

Fetal congenital heart diseases: Diagnosis by anatomical scans, echocardiography and genetic tests

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ABSTRACT

Objective: To determine the distribution of major fetal congenital heart diseases (CHDs) diagnosed antenatally during routine second-trimester obstetric anatomical scans in an unselected population at a single tertiary centre and to characterise and stratify risk factors, genetic diagnosis and long-term health at 4 years old.

Method: A single-centre cohort study of all major fetal CHDs detected on routine obstetric fetal anatomical ultrasound scans between January 2014 and December 2017 was performed in an unselected population. Demographic details, fetal echocardiogram reports, genetic test results, delivery outcomes and postnatal progress were stratified by CHD subtype.

Results: Of 20,031 screened pregnancies, 109 pregnancies (0.53%) had major fetal CHDs. The most common subtypes were coarctation of aorta (17.4%), transposition of great arteries (16.5%), and tetralogy of Fallot and univentricular hearts (13.8% each). Of the 60.5% that underwent confirmatory genetic testing—mostly conventional karyotyping and testing for 22q11 microdeletion—about a quarter had abnormalities, of which 22q microdeletion was the most common. We had complete obstetric data in 85 pregnancies (78%), of which 76.5% progressed to live birth. Among these, 92.1% of postnatal echocardiograms concurred with antenatal ones. At 4 years old, 43.2% of offspring had no medical or developmental issues, 20.0% had mild medical or developmental issues, 21.5% had major medical or developmental issues, and 12.3% had deceased.

Conclusion: Fetal echocardiograms accurately diagnose CHDs. Future studies should evaluate the roles of chromosomal microarray and next-generation sequencing in diagnosing CHD.

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INTRODUCTION

Congenital heart diseases (CHDs) are the most common major congenital anomaly at up to 28%¹ and are responsible for 5.7% of all infant mortality.² While earlier studies in developed countries reported an overall CHD birth prevalence of 3.7–5.54 per 1000 live births,^{3,4} more recent studies report a global and Asian birth prevalence of 9.1 and 9.3 per 1000 live births, respectively.⁵ This rise in prevalence over time has been

noted in other cohort studies^{5,6} and has been attributed to better antenatal and postnatal diagnoses through advancements in echocardiography and better screening protocols, as previously severe cases were only diagnosed on autopsy.^{5,7}

The birth prevalence of CHD in Singapore based on older studies is 9.07 per 1000 live births,^{8,9} which corresponds with reported global and Asian rates.⁵ In Singapore, standard antenatal ultrasound screening

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CLINICAL IMPACT

What is New

- The most common major fetal CHD subtypes were coarctation of aorta (17.4%), transposition of great arteries (16.5%), and tetralogy of Fallot and univentricular hearts (13.8% each).
- Upon confirmatory genetic testing, about a quarter were abnormal, where 22q microdeletion was the most common.
- Successful live births occurred in 76.5%. At age 4, 21.5% had major medical or developmental issues and 12.3% had deceased.

Clinical Implications

- Diagnosis of major fetal CHDs is associated with about a quarter having genetic abnormalities, major medical or developmental issues, or fetal demise.

includes a detailed second-trimester obstetric fetal anatomical scan between gestational age 18 and 22 weeks completed. This comprises cardiac assessment that incorporates a “5-transverse views” protocol visualising the abdominal situs, the 4-chamber view, each of the ventricular outflow tracts, and the 3-vessel and trachea view¹⁰ of the developing fetal heart. The addition of a 3-vessel and trachea view to the usual 4-chamber view increases the sensitivity from 65.6% to 81.3% according to a prospective observational cohort study of 8,025 pregnancies.¹¹ Where an abnormality is detected or in patients with higher baseline risk, a referral is made to a paediatric cardiologist for fetal echocardiography and counselling on the postnatal prognosis and management options. Studies have demonstrated high concordance (96.1%) between cardiac evaluations during second-trimester fetal anatomical scans and fetal echocardiograms, especially where the aforementioned views were obtained.¹² Additionally, recent studies have demonstrated that fetal echocardiography has a high degree of specificity (approaching 99%) and sensitivity (40–60%) in diagnosing CHDs.^{13,14}

This purpose of this study was to determine the distribution of major CHDs detected during routine antenatal fetal anomaly screening of a general obstetric population at a single centre, identify risk factors and genetic abnormalities and review their obstetric and postnatal outcomes.

METHOD

Population and disease

This was a single-centre retrospective cohort study that was approved by the Domain Specific Review Board, National Healthcare Group, Singapore with a waiver of informed consent (Reference Number 2018/00817). The study was performed between January 2014 and December 2017 at the Department of Obstetrics and Gynaecology, National University Hospital (NUH), a tertiary academic medical centre that conducts about 5000 deliveries per year. We reviewed the antenatal records for pregnancies where a major fetal CHD was diagnosed as part of routine screening. In cases with successful live birth at NUH, the child’s medical records were reviewed to compare postnatal echocardiographic findings with the antenatal diagnosis and to determine short- and intermediate-term outcomes up to 4 years of age. Major CHD was defined as malformations of the heart and great arteries that typically require surgery or catheter-based intervention within the first year of life.^{15,16} Included diagnoses were derived from Hoffman and Kaplan¹⁷ and comprised (1) univentricular hearts (UVH), including hypoplastic left heart syndrome (HLHS); (2) transposition of great arteries (TGA); (3) truncus arteriosus; (4) interrupted aortic arch; (5) double outlet right ventricle (DORV); (6) tetralogy of Fallot, including pulmonary atresia and absent pulmonary valve; (7) pulmonary atresia with intact ventricular septum; (8) critical pulmonary valve stenosis; (9) tricuspid atresia; (10) Ebstein anomaly; (11) atrioventricular septal defect (AVSD); (12) large ventricular septal defect (VSD); (13) critical or severe aortic stenosis; and (14) coarctation of the aorta.

For this study, minor findings that were not associated with a discrete structural abnormality (e.g. pericardial effusion of less than 4 mm, fetal arrhythmias, intracardiac echogenic foci and cardiac axis deviation) or were unlikely to have major clinical significance and which were frequently missed (e.g. small VSDs and ASDs of less than 3 mm) were excluded.

Antenatal detection of CHD

If a major CHD was detected during obstetric fetal anatomical scans, a referral was made to a paediatric cardiologist for a detailed fetal echocardiogram¹⁸ in the event that there was either (1) ambiguity in the initial diagnosis, or (2) the couple was keen to keep the pregnancy. This referral was not made if the couple was keen to terminate the pregnancy or follow-up elsewhere. Concordance between fetal echocardiograms and obstetric fetal anatomical scans was assessed after

reviewing scan reports and was defined as reaching the same major CHD diagnosis without missing any other major CHD. Discordance between obstetric fetal anatomic scans and fetal echocardiograms was defined as any discrepancy that may have an impact on postnatal clinical management and, therefore, affecting prenatal counselling.¹⁹ While the fetal echocardiogram diagnosis is a higher level and more specialised scan compared to the fetal cardiac assessment in second-trimester obstetric fetal anatomical scan and is routinely taken to be the overriding antenatal diagnosis, in practice, major discordances are usually reconciled after discussion by the obstetrician and fetal cardiologist at our centre as part of the multidisciplinary discussion required to plan the antenatal and immediate postnatal care of these patients. Additionally, the gold standard reference remains the anatomic diagnosis on postnatal transthoracic echocardiogram. In the event of a complex CHD, the most severe cardiac abnormality was selected as the CHD subtype.

Aneuploidy screening and genetic tests

Aneuploidy screening was performed in either the combined first trimester screening (FTS) (which comprises nuchal translucency measurement plus serum PAPP-A and free β -hCG) or the non-invasive prenatal testing (NIPT) depending on the patient's choice. In the event of a high-risk result, couples were counselled on the next step, which was typically confirmatory invasive genetic testing. Some couples opted for contingent testing (i.e. following up a high-risk FTS result with NIPT testing).

Confirmatory antenatal genetic testing by amniocentesis or chorionic villus sampling (CVS) would be offered to identify aneuploidies or microdeletion disorders, such as 22q deletion syndrome. In most cases, the genetic testing offered comprised conventional karyotyping with 22q11 fluorescence in-situ hybridisation (FISH). In the latter years of this study period, chromosomal microarray analysis (CMA) or next-generation sequencing was increasingly offered. These fetuses would be followed up with serial, interval fetal cardiac imaging to assess progression, refine the diagnosis and plan for postnatal management. Upon live birth, a postnatal echocardiogram was performed within 24 hours of birth and compared with fetal echocardiograms. Where antenatal genetic testing had not been done, postnatal genetic testing would be offered by paediatricians or neonatologists.

Data collection

Based on a medical record review, demographic data (e.g. age and ethnicity), maternal risk factors for fetal CHD (e.g. diabetes mellitus, exposure to teratogenic

drugs, family history), antenatal fetal anomaly scan and echocardiography results, aneuploidy screening details, and antenatal genetic testing results were collated. Data were presented either as means with standard deviation or frequencies with percentages (%), as appropriate. Outcomes of these pregnancies were classified as (1) termination of pregnancy (TOP), (2) pregnancy loss, (3) live birth, or (4) lost to follow-up. The mode of delivery was recorded. Postnatal echocardiograms, genetic tests and interventions were also collated. Outpatient records within NUH were reviewed for all offspring up to an age of 4 years to determine intermediate-term medical and developmental outcomes. These were classified as (1) no medical or developmental issues, (2) mild medical or developmental issues, or (3) major medical or developmental issues. Mild medical issues referred to situations where there was some medical impairment requiring continued regular outpatient monitoring but not requiring home oxygen therapy or frequent inpatient care. Major medical problems referred to situations where there was significant impairment to the child requiring either home oxygen therapy, non-invasive ventilatory support or frequent inpatient care. A mild developmental issue was a deficit in one developmental domain while major developmental issue referred to a deficit in 2 or more developmental domains.

RESULTS

Population

Over the study period, 20,031 pregnant patients (including multiple gestations) were screened for fetal anomalies at NUH, of which 109 (5.3 per 1000 pregnancies) were identified as having major CHDs (Fig. 1). Two cases were twin gestations. All cases were identified during standard obstetric fetal anatomical screening ultrasound scans between 18 and 22 gestational weeks. Findings on fetal echocardiograms by paediatric cardiologists were deemed concordant with fetal cardiac findings on obstetric fetal anatomical scans in 88.3% of cases. The mean age of women in the study population was 33.0 ± 4.5 years (Table 1). The rate of diabetes in pregnancy in the study population was 18.3%, of which 65% were gestational and 35% pre-existing. The rate of family or personal history of CHD was 4.6%. None of the patients in our cohort had other medical conditions (e.g. phenylketonuria) or teratogen exposure (e.g. anti-epileptics drugs, lithium or selective serotonin reuptake inhibitors) that predispose to fetal CHDs.

Among these 109 patients, we had complete obstetric data for 85 (78%) of cases, with the remaining 24 cases being lost to follow-up as their pregnancy outcomes

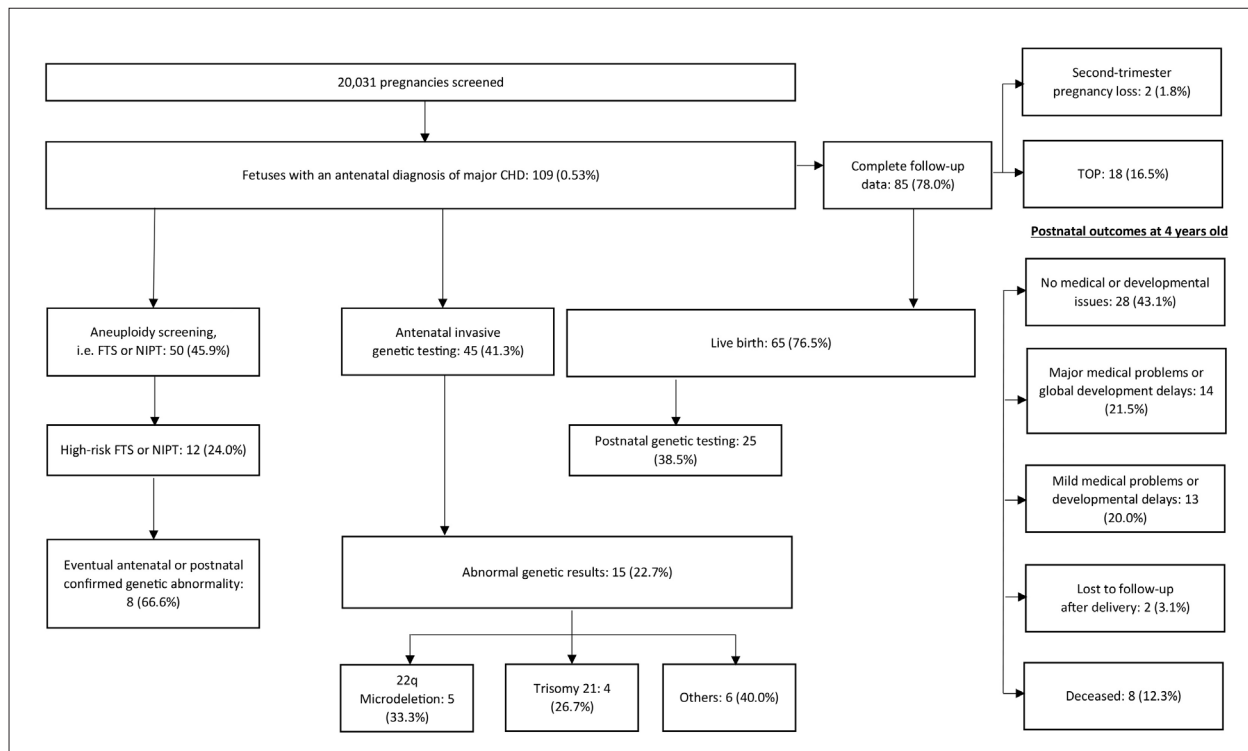


Fig. 1. Outcomes of the study population.

CHD: congenital heart disease, FTS: first trimester screening, NIPT: non-invasive prenatal testing, TOP: termination of pregnancy

Table 1. Demographic of the study population.

Sample size	109
Age, years	33.0 ± 4.5
Diabetes in pregnancy (%)	19 (17.4)
Gestational diabetes (%)	12 (63.2)
Pre-existing diabetes (%)	7 (36.8)
Family or personal history of CHD (%)	5 (4.6)
Ethnicity	
Chinese (%)	66 (60.6)
Malay (%)	18 (16.5)
Indian (%)	15 (13.8)
Others (%)	10 (9.2)
Complete follow-up data (until delivery or TOP)	85 (78.0)
Live birth (%)	65 (76.5)
TOP (%)	18 (21.2)
Second-trimester pregnancy loss (%)	2 (2.4)
Postnatal death (%)	8 (9.4)
Lost to follow-up	24 (22.0)

CHD: congenital heart disease, TOP: termination of pregnancy

Values represent mean ± standard deviation or frequency (percentage)

could not be ascertained (Table 1). Twenty of the 85 cases (23.5%) either underwent termination of pregnancy or resulted in pregnancy loss, and 65 (76.5%) progressed to live births. Of these 65 live births, 63 had postnatal echocardiograms of which 92.1% were deemed concordant with antenatal fetal echocardiograms.

Distribution of CHDs

Among the 109 CHDs (Table 2 and Fig. 2), 57.8% (63 in 109) were cyanotic. Specifically, 44.0% (48 in 109) were conotruncal abnormalities (i.e. TGA, tetralogy of Fallot, DORV and truncus arteriosus), 13.8% (15 in 109) were UVH (i.e. single ventricles, HLHS, or heterotaxy syndrome), 17.4% (19 in 109) were coarctation of aorta, 3.7% (4 in 109) were tricuspid atresia and 2.8% (3 in 109) were critical or severe aortic stenosis. Finally, critical pulmonary valve stenosis, AVSD and large isolated VSDs comprised 5.5% (6 in 109) each. Of the 109 cases, 12.8% (14 in 109) had extracardiac abnormalities. Genetic testing was performed in 11 of these 14 cases, of which 5 were abnormal (Table 3).

Obstetric and postnatal outcomes

Of the 85 cases that had complete follow-up data, 21.2% (18 in 85) underwent termination of pregnancy, 2.4% (2 in 85) sustained a second-trimester pregnancy loss, and

Table 2. Distribution of major congenital heart disease, genetic diagnoses and their outcomes (n=109).

Type of CHD	n (%)	Antenatal diagnosis						Outcome				
		Aneuploidy screening (i.e. FTS or NIPT)		Confirmatory genetic testing (i.e. Amniocentesis, CVS, or postnatal)		Extracardiac fetal anomaly (%)	Fetal echocardiography	Lost to follow-up (%)	Second-trimester pregnancy loss (%)	TOP (%)	Live birth	
		Number	High risk (%)	Number	Abnormal (%)							
						Number	Concordance* (%)	Number (%)	Neonatal outcome			
TGA	18 (16.5)	Total: 8 FTS: 2 NIPT: 3 Cont: 3	0 (0)	Total: 10 AN: 6 PN: 4	0 (0)	4 (22.2)	12	12 (100.0)	5 (27.8)	0 (0)	2 (11.1)	A: 0 B: 3 C: 0 D: 7 E: 1
DORV	10 (9.2)	Total: 5 FTS: 3 NIPT: 1 Cont: 1	2 (40)	Total: 5 AN: 3 PN: 2	1 (20.0)	0 (0)	4	3 (75.0)	3 (30.0)	0 (0)	2 (20.0)	A: 1 B: 0 C: 1 D: 3 E: 0
Tetralogy of Fallot	15 (13.8)	Total: 5 FTS: 2 NIPT: 3 Cont: 0	5 ^S (100)	Total: 14 AN: 11 PN: 6 [†] Cont: 0	6 (42.9) [‡]	4 (26.7)	13	10 (76.9)	2 (13.3)	0 (0)	3 (20.0)	A: 1 B: 2 C: 6 D: 1 E: 0
Truncus arteriosus	5 (4.6)	Total: 1 FTS: 1 NIPT: 0 Cont: 0	0 (0)	Total: 4 AN: 4 PN: 0	1 (25.0)	1 (20.0)	3	3 (100.0)	1 (20.0)	0 (0)	2 (40.0)	A: 2 B: 0 C: 0 D: 0 E: 0
Interrupted aortic arch	2 (1.8%)	Total: 0 FTS: 0 NIPT: 0 Cont: 0	-	Total: 0 AN: 0 PN: 0	-	0 (0)	0	-	1 (50.0)	0 (0)	1 (50.0)	A: 0 B: 0 C: 0 D: 0 E: 0
Coarctation of aorta	19 (17.4)	Total: 10 FTS: 5 NIPT: 3 Cont: 2	1 (10.0)	Total: 10 AN: 7 PN: 3	1 (10.0)	0 (0)	12	10 (83.3)	4 (21.1)	0 (0)	0 (0)	A: 2 B: 3 C: 4 D: 6 E: 0
UVH	15 (13.8)	Total: 6 FTS: 4 NIPT: 1 Cont: 1	0 (0)	Total: 6 AN: 5 PN: 1	1 (16.7)	1 (6.67)	7	7 (100.0)	5 (33.3)	1 (6.67)	6 (40.0)	A: 1 B: 1 C: 0 D: 0 E: 1

Table 2. Distribution of major congenital heart disease, genetic diagnoses and their outcomes (n=109). (Cont'd)

Type of CHD	n (%)	Antenatal diagnosis						Outcome					
		Aneuploidy screening (i.e. FTS or NIPT)		Confirmatory genetic testing (i.e. Amniocentesis, CVS, or postnatal)		Extracardiac fetal anomaly (%)	Fetal echocardiography		Lost to follow-up (%)	Second-trimester pregnancy loss (%)	TOP (%)	Live birth	
		Number	High risk (%)	Number	Abnormal (%)		Performed	Concordance* (%)					
						Number			Neonatal outcome				
Critical pulmonary valve stenosis	6 (5.5%)	Total: 4 FTS: 3 NIPT: 0 Cont: 1	0 (0)	Total: 4 AN: 3 PN: 1	0 (0)	0 (0)	5 (100.0)	5	1 (16.7)	0 (0)	0 (0)	5 (83.3)	A: 0 B: 2 C: 0 D: 3 E: 0
AVSD	6 (5.5%)	Total: 4 FTS: 1 NIPT: 3 Cont: 0	2 (40.0)	Total: 4 AN: 1 PN: 3	3 (75.0)	2 (33.3)	5 (100.0)	5	1 (16.7)	0 (0)	0 (0)	5 (83.3)	A: 1 B: 0 C: 1 D: 3 E: 0
Large ventricular septal defect	6 (5.5%)	Total: 2 FTS: 1 NIPT: 1 Cont: 0	0 (0)	Total: 4 AN: 2 PN: 2	1 (25.0)	1 (16.7)	5 (100.0)	5	1 (16.7)	0 (0)	0 (0)	5 (83.3)	A: 0 B: 1 C: 1 D: 3 E: 0
Tricuspid atresia	4 (3.7%)	Total: 3 FTS: 3 NIPT: 0 Cont: 0	2 (66.7)	Total: 3 AN: 2 PN: 2†	1 (33.3)	1 (25.0)	1 (50.0)	2	0 (0)	1 (25.0)	1 (25.0)	2 (50.0)	A: 0 B: 1 C: 1 D: 0 E: 0
Critical or severe aortic stenosis	3 (2.8%)	Total: 2 FTS: 1 NIPT: 1 Cont: 0	0 (0)	Total: 2 AN: 1 PN: 1	0 (0)	0 (0)	1 (50.0)	2	0 (0)	0 (0)	1 (33.3)	2 (66.7)	A: 0 B: 0 C: 0 D: 2 E: 0
Total	109	Total: 50 FTS: 26 NIPT: 16 Cont: 8	12 (24.0)	Total: 66 AN: 45 PN: 25†	15 (23.1)	14 (12.8)	62 (88.6)	70	24 (22.0)	2 (1.8)	18 (16.5)	65 (59.6)	A: 8 B: 13 C: 14 D: 28 E: 2

AN: antenatal, AVSD: atrioventricular septal defect, Cont: contingent, CVS: chorionic villus sampling, DORV: double outlet right ventricle, FTS: first trimester screening, NIPT: non-invasive prenatal testing, PN: postnatal, TGA: transposition of great arteries, TOP: termination of pregnancy, UVH: univentricular hearts

A: infant death, B: mild medical/developmental issues, C: major medical or developmental issues, D: no medical or developmental issues, E: postnatal lost to follow-up

* Where formal fetal echocardiography was performed, concordance was met when the obstetric fetal anatomical scan made the same major CHD diagnosis without missing any other major CHD

† Three cases of tetralogy of Fallot and one case of tricuspid atresia underwent postnatal genetic testing in addition to antenatal invasive genetic testing for the purpose of additional genetic testing

‡ One case of tetralogy of Fallot had a variant of unknown significance on chromosomal microarray analysis. These variants were also detected in phenotypically normal parents.

§ One aneuploidy screening result was deemed high risk, as it comprised two consecutive inconclusive NIPT results.

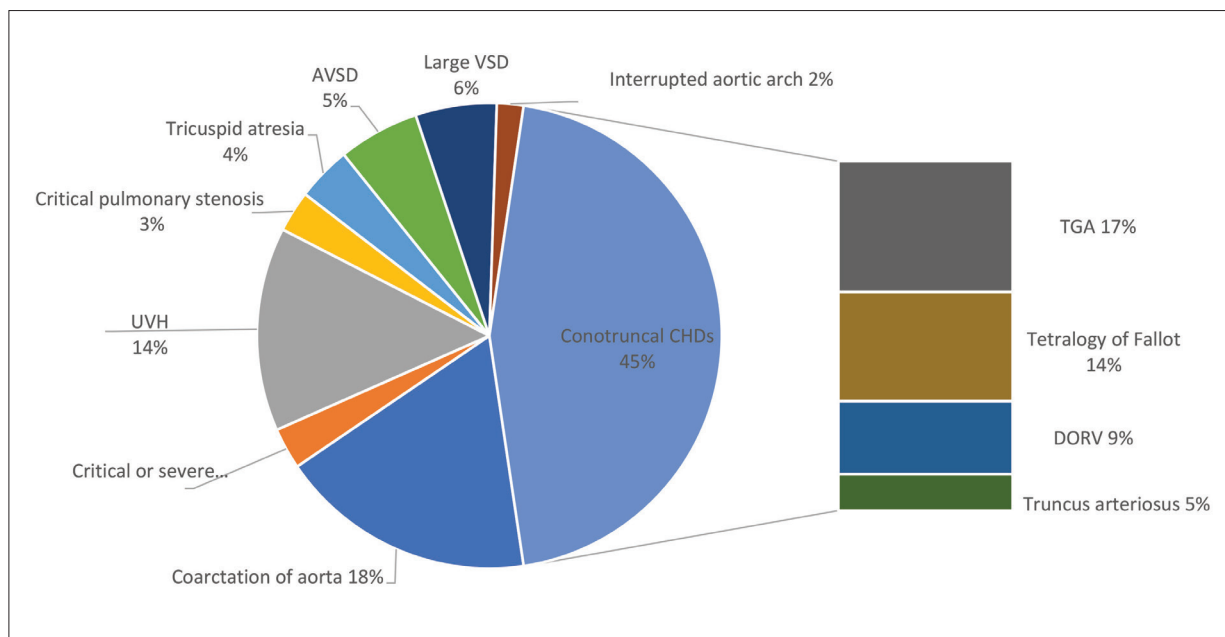


Fig. 2. Distribution of major congenital heart diseases.

AVSD: atrioventricular septal defect, CHD: congenital heart disease, DORV: double outlet right ventricle, TGA: transposition of great arteries, UVH: univentricular hearts, VSD: ventricular septal defect

Table 3. Fetuses with congenital heart diseases and extracardiac abnormalities.

Congenital heart disease	Extracardiac anomaly	Genetic diagnosis
TGA	CCAM	Amniocentesis karyotype: normal
TGA	CCAM	Amniocentesis karyotype: normal
TGA	Abdominal situs inversus	Genetic test not performed
TGA	Bilateral cleft lip and palate	Postnatal karyotype: normal
Truncus arteriosus	Left multicystic kidney	Amniocentesis karyotype: normal
Tetralogy of Fallot	Ectopia cordis	Amniocentesis karyotype: normal
Tetralogy of Fallot	Bilateral talipes equinovarus	Lost to follow-up
Tetralogy of Fallot	Epilepsy, global developmental delay, central hypotonia, central apnoea, nystagmus, hepatomegaly, supernumerary nipples	Postnatal chromosomal microarray: benign familial copy variants WES: homozygous mutation in PIGN causing congenital disorder of glycosylation
Tetralogy of Fallot	1) Spinal angulation at thoracic level 2) Dysmorphic facies with micrognathia	Amniocentesis karyotype: normal
Tricuspid atresia	Right ulnar dysplasia/hypoplasia with intrauterine growth restriction	Amniocentesis karyotype: 46,XX,der(21)ins(21;?)(q11.2;?)
UVH	Abdominal situs inversus	Lost to follow-up
AVSD	Inferior cerebellar vermis hypoplasia	Postnatal karyotype: trisomy 21
AVSD	Bilateral adducted fourth toe	Postnatal CMA: duplication of 444 kb in 15q13.3 encompassing part of <i>CHRNA7</i> gene. Known to have variable penetrance and not associated with AVSD. No parental CMA.
Large ventricular septal defect	Right talipes equinovarus	Postnatal karyotype: normal. FISH: 22q deletion detected

AVSD: atrioventricular septal defect, CCAM: congenital cystic adenomatoid malformation, CMA: chromosomal microarray analysis, FISH: fluorescence in-situ hybridisation, TGA: transposition of arteries, UVH: univentricular hearts, WES: whole exome sequencing

76.4% (65 in 85) were live births (Table 2 and Fig. 1). Of the 65 live births, 56.9% (37 in 65) were delivered via caesarean section with the remaining via normal vaginal delivery. Among the liveborn neonates, 72.3% (47 in 65) underwent postnatal cardiac intervention. At 4 years of follow-up, 43.2% (28 in 65) had no medical or developmental issues, 20.0% (13 in 65) had mild medical or developmental issues, and 21.5% (14 in 65) had major medical or developmental issues. Additionally, 12.3% (8 in 65) had deceased (at an average of 6.9 months of age), and 3.1% (2 in 65) were lost to follow-up.

Aneuploidy screening

Aneuploidy screening by FTS or NIPT had been performed in 45.9% (50 in 109) cases prior to the diagnosis of a major fetal CHD (Fig. 1). Of these, 52% (26 in 50) and 32% (16 in 50) were primary screening by FTS and NIPT, respectively, while 16% (8 in 50) were cases where couples opted for contingent NIPT as opposed to invasive testing after being counselled for high-risk or inconclusive FTS results. Of the 50 aneuploidy screenings performed, 24.0% (12 in 50) were high-risk results (FTS: 6; primary NIPT: 5; contingent NIPT: 1). Among these 12 high-risk results, 9 underwent confirmatory antenatal genetic testing with conventional karyotyping and FISH (2 CVS and 7 amniocentesis). Three were abnormal: 1 trisomy X, 1 22q11 microdeletion and 1 46 XX with an unbalanced insertion of unidentifiable material in the long arm of chromosome 21. Among the remaining 3 high-risk results where confirmatory antenatal testing was not performed, postnatal genetic testing revealed 2 cases of trisomy 21. Thus, among the 12 high-risk aneuploidy screening results, a total of 5 (41.7%) had a confirmed abnormal genetic diagnosis.

Confirmatory genetic testing

Out of the 109 cases of fetal CHD, 41.3% (45 in 109) had antenatal genetic testing by amniocentesis (43) or CVS (2). Nine were indicated by high-risk aneuploidy screening results, as described above, while the rest were indicated by findings of fetal CHD. Of the cases that underwent antenatal genetic testing, 60.0% (27 in 45) had not undergone any aneuploidy screening prior and 17.8% (8 in 45) had high-risk results. Of the 45 who had antenatal genetic testing, 42 underwent conventional karyotyping with FISH for 22q, 2 underwent CMA only and 1 underwent both CMA and whole exome sequencing (WES). Abnormal results were obtained in 17.7% (8 in 45) and are discussed below. Of the 65 live births, 38.4% (25 in 65) had postnatal genetic testing of which all except 3 were conventional karyotyping with

FISH for 22q (2 were CMA, 1 was both CMA and WES). Seven had abnormal results and are discussed below.

Out of the 109 cases of fetal CHD, 60.5% (66 in 109) underwent either antenatal or postnatal confirmatory genetic testing (4 cases with antenatal genetic testing also underwent other forms of postnatal genetic testing). Of these, 22.7% (15 in 66) had abnormalities. The following abnormalities were found on conventional karyotyping and FISH for 22q11 microdeletion: 5 22q11 microdeletion (2 tetralogy of Fallot, 1 DORV, 1 truncus arteriosus, 1 VSD); 4 trisomy 21 (2 AVSD, 1 tetralogy of Fallot, 1 univentricular heart); 1 trisomy X (tetralogy of Fallot); 1 monosomy X (coarctation of aorta); and 1 unbalanced insertion of unidentifiable material in long arm of chromosome 21 (tricuspid atresia in 1 twin of a dichorionic, diamniotic pair). Three cases had abnormal CMA results. One fetus with an AVSD had an abnormal postnatal CMA result showing a pathogenic 444 kb duplication in 15q13.3, encompassing part of the *CHRNA7* gene. While AVSDs have been reported with 15q13.3 microdeletions,²⁰ it has a highly variable clinical spectrum ranging from non-pathogenic to severe phenotype. Parental testing was not performed in this case to determine if it was de novo or inherited. For 1 fetus with tetralogy of Fallot, while antenatal CMA was normal, additional postnatal genetic testing was performed due to the presence of extracardiac defects, such as spinal angulation at the thoracic vertebrae and micrognathia and a previous termination of pregnancy for a fetus with multiple fetal anomalies. A postnatal expanded CMA panel only revealed benign familial copy number variants, and a next-generation sequencing panel for genes associated with skeletal malformations was unremarkable. WES revealed an inherited homozygous mutation in the *PIGN* gene causing a congenital disorder of glycosylation. Finally, 1 fetus with a tetralogy of Fallot had a variant of unknown significance with a 7p21.2 duplication of 453 kb and 7p21.1 duplication of 434 kb. While associated with macrosomia and a mild bowel dilation in literature, this was deemed to be a benign familial copy number variant as this was seen in the normal parent as well.

DISCUSSION

This is a longitudinal cohort study in 109 pregnancies with antenatally diagnosed major CHDs that stratifies outcomes by the type of CHD and reports their associated extracardiac anomalies, genetic tests, obstetric outcomes and developmental and health outcomes at 4 years of age. There was a high concordance between obstetric fetal anatomical scans and fetal echocardiography in the characterisation of major CHDs (88.6%). At 4 years

postnatally, 43.2% had no medical or developmental issues while 21.5% had major medical problems of global developmental delay and 12.3% deceased. Such data have been further presented stratified by the type of major CHD. We were able to report on the results of genetic testing, mostly via conventional karyotyping and FISH for 22q microdeletion in 60.5% of cases, and found that about a quarter had abnormalities. The most common genetic abnormality was a 22q microdeletion followed by trisomy 21. Overall, 59.6% of our patients progressed to live birth. Our cohort had a loss to follow-up rate of 22.0% (24 in 109) at the time of delivery and a further 3.1% after delivery (2 in 65).

The prevalence of major CHD in our study cohort was 5.3 per 1000 pregnancies screened at our centre. This value lies within figures provided by two other studies in Singapore; a single-centre study involving euploid fetuses conducted between 2008 and 2009 (2.7 per 1000 fetuses)¹⁸ and a national study involving live births between 1994 and 2000, which also included minor CHDs (8.12 per 1000 live births and stillbirths)⁹ (Fig. 3). Comparison with the former study was limited, as it excluded fetuses with abnormal karyotype, which accounted for 9.09% (6 in 66) of our cases that underwent

confirmatory genetic testing. The latter study evaluated birth defects reported to the National Birth Defects Registry after delivery (live birth or stillbirth) and abortion. A likely reason for an overall higher incidence of CHDs in Tan KH et al. (3.37 vs 0.30 per 1000) may be the inclusion of small VSDs based on postnatal diagnosis which are frequently not seen on fetal echocardiogram, do not require intervention, and may spontaneously close. Among other major CHDs, such as tetralogy of Fallot, HLHS or UVH, transposition of great arteries and truncus arteriosus, our cohort reported higher incidence rates than Tan KH et al., although this may be due to a selection bias as our hospital is a national referral centre for complicated fetal anomalies.

While Asia has the highest birth prevalence of CHDs (9.3 per 1000 live births⁵), significant inter-regional variations exist likely due to differences in factors such as nutrition,⁶ consanguinity,²¹ maternal age,²² ethnicity,⁹ abortion patterns,²³ temporal changes in CHD prevalence,⁵ availability of accurate fetal diagnosis^{5,7} and differences in reporting standards. Our cited prevalence is similar to that cited in a recent

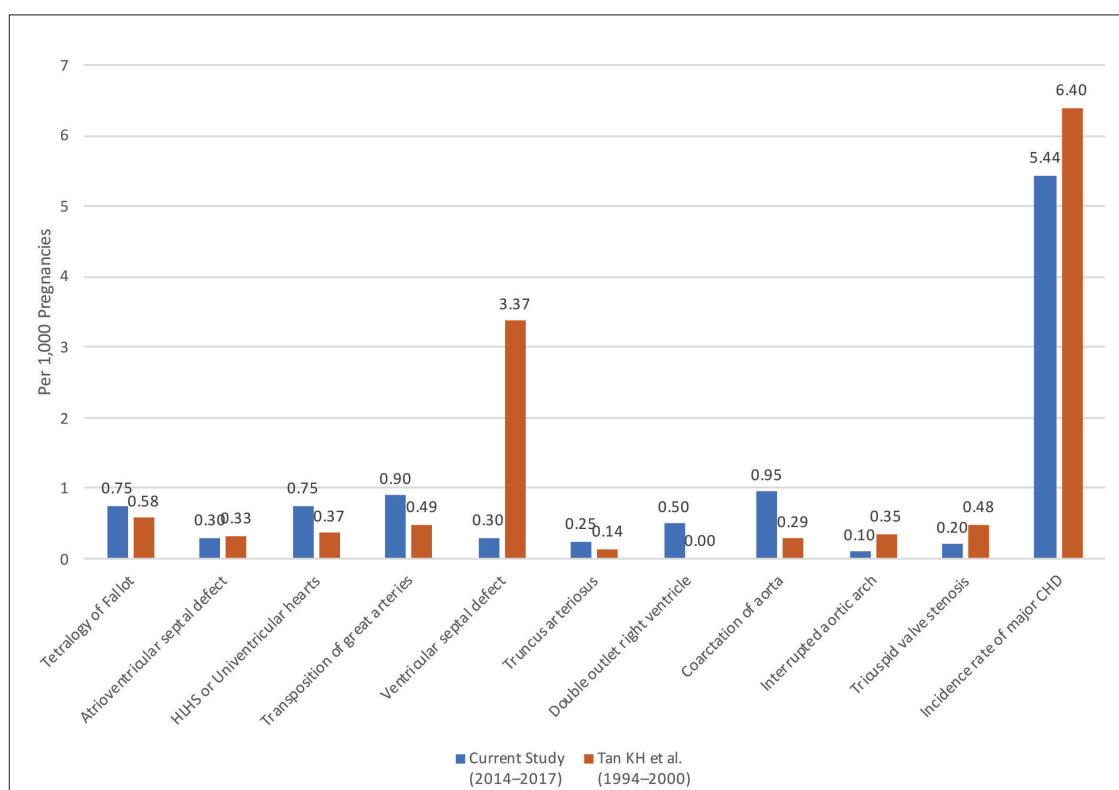


Fig. 3. Comparisons in prevalence of major congenital heart disease between the current study (2014–2017) and a historical cohort, i.e. Tan KH et al.⁹ (1994–2000).

extensive systematic review involving European populations (6.51 per 1000 fetuses⁶), although a higher proportion were conotruncal abnormalities (44.0% vs 20%). This may also be due to a selection bias arising from our centre's status as a major referral centre for complicated fetal anomalies.

While confirmatory antenatal genetic testing is recommended when 1 or more major fetal structural abnormalities are detected,²⁴ only 41.3% of our cohort underwent this, of which 18.3% found genetic abnormalities. CMA is currently recommended as a first-line test for isolated fetal anomalies, as submicroscopic copy number variants have been detected in up to 4.6% of cases with isolated fetal CHDs and normal karyotypes.²⁵ In our cohort, while only 6.67% of fetuses underwent antenatal CMA, this is likely because professional consensus for this was only formed from 2016 onwards,²⁴ i.e. in the latter years of this study's inclusion window. Two other factors, which may have lowered uptake during the timeframe of this study, included lack of government subsidy for CMA and NGS as well as minimal access to professional genetic counsellors, a feature that has been associated with increased uptake of genetic tests.^{26–28} Both factors have been addressed in recent years, and a more updated study would likely give a better understanding of the genetic diagnosis associated with major fetal CHDs.

With increasing affordability and accessibility, next-generation sequencing techniques, such as WES, will grow in importance in evaluating fetal anomalies.²⁹ Two large cohort studies^{30,31} and a recent meta-analysis³² have demonstrated that 5–11.1% of fetuses with CHDs and a normal karyotype or CMA had diagnostic genetic variants on WES with higher yields in multisystem fetal anomalies. As 65.4% of the diagnostic genetic variants were associated with learning disability,³¹ genetic counselling is increasingly a critical adjunct to a fetal cardiac service^{33,34} to help weigh decisions for the nature of tests to be done, continue pregnancy and allay parental anxiety.³⁵

While earlier studies did not demonstrate a significant mortality benefit associated with the antenatal diagnosis of CHDs,^{36,37} more recent meta-analyses^{38,39} have shown significant reduction in invasive respiratory support,⁴⁰ fewer perioperative neurologic events and earlier initiation of prostaglandin E1 therapy⁴¹ likely as a result of improved antenatal counselling, better coordination of delivery resources⁴² and facilitation of postnatal care and procedural planning.⁴³ Furthermore, accurate antenatal diagnosis will play an important role in facilitating nascent fetal cardiac interventions, such as aortic

valvuloplasties, to prevent progression of aortic stenosis into HLHS.⁴⁴

Strengths and limitations

To the best of the researchers' knowledge, this historical cohort study of fetal CHDs is the largest reported in Singapore. While local birth registry data have been published,^{8,9} we have not explored other aspects, such as demographics, sonographic and echocardiographic diagnoses; aneuploidy screening results; extracardiac abnormalities; genetic testing results; postnatal findings; and intermediate-term outcomes. We believe this study serves as a baseline for other local and regional studies. As the outcomes of our study have been stratified according to the type of CHD, this research aids counselling of parents at the time of fetal CHD diagnosis. We also believe our study establishes a framework for reporting and auditing outcomes of a fetal CHD screening programme with charting of outcomes from the time of antenatal diagnosis until 4 years old.

A major limitation in our cohort was the low uptake of CMA despite being a first-line test for fetal anomalies. This was likely due to cost considerations, as CMA was not covered by either government co-payment or most insurance providers. Utilising CMA, as opposed to karyotyping as a first-line test, was also gaining acceptance around the duration of this cohort. Further studies to understand factors that contribute to patient acceptability of antenatal genetic tests should be conducted. Another limitation of our study was a significant loss to follow-up rate of 22.0% during pregnancy. Given that the expertise and availability of advanced paediatric cardiac services are primarily in tertiary public health institutions in Singapore, it is likely that most of these cases switched to local private or overseas healthcare institutions for terminations of pregnancy either for privacy reasons, cost savings, or due to Singapore's legal limits for termination of pregnancy being at the gestational age of 24 weeks completed. As our study's inclusion criteria involved fetal CHDs detected antenatally, there is a possibility that missing CHDs are detected only postnatally either due to false negative screening at the fetal anatomical scan or poor antenatal follow-up. While this population is anecdotally thought to be very small, reviewing paediatric cardiac databases would eliminate this ambiguity and contribute to more holistic conclusions. Finally, given that single-centre studies are susceptible to selection biases, major CHDs may also have been potentially over-represented at our centre, which is a referral site for complex fetal FAs. Nevertheless, to our knowledge,

there is no formal database at a national-level or in other health institutions that includes our study parameters to readily facilitate comparison.

Further research

To mitigate some of the above limitations, a population-based approach involving a national birth defect registry linking genetic diagnosis, extracardiac findings and long-term outcomes would permit a more holistic understanding of the true distribution of fetal CHDs and temporal epidemiologic trends. Additionally, multicentre data would facilitate an audit assessing the effectiveness of current antenatal birth defect screening policies in detecting fetal CHDs early and accessibility of confirmatory genetic tests to interested parents. A more contemporary study would also capitalise on the rising accessibility to antenatal genetic diagnosis through CMA and NGS as well as professional genetic counselling to allow a more updated understanding of the relationship between fetal CHD and genetic abnormalities.

CONCLUSION

The accuracy of fetal anatomical scans and echocardiography in detecting major CHD is high with good concordance between prenatal and postnatal diagnoses. As CHD is the most common fetal anomaly¹ and with 22.7% being associated with genetic abnormalities, fetal CHD diagnosis may increasingly serve as a gateway for antenatal genetic diagnoses. High-quality genetic counselling and testing is essential. A repeat study with a similar format involving present-day practices, which include CMA and next generation sequencing (e.g. WES), would help develop local guidelines for counselling CHD.

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