

Maternal and foetal angiogenic imbalance in congenital heart defects

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Received 17 April 2013; revised 23 July 2013; accepted 26 August 2013; online publish-ahead-of-print 24 October 2013

See page 680 for the editorial comment on this article (doi:10.1093/eurheartj/ehz485)

Aims

Animal models showed that angiogenesis is related to abnormal heart development. Our objectives were to ascertain whether a relationship exists between congenital heart defects (CHDs) and angiogenic/anti-angiogenic imbalance in maternal and foetal blood and study the expression of angiogenic factors in the foetal heart.

Methods and results

Maternal and cord blood placental growth factor (PLGF), soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) were compared in 65 cases of CHD and 204 normal controls. Angiogenic factor expression and markers of hypoxia were measured in heart tissue from 23 CHD fetuses and 8 controls. In the CHD group, compared with controls, plasma PLGF levels were significantly lower (367 ± 33 vs. 566 ± 26 pg/mL; $P < 0.0001$) and sFlt-1 significantly higher (2726 ± 450 vs. 1971 ± 130 pg/mL, $P = 0.0438$). Fetuses with CHD had higher cord plasma sFlt-1 (442 ± 76 vs. 274 ± 26 pg/mL; $P = 0.0285$) and sEng (6.76 ± 0.42 vs. 4.99 ± 0.49 ng/mL, $P = 0.0041$) levels. Expression of vascular endothelial growth factor (VEGF), sFlt-1, markers of chronic hypoxia, and antioxidant activity were significantly higher in heart tissue from CHD fetuses compared with normal hearts (VEGF, 1.59-fold; sFlt-1, 1.92-fold; hypoxia inducible factor (HIF)-2 α , 1.45-fold; HO-1, 1.62-fold; SOD1, 1.31-fold).

Conclusion

An intrinsically angiogenic impairment exists in CHD that appears to be present in both the maternal and foetal circulation and foetal heart. Our data suggest that an imbalance of angiogenic-antiangiogenic factors is associated with developmental defects of the human heart.

Keywords

Congenital heart defects • Placental growth factor • sFlt-1 • Vascular endothelial growth factor • Soluble endoglin

Introduction

Congenital heart defects (CHDs) are present in 8 per 1000 live births.¹ Abnormalities of the heart and great arteries are the most common congenital defects, accounting for ~20% of all stillbirths and 30% of neonatal deaths.² Despite significant advances in the understanding of mechanisms determining heart formation, the

causes of CHD in humans remain undefined in the vast majority of cases³ and probably depend on the interplay of multiple genetic and environmental factors.⁴

Pregnancy requires both vasculogenesis and angiogenesis in the foetal compartment and angiogenesis in the maternal compartment.⁵ Abnormal angiogenesis in the placenta determines impaired remodelling of the maternal spiral arteries and placental underperfusion that

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may ultimately lead to the development of foetal growth restriction and maternal pre-eclampsia.^{6–8} In the foetal compartment, a heterozygous deletion of vascular endothelial growth factor (VEGF), one of several angiogenic factors, causes death of the embryo owing to its inability to form the vascular tree.^{7,9} In zebra fish embryos, the blockage of VEGF receptors resulted in a functional and structural defect in cardiac valve development, suggesting that these receptors are involved in heart valve formation.¹⁰

The soluble form of fms-like tyrosine kinase-1 (sFlt-1) is a potent antagonist of both VEGF and placental growth factor (PlGF) that prevents their interaction with cell receptors.¹¹ Placental growth factor, a glycoprotein of the family of VEGFs, is produced by the placenta and induces proliferation, migration, and activation of endothelial cells.^{6,7} We recently reported that, in isolated major foetal heart defects, maternal serum PlGF levels at 11–13 weeks' gestation were decreased, suggesting impaired placental angiogenesis.¹² However, to what extent angiogenesis is involved in human heart development remains to be determined.

This study aimed to ascertain whether, in euploid fetuses with major heart defects, there is evidence of altered angiogenesis in the foetal heart and maternal and foetal blood. To test our hypothesis, we first evaluated the expression of angiogenic factors and hypoxia markers in foetal heart tissue and, secondly, we determined maternal serum levels of PlGF, sFlt-1 and soluble endoglin (sEng) in the second and third trimesters of pregnancy. Finally, we sought to ascertain whether such changes are related to foetal cord angiogenic and anti-angiogenic markers and perinatal results.

Methods

Study population and inclusion criteria

This was a case–control study for major congenital cardiac defects. All pregnant women attending the Foetal Medicine Unit of Vall d'Hebron University Hospital between June 2010 and September 2012 for suspected foetal cardiac defect were eligible for the study. All cases were examined by a foetal cardiologist and a foetal medicine specialist. A termination of pregnancy (TOP) was considered by some of the couples. Each case was matched according to gestational age with three healthy pregnant complication-free controls who gave birth to phenotypically normal infants. Maternal venous blood was drawn for routine blood tests and samples processed within 1 h. Plasma was separated by centrifugation at 1400 g for 10 min at 4°C, and sample aliquots were immediately stored at –80°C until assayed. Cord blood samples from fetuses with CHD were obtained at birth when possible.

In TOP cases, heart tissue from the apical area of the left ventricle was obtained prospectively from abnormal and normal foetal hearts after necropsy and samples stored at the Foetal Tissue Bank of the Vall d'Hebron University Hospital. Euploid fetuses without CHDs (Non-CHD) were used as controls. All samples stemmed from pregnancies terminated at 17–22 weeks of gestation in otherwise healthy women.

Exclusion criteria

All aneuploidic fetuses and those with associated non-cardiac defects diagnosed prenatally or in the neonatal period were excluded as were those that developed foetal growth restriction, defined by low birth weight (below the 10th centile for the study population) and abnormal

umbilical Doppler assessment on pre-delivery ultrasound. In addition, mothers who developed pre-eclampsia were also excluded.

Plasma levels of angiogenic and anti-angiogenic factors

Soluble fms-like tyrosine kinase, free PlGF and sEng concentrations were measured by ELISA kits (R&D Systems).

RT–PCR analysis

mRNA of foetal heart tissue samples was extracted using RNeasy Fibrous Tissue (Qiagen) according to the manufacturer's protocol, and used for cDNA synthesis (Fermentas). Inventoried and customized (sFlt-1)¹³ TaqMan Gene Expression Assays were obtained from Applied Biosystems. mRNA expression levels were normalized to the levels of human DNA-directed RNA-polymerase II. Relative expression was calculated using a Prism 7000 Sequence Detection System (ABI).

Statistical analysis

Comparisons of maternal characteristics between outcome groups were measured by Fisher's exact test for categorical variables. The D'Agostino and Pearson omnibus K2 tested the distribution of the continuous variables for normality. When the assumption of normality was satisfied, data were analysed by the two-sided Student's unpaired *t*-test; otherwise, the non-parametric Mann–Whitney *U*-test was used. The *P*-value threshold used for significant differences was <0.05.

For the Doppler indices, the Z-scores were calculated with respect to the expected Doppler values in normal pregnancies after log-transformation of the indices.^{14,15} A Z-score of 0 is at the mean of the normal data and a Z-score of ± 1 and ± 2 is at 1 and 2 SDs from the mean, respectively. The Doppler information of the last scan before delivery was used for the analysis.

In each case and control, the measured PlGF, sFlt-1, and sEng were converted into multiples of the median (MoM) after adjustment for gestation, maternal age, race, body weight, parity, and method of conception, as previously described.^{16,17}

Student's unpaired *t* test or Mann–Whitney *U*-test, as appropriate, or Kruskal–Wallis test and Dunn's multiple comparison post tests were used to determine the significance of differences in plasma concentrations of angiogenic factors or the median MoM among cases and controls.

Spearman's correlation analysis was undertaken to relate maternal MoM PlGF to birth weight, length, and head circumference of fetuses in the study groups.

Statistical analyses were made using the GraphPad Prism software (GraphPad, version 5.0b, San Diego, CA, USA) and Statistical Package for Social Sciences software (SPSS 17.0; Chicago, IL, USA).

Results

Angiogenesis and hypoxia markers in heart tissue of fetuses with congenital heart defect

Heart tissue analysis was carried out in 23 euploid fetuses with CHDs and 8 fetuses with isolated cerebral or gastrointestinal malformations were used as controls.

Genes differentially expressed in foetal cardiac tissues are shown in Figure 1. sFlt-1 and VEGF-A mRNA expressions were significantly increased in the CHD group compared with controls (92%, *P* = 0.005 and 59%, *P* = 0.016, respectively). Significant increases in the transcript level of hypoxia inducible factor (HIF)-2 α , HO-1 and

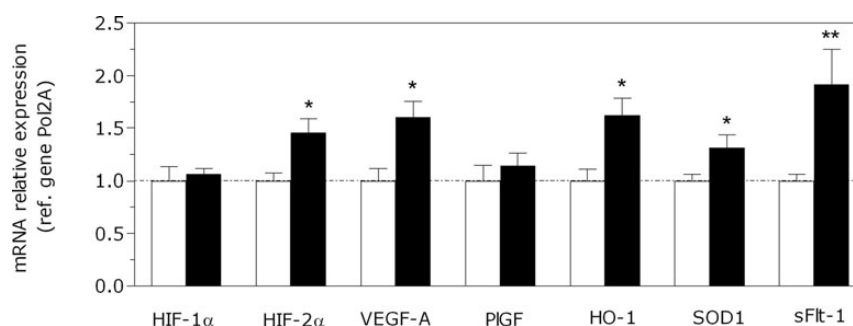


Figure 1 HIF-1α, HIF-2α, vascular endothelial growth factor-A, placental growth factor, HO-1, SOD1, and sFlt-1 expression genes in foetal cardiac tissue in congenital heart defect (black columns) and controls (white columns). Differences were analysed by Student's *t*-test or the non-parametric Mann–Whitney *U*-test, as appropriate. Data are presented as mean with SEM. **P* < 0.05 and ***P* < 0.01.

SOD1 were also observed in the CHD group compared with controls (non-CHD) (45%, *P* = 0.019; 62%, *P* = 0.015; and 31%, *P* = 0.049, respectively). However, no significant changes in HIF-1α and PlGF mRNA expressions were observed between groups.

Angiogenic/anti-angiogenic factors in blood of women carrying a congenital heart defect foetus

Seventy-six women carrying a foetus with a major CHD were included. Three patients who developed severe pre-eclampsia, one women with a foetus that developed intrauterine growth restriction and seven women who were found to be carrying a foetus with a chromosomal/genetic abnormality (four trisomy 21, one trisomy 18, one Jarcho Levin syndrome, and one DiGeorge syndrome) were excluded from the final analysis, leaving 65 cases with isolated major cardiac defects. Each case was matched for gestational age with three pregnant complication-free controls who delivered phenotypically normal infants. Maternal characteristics are compared in Table 1. Type of cardiopathy, prenatal Doppler, and perinatal characteristics of the study groups are listed in Table 2.

In the heart defect group, compared with controls, plasma PlGF levels were significantly lower (367 ± 33 vs. 566 ± 26 pg/mL; *P* < 0.0001), sFlt-1 significantly higher (2726 ± 450 vs. 1971 ± 130 pg/mL, *P* = 0.0438), and no differences were found in sEng (7.71 ± 0.54 vs. 7.75 ± 0.34 ng/mL; *P* = 0.4612).

Cardiac defects were classified as: (i) left ventricular outflow tract obstruction (LVOT): aortic valve stenosis, Shone syndrome, coarctation of the aorta and hypoplastic left heart syndrome (23 cases); (ii) conotruncal abnormalities: transposition of the great arteries, tetralogy of Fallot and double-outlet right ventricle (25 cases); and (iii) atrioventricular valve defects (A-V): septal defects, atrioventricular septal defect, complex atrioventricular septal defect, Ebstein's anomaly, and tricuspid valve atresia without right outflow obstruction (17 cases).

Placental growth factor levels according to cardiac defect subgroups are shown in Figure 2A. Lower PlGF concentrations were observed in the subgroup with atrioventricular valve defects and in those with conotruncal abnormalities compared with controls (263 ± 38 vs. 566 ± 26 pg/mL, *P* < 0.001) and (388 ± 55 vs. 566 ± 26 pg/mL, *P* < 0.05), respectively. However, no differences

Table 1 Clinical characteristics of pregnant women in the case–control study population

	Control (n = 204)	CHD (n = 65)	P-value
Maternal age (years)	32.5 ± 4.85	32.2 ± 5.26	0.6812
GA at sampling (weeks)	26.4 (8.8)	26.3 (9.6)	0.9949
Maternal weight (kg)	63 (13)	64 (14)	0.9927
Caucasian	198 (97)	57 (88)	0.0068
Conception			
Spontaneous	198 (97)	61 (94)	0.2609
Assisted	6 (3)	4 (6)	
Smoker	172 (84)	55 (84)	1.000
Parity			
Nulliparous	93 (46)	34 (52)	0.3927
Parous	111 (54)	31 (48)	

Values expressed as means ± SD, median (SD), or as number (percentage). Comparisons with the control group by Fisher's exact test for categorical variables and independent-sample Student's *t*-test or Mann–Whitney *U*-test, as appropriate, for continuous variables. GA, gestational age.

were found between the LVOT group and controls (422 ± 64 vs. 566 ± 26 pg/mL, *P* > 0.05). sFlt-1 concentrations were significantly higher in the whole cardiac defect group than in controls (2726 ± 450 vs. 1971 ± 130 pg/mL, *P* < 0.05), although no differences were found when CHD were subclassified according to type of defect (Figure 2B). The same results were obtained in the study groups when PlGF, sFlt-1, and sEng levels were corrected for gestational age, maternal age, race, body weight, parity and method of conception and expressed in MoM values (Table 3).

A cross-sectional analysis of samples obtained within gestational age intervals of 4 to 5 weeks was made to evaluate gestational patterns of angiogenic and anti-angiogenic factors. Placental growth factor levels were lower throughout gestation in women carrying a foetus with CHD than in those with a healthy foetus (Figure 3).

A positive and significant correlation between maternal MoM PlGF values and birth weight percentile was observed in the CHD group but not in the control group (Figure 4A and B, respectively).

Table 2 Type of cardiac defect, feto-placental Doppler and perinatal outcome in the study groups			
	Control (n = 204)	Congenital heart defects	
		Alive (n = 42)	TOP (n = 23)
CHD type			
A-V		10 (24)	7 (30)
Conotruncal		19 (45)	6 (26)
LVOT		13 (31)	10 (44)
Doppler parameters ^a			
UA Z-Score	0.050 (1.382)	0.405 (1.973)	
MCA Z-Score	−0.097 (1.094)	−0.257 (1.514)	
CPR Z-Score	0.173 (1.612)	−0.594 (1.310)	
Mean UtA Z-Score	−0.200 (1.124)	−0.135 (0.780)	
GA at delivery (week)	39.4 (2.2)	38.5 (3.0)*	22 (1.9)
Birth weight (g)	3210 (589)	2730 (855)***	420 (145)
Birth weight (p)	53 (51)	20 (35)***	
Length (cm)	50 (2)	48 (4)**	
Length (p)	50 (43)	50 (68)*	
Head circumference (cm)	34.0 (1.5)	33.5 (2.7)**	
Head circumference (p)	44 (59)	36 (43)	
Placental weight (g)	448 (122)	420 (80)	
5 min Apgar <7		8 (19)	
Days NICU		16.0 (7.0–29.2)	
Days hospital		22 (13.0–46.0)	

TOP, termination of pregnancy; UA, umbilical artery; PI, pulsatility index, MCA, middle cerebral artery; CPR, cerebral-to-placental ratio (MCA-PI/UA-PI); UtA, uterine artery; GA, gestational age; p, percentile; CHD, congenital heart defect; A-V, atrioventricular valve; LVOT, left ventricular outflow tract obstruction; NICU, neonatal intensive care unit.

^aDoppler results available in 125 controls and all cases.

Values expressed as median (SD) or as number (percentage).

Differences between the control group and the congenital heart defects (Alive) group were analysed by the Mann–Whitney U-test.

**P* < 0.05.

***P* < 0.01.

****P* < 0.001.

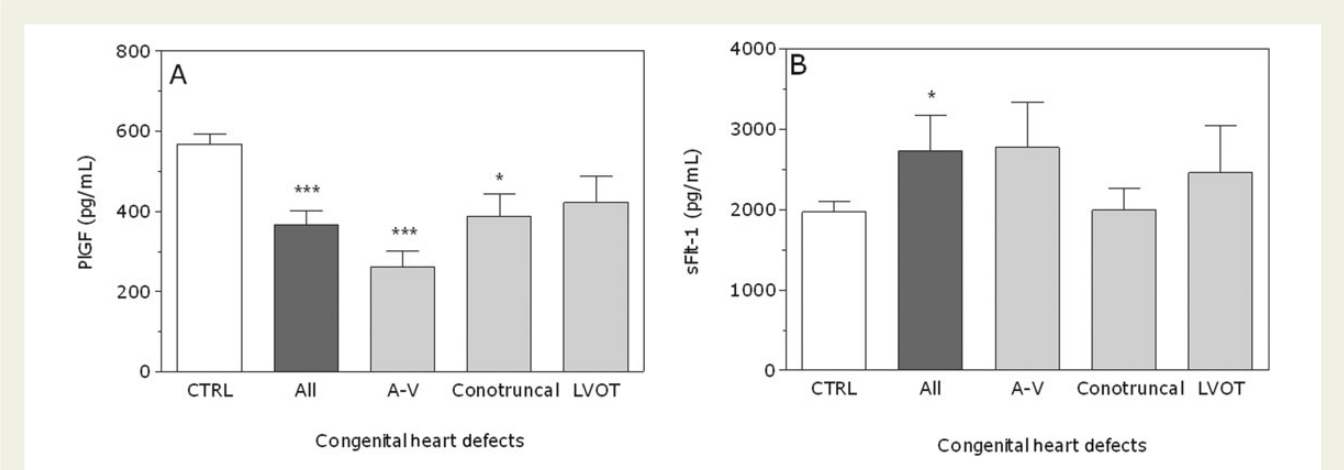


Figure 2 Maternal plasma placental growth factor (A) and soluble fms-like tyrosine kinase (B) concentrations in the study groups. Differences between CTRL (*n* = 204) and congenital heart defect (All) (*n* = 65) groups were analysed by the Mann–Whitney *U*-test. Differences between the CTRL group (*n* = 204) and type of the congenital heart defect atrioventricular valve (A-V) group (*n* = 17), conotruncal group (*n* = 25), or LVOT group (*n* = 23) were analysed by the Kruskal–Wallis test and Dunn’s multiple comparison post tests. Data are presented as mean with SEM. **P* < 0.05 and ****P* < 0.001.

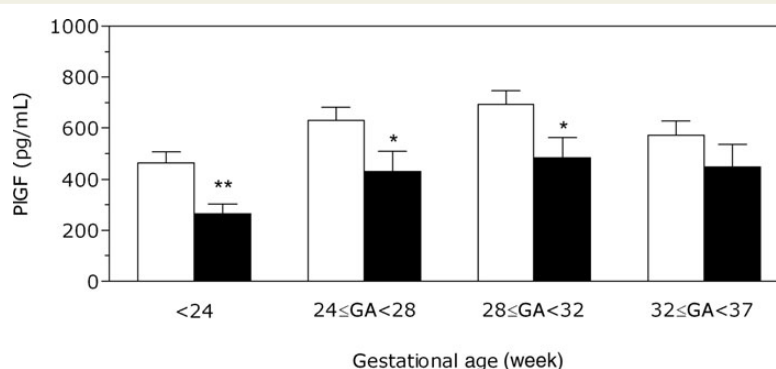
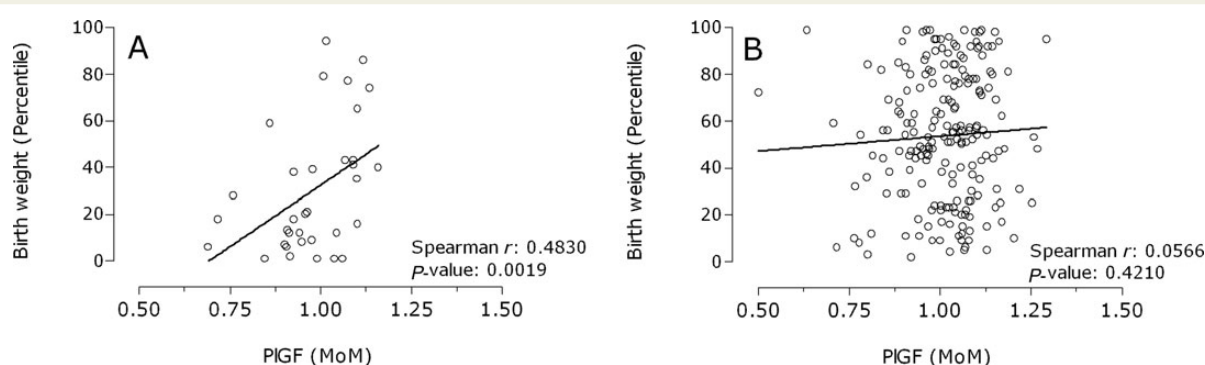
Table 3 Multiple of median values of placental growth factor, soluble fms-like tyrosine kinase-1-1, and soluble endoglin in the study groups

	Control (n = 204)	Congenital heart defects			
		All (n = 65)	A-V (n = 17)	Conotruncal (n = 25)	LVOT (n = 23)
PlGF MoM	1.03 (1.08–0.95)	0.94*** (1.03–0.79)	0.92*** (0.98–0.89)	0.95* (1.05–0.84)	0.98 (1.09–0.90)
sFlt-1 MoM	0.99 (1.07–0.93)	1.03* (1.08–0.95)	1.02 (1.09–0.91)	1.02 (1.08–0.94)	1.04 (1.10–0.96)
sEng oM	1.005 (1.18–0.83)	1.01 (1.17–0.88)	1.03 (1.13–0.91)	0.98 (1.18–0.76)	1.06 (1.21–0.88)

Values expressed as median (IQR). Differences between the control group and the congenital heart defects (All) group were analysed by the Mann–Whitney *U*-test. Differences between the control group (n = 204) and type of CHD (atrioventricular valve group, conotruncal group, or LVOT group) were analysed by the Kruskal–Wallis test and Dunn's multiple comparison post tests.

**P* < 0.05.

****P* < 0.001.

**Figure 3** Maternal plasma concentrations of placental growth factor (pg/mL) in the control group (white columns) and congenital heart defects group (black columns). Differences were analysed by the non-parametric Mann–Whitney *U*-test. Data are presented as mean with SEM. **P* < 0.5 and ***P* < 0.01.**Figure 4** Correlation between maternal placental growth factor MoM and birth weight (percentile) of foetuses in the congenital heart defect group (A) and the control group (B).

Angiogenic/anti-angiogenic factors in cord blood from congenital heart defect foetuses

During the study period, 39 cord blood samples were obtained from pregnant women carrying a foetus with CHD. Thirty-two control

patients who delivered a phenotypically normal foetus during the same period were invited to participate in the study.

Soluble fms-like tyrosine kinase-1 and sEng levels were significantly higher in cord blood from foetuses with CHD than in controls (442 ± 76 vs. 274 ± 26 pg/mL; *P* = 0.0285 and 6.76 ± 0.42 vs.

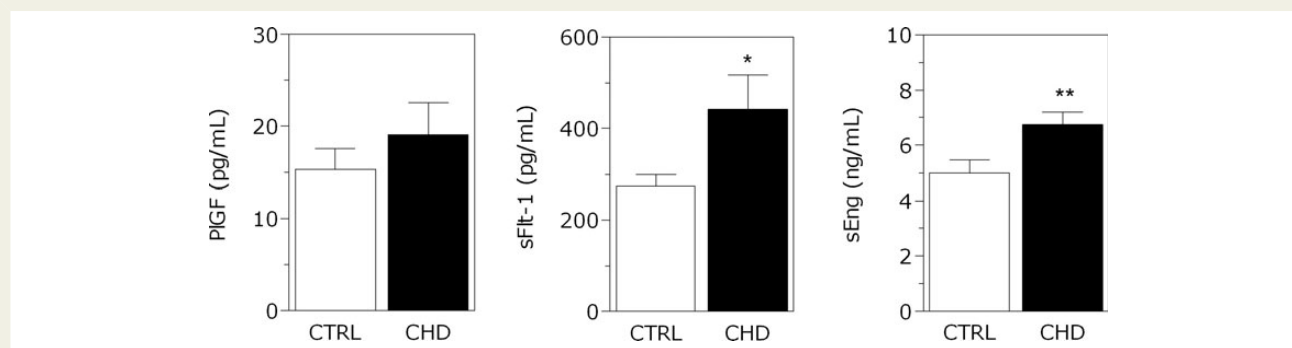


Figure 5 Levels of placental growth factor (pg/mL), soluble fms-like tyrosine kinase-1-1 (pg/mL) and soluble endoglin (ng/mL) in cord blood from congenital heart defect fetuses ($n = 39$) and controls (CTRL) ($n = 32$). Differences were analyzed by Student's *t*-test or the non-parametric Mann–Whitney *U*-test, as appropriate. Data are presented as mean with SEM. * $P < 0.05$ and ** $P < 0.01$.

4.99 ± 0.49 ng/mL, $P = 0.0041$, respectively). However, no changes in PIGF levels were found between cases and controls (19.07 ± 3.47 vs. 15.35 ± 2.22 pg/mL, $P = 0.4712$) (Figure 5).

Discussion

Angiogenesis and hypoxia markers in heart tissue of fetuses with congenital heart defect

This study presents the first evidence of abnormal angiogenesis in heart tissue of human fetuses with CHD compared with normal heart controls which showed increased VEGF-A and sFlt-1 expression and overproduction of proteins such as HIF-2 α , HO-1 and SOD1 as a result of chronic hypoxia.

Vascular endothelial growth factor -A expression is dynamic during embryonic heart development and is involved in both long-range and short-range signalling that influences vessel patterning. In mutant mouse embryos, two to three-fold overexpression of VEGF-A resulted in severe abnormalities in heart development, including attenuated compact layer of the myocardium, overproduction of trabeculae, defective ventricular septation, and remodelling of the outflow tract.¹⁸ Interestingly, sFlt-1 expression in our study was increased two-fold in CHD samples compared with controls. Flt-1 has also been implicated in the regulation of embryonic heart function and cardiac morphogenesis.¹⁹

Placental growth factor has emerged as a central mediator in both coordination of cardiomyocyte growth and neo-angiogenesis.²⁰ Genetic and pharmacological studies identified PIGF as a novel cardioprotective factor.²¹ In our study, the lack of increased PIGF production and overproduction of sFlt-1 and VEGF suggests that CHDs might be caused by the combination of a heart-specific vascular defect and a placental anti-angiogenic environment.

This study also evaluated the presence of hypoxia in abnormal human foetal hearts by analysing the expression of HIF-1 α , HIF-2 α , HO-1, and SOD1. In CHD heart tissue, an overexpression of HIF-2 α and HO-1 was observed, probably as a consequence of chronic hypoxia. Previous *in vitro* studies demonstrated that HIF-2 α preferentially activates VEGF expression.²² Some

experimental models also showed the importance of HO-1 in heart development.²³ Increased SOD1 expression in CHD cases, as a cell response to tissular hypoxia, would be consistent with the presence of oxidative stress in hearts from CHD fetuses. In our study, the increased expression of hypoxia-inducible genes suggests that a certain degree of hypoxia might be present in CHD. Although chronic hypoxia might up-regulate the expression of pro-angiogenic factors, an overall deregulation of angiogenesis with a net balance towards an anti-angiogenic environment was observed in heart tissue from fetuses with CHD, pointing to an intrinsic angiogenic impairment in these cases. However, as the evaluation of these factors was made in the second trimester of pregnancy, and not during embryogenesis, we are unable to confirm whether angiogenic imbalance leads to abnormal heart development or rather is a consequence of the heart defect.

Angiogenic/anti-angiogenic factors in blood of women carrying a congenital heart defect fetus

The findings of this study showed that, in isolated major foetal heart defects, maternal serum PIGF was decreased and sFlt-1 increased at 18–37 weeks' gestation, suggesting impaired placental angiogenesis. Such impairment was observed in the presence of conotruncal and septal-valve defects but not of left heart defects. In very early foetal mouse development, VEGF-A expression was found in most endocardial cells of the heart tube, whereas at Day 9.5 this expression became restricted to endocardial cells located at points of cushion formation.²⁴ VEGF-A is required for the activation, proliferation, and remodelling of cushion cells into valve leaflets. Our results appear to show that abnormal angiogenesis may also be deleterious for septal-valve and outflow track formation in human heart embryogenesis.

The aetiologies of left-sided heart malformations are likely complex, with environmental exposures, chromosomal abnormalities, such as Turner's syndrome²⁵ and Jacobsen's syndrome,²⁶ and they are strongly determined by genetic factors.²⁷ A genetic component in the NOTCH1 pathway has been described for patients with LVOT malformations.^{27–29} A family-based study in patients with left congenital cardiac defects identifies both cosegregating and de novo

copy number variants enriched in angiogenesis that accounted for up to 10% of left-sided heart disease.³⁰ The presence of a non-significant trend towards low PIGF levels in cases with LVOT defects in this study might indeed indicate the presence of angiogenic imbalance in a specific subset of these patients, and deserves further investigation.

Several explanations exist for the abnormal angiogenic pattern in maternal blood in pregnant women carrying a foetus with CHD. Our data suggest that these foetuses may have an intrinsic altered angiogenesis leading to abnormal formation of the heart that may also be present in trophoblastic cells. Alternatively, low PIGF may be associated with a lesser degree of trophoblast invasion of the spiral arteries and ensuing placental hypoxia. Placental hypoxia due to abnormal angiogenesis may cause a certain degree of foetal hypoxia that is related to abnormal heart development.

The association of complex CHD, low birth weight, and small head circumference is well established.^{31,32} Disease-specific biometrics are thought to be a consequence of blood flow disturbance to the body caused by abnormal morphology and oxygen distribution in foetuses with heart defects. However, a correlation between lower maternal PIGF levels and birth weight percentile in CHD babies was found in our study, suggesting that placental impairment contributes to diminished growth potential of foetuses with CHD. In addition to low birthweight, foetuses with CHD showed a trend towards a higher umbilical and lower middle cerebral artery Doppler resistance compared with controls. These findings were in line with previous studies that consistently showed that CHD have significantly reduced blood flow resistance in the brain.^{33–38} However, in contrast to common findings in patients with placental complications of pregnancy, uterine Doppler and placental weight were within the normal range in CHD cases. These results are in line with a previous study in the first trimester of pregnancy, reporting that uterine artery Doppler and pregnancy associated plasma protein-A levels in women with a CHD foetus were similar to those of controls.¹² In both this and the current study, clinical cases of placental dysfunction, i.e. intrauterine growth restriction and pre-eclampsia, were excluded, which may explain the absence of clear differences in Doppler and placental parameters among the study groups. We acknowledge that placental histology information might have offered important insights in relation with the interpretation of this potential association, and that future studies including this information are warranted. We believe that evaluating the relationship between CHD and placental-related complications is an important hypothesis to explore as the subject of future research.

Angiogenic/anti-angiogenic factors in cord blood from congenital heart defect foetuses

A net anti-angiogenic imbalance with significantly higher sFlt-1 levels and sEng was found in cord blood of CHD compared with control foetuses. Exogenous sFlt-1 alone caused diastolic dysfunction in wild-type mice.³⁹ Anti-angiogenic therapies, including antibodies that neutralize VEGF and small-molecule VEGF receptor inhibitors, are being increasingly used in the oncological and ophthalmological settings in which cardiomyopathy and heart failure have recently been recognized as major side effects.^{40,41} Peripartum cardiomyopathy, often

a fatal disease that affects pregnant women, was recently associated with a systemic angiogenic imbalance.⁴² Angiogenic factors play an important role in the development of atherosclerosis and show pronounced changes during acute myocardial infarction (AMI).⁴³ High sFlt-1 levels proved to be a good predictor of mortality during a 1-year follow-up of AMI, regardless of information provided by troponin T and N-terminal pro-B-type natriuretic peptide.⁴⁴ Overall, these studies indicated that an anti-angiogenic state could be harmful to the human heart.

To what extent anti-angiogenic imbalance in the uterus determines cardiac function and neurodevelopment in infants with CHD remains to be determined. Although it is becoming well established that prenatal factors may contribute to perinatal mortality⁴⁵ and neurodevelopment morbidity⁴⁶ associated with CHD, the specific prenatal causes and mechanisms of insult are largely unknown. The main hypothesis is that major circulatory disorders and ensuing chronic hypoxia may lead to abnormal neurological development in foetuses with CHD⁴⁷; however, our study also revealed a certain degree of placental impairment in these cases which may contribute to a deficient supply of oxygen and metabolic substrate to the developing brain. Moreover, this anti-angiogenic environment during foetal life could adversely impact on the adaptative capabilities of the heart later in life.

Conclusions

This study provides first evidence of abnormal angiogenesis in human CHD. The data are in line with previous results from animal studies, and strongly suggest an excessive anti-angiogenic signalling in the embryogenic period that could contribute to the development of some CHDs. To what degree the anti-angiogenic state determines cardiac function and clinical prognostic value, and the relationship between cardiac disease and placental dysfunction remains to be determined. This newly described relationship between angiogenesis and human CHD may allow the development of novel predictive strategies and open a window of research opportunities into novel preventive therapeutics in these patients.

Acknowledgements

The authors are grateful to Christine O'Hara for her help with the English version of the paper.

Ethics committee approval

Written informed consent was obtained from mothers agreeing to participate in this study on CHDs approved by the Ethics Committee of the Vall d'Hebron University Hospital. Heart tissue samples were provided by the Foetal Tissue Bank of the Vall d'Hebron University Hospital Biobank with appropriate ethics committee approval.

Funding

This study was supported by research grants (07/1095) from the Fondo de Investigación Sanitaria and Maternal and Child Health and Development Network SAMID (RD 08/0072 and RD12/0026) and Dr. O.S. salary is supported by SAMID (RD08/0072 and RD12/0026), financed by the Carlos III Institute of Health in Spain.

Conflict of interest: none declared.

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