This study shows that fetuses with isolated cases of congenital heart defects show some delay in cortical maturation as compared to healthy control cases. The clinical relevance of this small difference is debatable. This finding was consistent throughout pregnancy and did not progress during the third trimester.

Keywords:

Congenital Heart Defects, Malformations of cortical development,| Ultrasonography, Fetus, Neurodevelopmental outcome

Key Message:

Fetuses with congenital heart defects are shown to have a slight delay in cortical maturation when compared to controls, using a novel brain-age prediction algorithm.

Abbreviations

CHD	congenital heart defect
MRI	magnetic resonance imaging
GA	gestational age
POF	parieto-occipital fissure
SF	sylvian fissure

INTRODUCTION

Improvements over time in the quality of neonatal care and cardiothoracic surgery in children with congenital heart defects (CHD) have resulted in an increased survival of children with severe CHD. This has stimulated longer term follow up and a recognition that there is an association between CHD and impaired neurodevelopmental outcome.[1, 2] Developmental delay, decreased IQ and behavioral disorders have been reported, even in non-syndromic CHD children.[1]

Previously, these sequelae were attributed to perioperative hypoxia or thrombo-embolic events during surgery. Recent studies suggest, however, that signs of abnormal neurological development may be present prior to surgery.[3-5] Imaging studies in pregnancy using magnetic resonance imaging (MRI)[6, 7] and ultrasound [8, 9], have shown signs of delayed fetal brain development. It has been suggested that it is these abnormal findings that result in the altered neurological outcome later in life.[5, 6] The hypothesized mechanism is that the abnormal development of the brain is the result of altered brain oxygenation in fetal life.[10, 11]

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

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

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

MATERIAL AND METHODS

Data acquisition

All consecutive pregnant women, diagnosed with a fetal CHD before 32 weeks gestation at the Leiden University Medical Center between March 2014 and December 2016, were approached to participate in the Heart And Neurodevelopment (HAND)-study. To account for natural variation of cortical development in the healthy population, we constructed a control group by the recruitment of unselected pregnant women after a normal structural anomaly scan. Control cases were not offered additional genetic testing but had a postnatal visit in which dysmorphic featured were assessed. Gestational age (GA) in both the CHD cases and the control cases, was based on first-trimester ultrasound at approximately 10 weeks gestation, according to Dutch national guidelines. For both cases and controls we excluded: Maternal age <18 years, multiple gestation, genetic or syndromic defects (prenatally diagnosed or postnatally apparent up to the age of six months), cases with placental pathology (pre-eclampsia, severe growth restriction) and cases that showed normal cardiac anatomy after birth. In the CHD group, non-isolated cases were excluded. The reasons for only including isolated CHD was that altered neurodevelopment could otherwise be attributed to genetic or syndromic defects. Furthermore, cases with aortic valve stenosis that underwent fetal balloon valvuloplasty were excluded, since fetal brain oxygenation may have changed due to the intervention during pregnancy.[12] Also, strictly minor cases (persistent left caval vein, mild pulmonary stenosis and restrictive foramen ovale) were also excluded, since in these cases, blood flow towards the brain is expected to be uncompromised. The sample size calculation for the normal reference population was based on the available evidence from two MRI-studies[7, 13] that compared hypoplastic left heart syndrome (HLHS)-fetuses to controls, to detect a difference in mean brain age of two weeks. The normal reference population was calculated to consist of 60 fetuses. The CHD-group contains all the women that met the inclusion criteria and were referred between March 2014 – December 2016.

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

A detailed neurosonographic examination was performed in cases and controls every four weeks after the diagnosis or in the case of controls after normal standard anomaly scan.

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

Brain-Age prediction algorithm

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

gyrification of the fetal cortex, which varies during gestation (figure 2). Furthermore, since the true GA was known for each case, we were able to compare the brain-age with the true age to determine any delay in cortical maturation. A more extensive description of the algorithm is available as Supporting information Appendix S1.

Data handling

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

Statistical analyses

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

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

Ethical Approval

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

RESULTS

In the study period, 90 consecutive CHD cases and 75 controls were included (see Table 1 for study characteristics). The groups were not prospectively matched for baseline characteristics, however the groups did not differ significantly in maternal age, parity, body mass index or maternal diabetes. We excluded 14 cases according the defined exclusion criteria, of which eight were postnatal diagnoses of genetic syndromes (three CHARGE syndrome, two Kabuki syndrome, three with postnatal multiple dysmorphic features, final genetic diagnosis pending). The genetic diagnosis of the CHD-cases was followed up until one year postnatally. 30 % of control cases opted for first-trimester screening. No genetic or structural abnormalities were found in the control-group up to six months postnatally. Thus, a total of 152 CHD cases and controls were eligible for analysis. From these 152 women, in 493 scanning sessions, volume recordings were made. Of these volumes, 16 (3.2%) were excluded due to ultrasonographic factors (oblique insonation, fetal movement artifacts or very poor image quality), resulting in 477 volumes suitable for analysis by the age-prediction algorithm. In total, 199 volumes in 77 cases (mean of 2.4 recorded volumes per woman) and 278 volumes in 75 controls (mean of 3.7 recorded volumes per woman) were analyzed using the automated age prediction algorithm. The CHD cases were scanned at 1-5 different time points during pregnancy, with 63% of the women scanned more than once. For the control group, all cases were measured more than once.

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

The overall test indicated that the time trend significantly differed between CHD-cases and controls ($p=0.005$) indicating that indeed there was a group effect. When comparing CHD

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

DISCUSSION

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

Neurodevelopmental delay in CHD children has been recognized for decades, even with optimized pre-operative and neonatal care.[20] prenatal brain damage is hypothesized to result from the altered hemodynamics caused by the cardiac defect, which may result in decreased flow or oxygenation of the blood directed towards the brain [21, 22], resulting in delayed brain development. Increased N-acetylaspartate to choline (NAA:Cho)-ratio and increased lactate levels in MRI and spectroscopy studies support a decrease in brain oxygenation in the developing fetal brain of fetuses with CHD.[19, 23]

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

The differences in the results of these studies can be explained by the small sample sizes, different methodologies, and the differences in statistical analysis of the data.[9]

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

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

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

Whether a delay of three days is clinically relevant, is debatable. On the other hand one could argue that even though differences are small, they could still have an impact on long term

outcome, since the detected delay is visible in early life.[27] Two of the studies mentioned above[6, 8] found significant differences when correlating cortical maturation and neurodevelopmental outcome by performing Bayley Scales of Infant and Toddler Development (BSID). However, authors performed BSID in a minority of infants and, paradoxically, only in milder CHD cases. With this sparse evidence, it is undisputable that there is an urgent need to explore the relation between altered fetal brain maturation and neurodevelopmental outcome further. As this is a limitation of the current study, we are planning to correlate the findings in this cohort to postnatal neurodevelopment.

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

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

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

CONCLUSION

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

REFERENCES

1. Marino BS, Lipkin PH, Newburger JW, et al. Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association. *Circulation*. 2012;126(9):1143-72.
2. Paladini D, Alfirevic Z, Carvalho JS, et al. ISUOG consensus statement on current understanding of the association of neurodevelopmental delay and congenital heart disease: impact on prenatal counseling. *Ultrasound Obstet Gynecol*. 2017;49(2):287-8.
3. Licht DJ, Shera DM, Clancy RR, et al. Brain maturation is delayed in infants with complex congenital heart defects. *J Thorac Cardiovasc Surg*. 2009;137(3):529-36; discussion 36-7.
4. Jansen FA, Everwijn SM, Scheepjens R, et al. Fetal brain imaging in isolated congenital heart defects - a systematic review and meta-analysis. *Prenat Diagn*. 2016;36(7):601-13.
5. Majnemer A, Limperopoulos C, Shevell MI, Rohlicek C, Rosenblatt B, Tchervenkov C. A new look at outcomes of infants with congenital heart disease. *Pediatr Neurol*. 2009;40(3):197-204.
6. Masoller N, Sanz-Cortes M, Crispi F, et al. Mid-gestation brain Doppler and head biometry in fetuses with congenital heart disease predict abnormal brain development at birth. *Ultrasound Obstet Gynecol*. 2016;47(1):65-73.
7. Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. Delayed cortical development in fetuses with complex congenital heart disease. *Cereb Cortex*. 2013;23(12):2932-43.
8. Peng Q, Zhou Q, Zang M, et al. Reduced fetal brain fissures depth in fetuses with congenital heart diseases. *Prenat Diagn*. 2016;36(11):1047-53.
9. Koning IV, van Graafeiland AW, Groenenberg IAL, et al. Prenatal influence of congenital heart defects on trajectories of cortical folding of the fetal brain using three-dimensional ultrasound. *Prenat Diagn*. 2017;37(10):1008-16.
10. Sun L, Macgowan CK, Sled JG, et al. Reduced fetal cerebral oxygen consumption is associated with smaller brain size in fetuses with congenital heart disease. *Circulation*. 2015;131(15):1313-23.
11. Al NB, van Amerom JF, Forsey J, et al. Fetal circulation in left-sided congenital heart disease measured by cardiovascular magnetic resonance: a case-control study. *J Cardiovasc Magn Reson*. 2013;15:65.
12. Prosnitz AR, Drogosz M, Marshall AC, et al. Early hemodynamic changes after fetal aortic stenosis valvuloplasty predict biventricular circulation at birth. *Prenat Diagn*. 2018;38(4):286-92.
13. Mlczoch E, Brugger P, Ulm B, et al. Structural congenital brain disease in congenital heart disease: Results from a fetal MRI program. *Eur J Paediatr Neurol*. 2012.
14. Clouchoux C, Kudelski D, Gholipour A, et al. Quantitative in vivo MRI measurement of cortical development in the fetus. *Brain Struct Funct*. 2012;217(1):127-39.

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

16. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006;31(3):1116-28.

17. Namburete AI, Stebbing RV, Kemp B, Yaqub M, Papageorghiou AT, Alison Noble J. Learning-based prediction of gestational age from ultrasound images of the fetal brain. *Med Image Anal*. 2015;21(1):72-86.

18. van Velzen CL, Clur SA, Rijlaarsdam ME, et al. Prenatal diagnosis of congenital heart defects: accuracy and discrepancies in a multicenter cohort. *Ultrasound Obstet Gynecol*. 2016;47(5):616-22.

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

20. Dittrich H, Buhrer C, Grimmer I, Dittrich S, Abdul-Khaliq H, Lange PE. Neurodevelopment at 1 year of age in infants with congenital heart disease. *Heart*. 2003;89(4):436-41.

21. Zeng S, Zhou J, Peng Q, et al. Assessment by three-dimensional power Doppler ultrasound of cerebral blood flow perfusion in fetuses with congenital heart disease. *Ultrasound Obstet Gynecol*. 2015;45(6):649-56.

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

23. Azpurua H, Alvarado A, Mayobre F, Salom T, Copel JA, Guevara-Zuloaga F. Metabolic assessment of the brain using proton magnetic resonance spectroscopy in a growth-restricted human fetus: case report. *Am J Perinatol*. 2008;25(5):305-9.

24. Alonso I, Borenstein M, Grant G, Narbona I, Azumendi G. Depth of brain fissures in normal fetuses by prenatal ultrasound between 19 and 30 weeks of gestation. *Ultrasound Obstet Gynecol*. 2010;36(6):693-9.

25. Alves CM, Araujo Junior E, Nardozza LM, et al. Reference ranges for fetal brain fissure development on 3-dimensional sonography in the multiplanar mode. *J Ultrasound Med*. 2013;32(2):269-77.

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

27. Miller SL, Huppi PS, Mallard C. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *J Physiol*. 2016;594(4):807-23.

28. Griffiths PD, Bradburn M, Campbell MJ, et al. Use of MRI in the diagnosis of fetal brain abnormalities in utero (MERIDIAN): a multicentre, prospective cohort study. *Lancet*. 2017;389(10068):538-46.

29. Kelly CJ, Makropoulos A, Cordero-Grande L, et al. Impaired development of the cerebral cortex in infants with congenital heart disease is correlated to reduced cerebral oxygen delivery. *Sci Rep*. 2017;7(1):15088.

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

SUPPORTING INFORMATION legend

Appendix S1. Description of the algorithm

FIGURE LEGENDS

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

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

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

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

422 **Table 1.** Baseline characteristics of included cases.

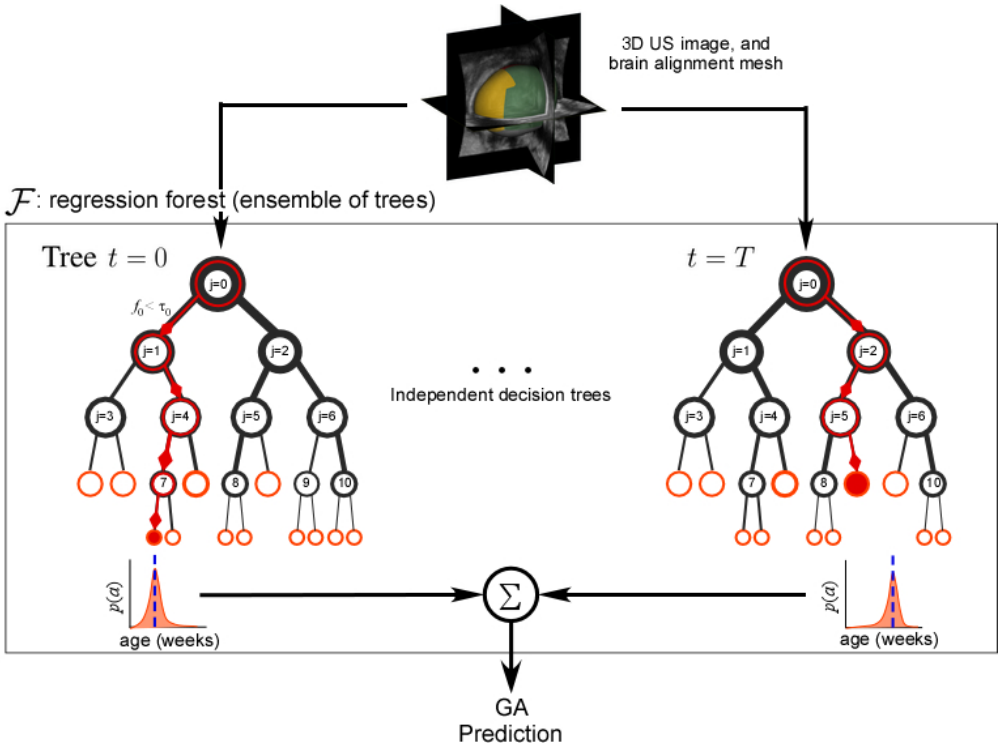
423

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

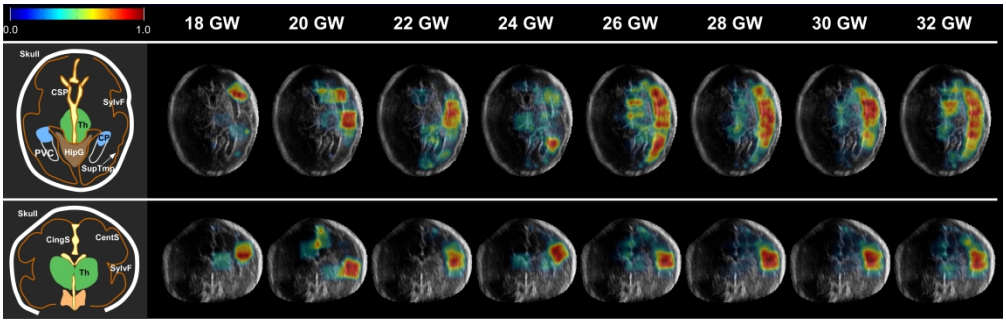
Fetal Intervention	3		
Postnatal non-isolated/syndromic	8		
Postnatal normal heart	3		
Pregnancy outcome			n.a.
Live birth	75 (83%)	75(100%)	
Termination of Pregnancy	15 (17%)	0(0%)	

Abbreviations: CHD, Congenital Heart Defect; SD, Standard Deviation; BMI, Body Mass Index; HLHS, Hypoplastic Left Heart Syndrome; TGA, Transposition of the Great Arteries;

[a] Other major CHD include: Truncus Arteriosus, Multiple level left obstruction syndrome (Shone's complex), Double Outlet Right Ventricle-TGA, Congenitally Corrected TGA.



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



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

1863x587mm (96 x 96 DPI)

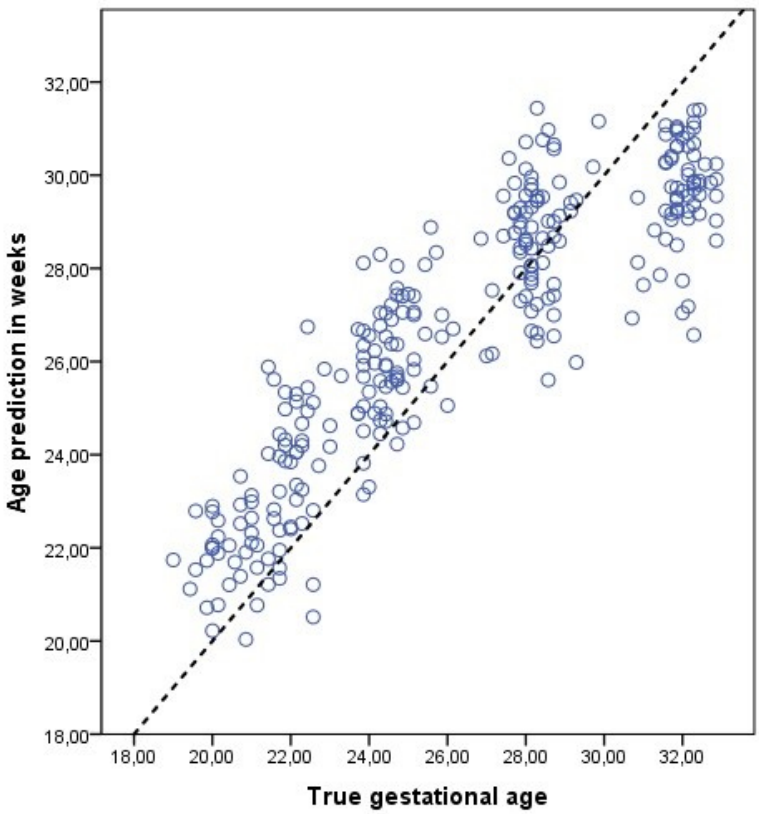


Figure 3. Regression plot for 75 control cases: gestational age('true age') on the x-axis and age prediction on the y-axis.

159x134mm (96 x 96 DPI)

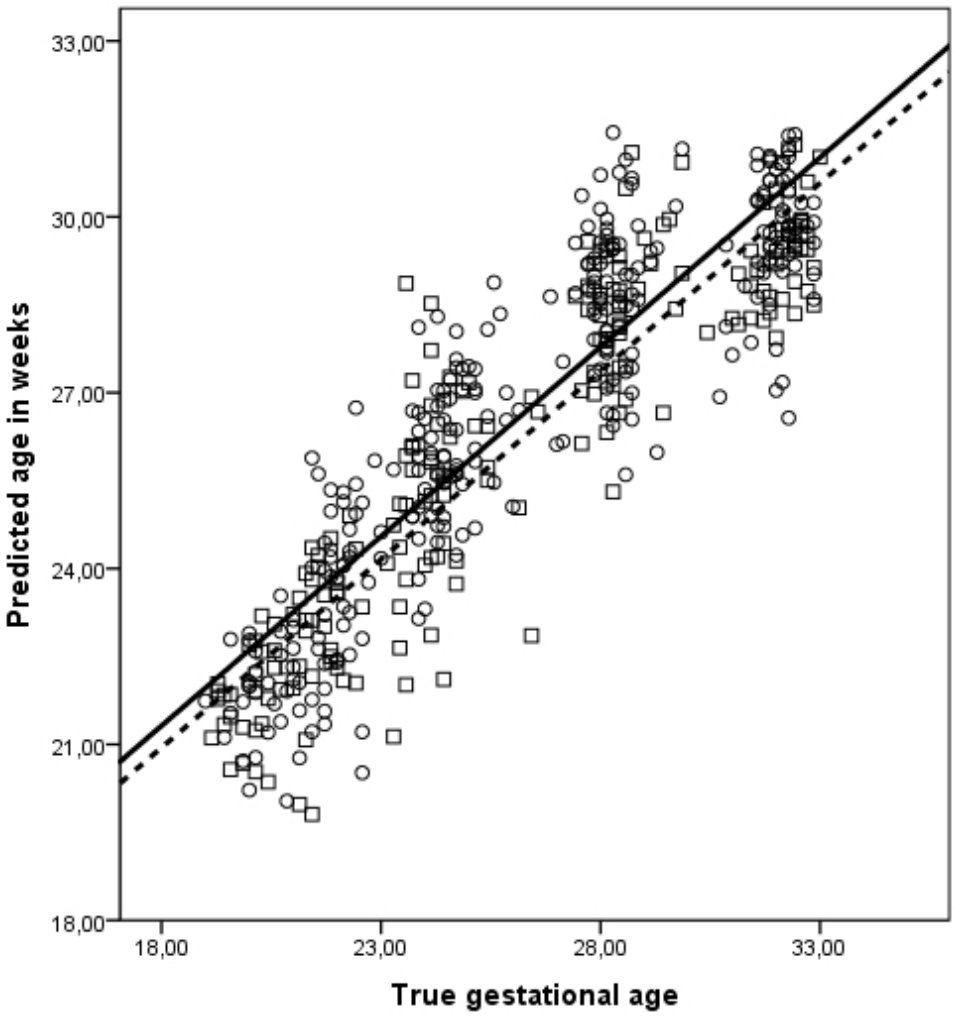


Figure 4. The x-axis shows the gestational age at ultrasound('true age'), the y-axis shows age as predicted by the algorithm. Legend: □ CHD cases, - - (interrupted line), ○ control cases. ── (continuous line).

133x135mm (96 x 96 DPI)

Supplement

Estimation of gestational age is an essential part of safe obstetrical care. In order to provide an accurate age estimation, an ultrasonographic examination is performed around 10 weeks' gestation.
According to the 'ISUOG Practice Guideline: Performance of first-trimester fetal ultrasound scan': *Accurate dating provides valuable information for the optimal assessment of fetal growth and appropriate obstetric management. Dating a pregnancy by ultrasound between 10+0 and 13+6 weeks appears to be the most reliable method with which to establish true gestational age.*
The age prediction algorithm which we have used in a clinical group of CHD-fetuses, originates from the idea that not all women have access to a first-trimester ultrasound scan to accurately date their pregnancy. Especially women living in rural settings are at risk for erroneous obstetrical management, resulting from inaccuracies in gestational age.

The age prediction algorithm consists of an automated machine learning-based predictive model which has the ability to learn patterns of fetal brain changes over time. This model was 'trained' by 448 3D US images (GA range 18+0-33+6 weeks) from the INTERGROWTH-21st database (Papageorghiou et al. Lancet. 2014). This database contains a group of healthy volunteers from a low risk setting, for which pregnancy dating was performed following the ISUOG practice guideline as mentioned above. In addition, to account for natural variation in left and right hemispheres, only abdominal 3D Ultrasound(US) volumes were used with both visible hemispheres.
This supplement provides a more detailed description of the algorithm used in the main article.

Brain Feature Selection
Three-dimensional US volumes contain a large amount of information and possibly several neighbouring image patches containing similar information. Reducing the number of surface/image 'points' included in the search space reduces redundancy which in turn improves the computational cost. To this end, the cranial surface was densely evaluated with a pre-selected number of points to represent anatomical regions of interest, P (Figure S.1).

However, due to the effects of cranial calcification, the brain hemisphere proximal to the US probe is typically occluded, leaving only the distal hemisphere with visible and discernible intracranial structures. As such, feature extraction is confined to points on half of the cranial surface corresponding to the distal cerebral hemisphere in the image volume (Figure S.1). For simplicity, the surface is split by the midsagittal plane— defined by a normal vector and the centre point of the plane.

Appearance-Based Features

The appearance-based features used by the model comprise of two groups: sulcal and intracranial VOIs. Sulcal features are evaluated by affixing the VOI to the inner cranial surface (Figure S.2). They are designed to capture the sonographic image appearance related to changes in shape and morphology of the sulci and gyri on the cortical surface across gestation.

Intracranial features, on the other hand, are evaluated by displacing the VOI along the vector normal to $\boldsymbol{Y}^m$ (Figure S.1). For these features, the VOI can be placed anywhere in the trajectory between the inner skull and the falx cerebri (or midsagittal plane, $\boldsymbol{Y}^m$). This ultimately covers the entire space in the cerebral hemisphere of choice.

Biometry-Based Features

Guided by prenatal assessment of fetal growth, the biometric feature is akin to the clinical head circumference (HC) measurement acquired from the standard transthalamic (TT) plane of the head. In this case, the feature is evaluated as the perimeter of the inner contour of the deformed cranial surface at the level of the diagnostic TT plane. The biometric HC feature captures global changes in head size in a manner similar to the current clinical method of GA estimation, emulating global (rigid) transformations related to fetal head growth.

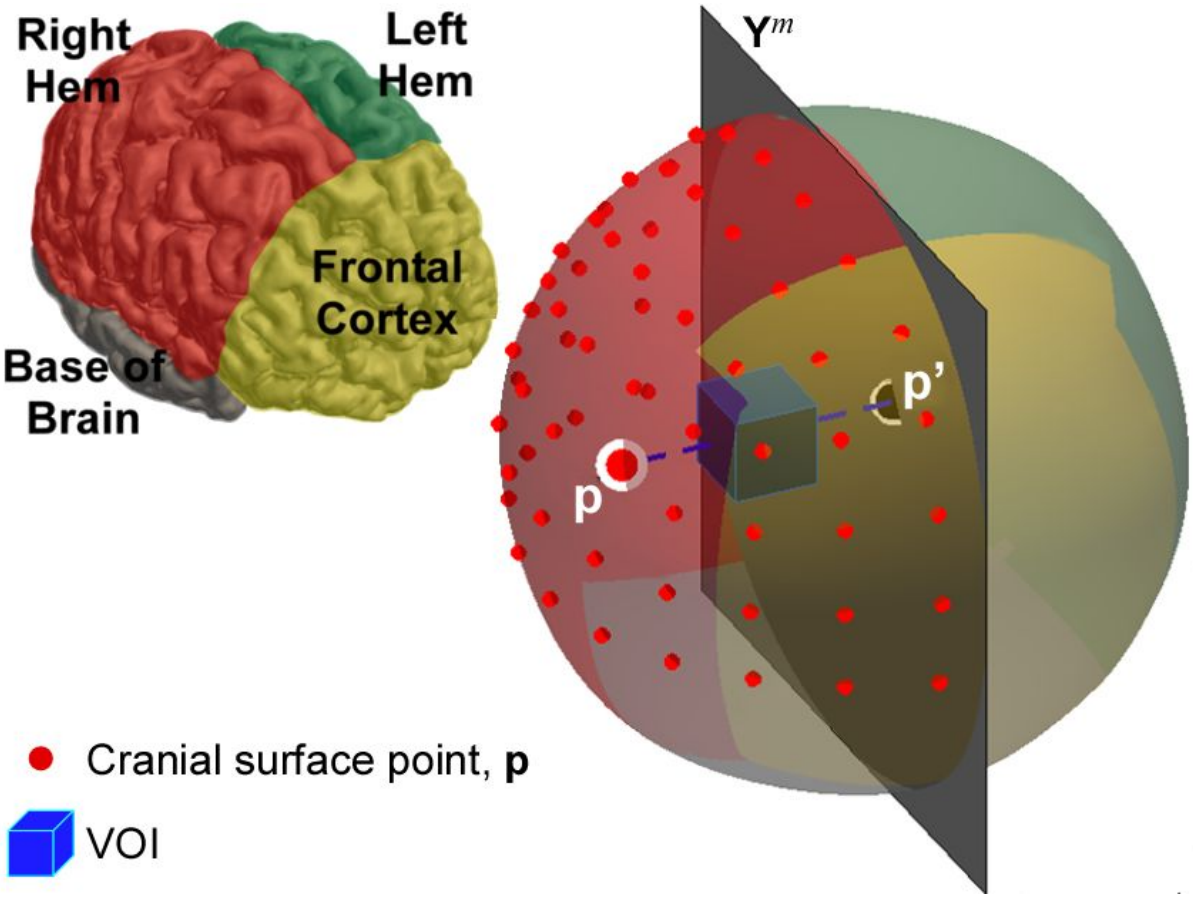


Figure S.1.

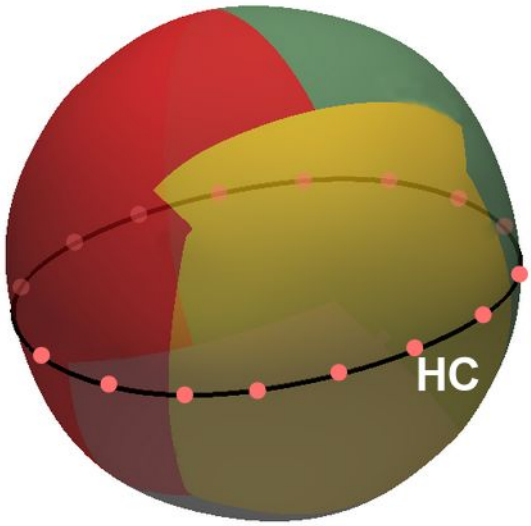


Figure S.2.

GA Prediction

During age prediction, an unseen data sample (i.e. brain volume and corresponding surface) of unknown GA traverses the nodes in each tree of the trained forest model, and the binary test associated with each split node evaluates whether to send the data to the left or right child nodes, until the sample eventually reaches a leaf node (Figure S.3). For each tree, the leaf node reached provides a mean age estimate with an associated variance. Leaf nodes with high variance values have lower age certainty, so they are assumed to be less informative and likely to add noise to the output predictions. Therefore, a single prediction $\bar{a}$ is generated by taking the mean of the GA estimated by all trees in the regression forest. Specifically:

$$\bar{a} = \sum_{t \in T} a_t$$

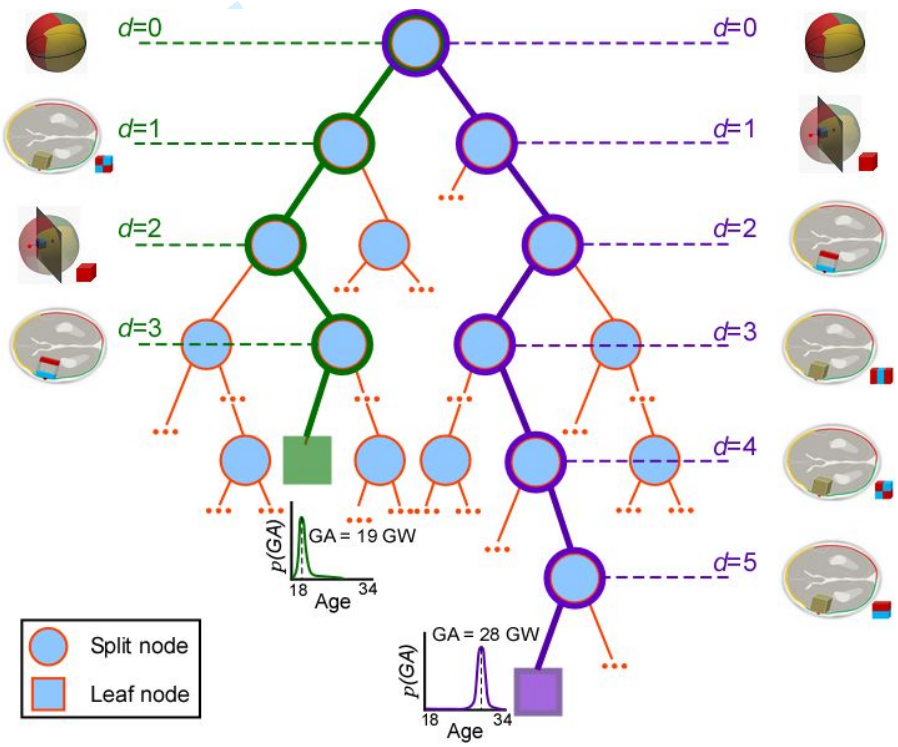


Fig S.3. GA Determination (Data used for model training)

In the INTERGROWTH-21st study, GA was defined by the known last menstrual period (LMP) and confirmed by a first-trimester (acquired between 9+0 and 13+6 GW) ultrasonographic measurement of the fetal crown-rump length. For added confidence, true GA was defined as the LMP- and CRL-based ages agreeing within 7 days, where the CRL-based GA is accurate within 2.7 days, as determined from 3 independent clinical measurements. All women included in the study were screened according to the INTERGROWTH-21st criteria and scanning protocol, and were thus identified as having low risk of impaired fetal growth and absence of fetal brain anomalies. Consequently, it was assumed that there were no delays in cortical development and that the fetal brains included in the study exhibited age-appropriate structural composition.

Image Selection for Model Training

Due to the fact that fetal head US images are typically challenged by reverberation artefacts (a consequence of increasing calcification of the cranial bones), the cerebral hemisphere proximal to the ultrasound probe is partially obstructed. Thus, the images selected for model training were included based on the following criteria:

1. Cranium occupies ≥ 50% of the image
2. Distal cranial hemisphere is unobstructed
3. Interhemispheric fissure and falx cerebri are clearly visible in the entire supratentorial region
4. Sylvian fissure is visible in the distal hemisphere
5. Thalami are visible
6. Cavum septum pellucidi are visible

The images were converted to an isotropic spatial resolution whilst preserving anatomical boundaries, speckle, and texture profiles obtained during image acquisition.