



OPEN Chromosomal analysis and short-term outcome of prenatally diagnosed congenital heart disease

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Congenital structural heart disease (CHD) is the leading cause of infant death from birth defects. Postnatal survival primarily depends on the type and severity of the defect. In addition, worse cardiac prognosis is observed when extra-cardiac anomalies (ECA) are associated. This retrospective chart review was aimed at finding markers for short-term outcome prediction of prenatally-diagnosed complex CHD, focusing in particular on the impact of CHD category, of CHD severity score and of prenatal or postnatal diagnosis of ECA or chromosomal anomalies on 4 primary outcomes: termination of pregnancy (TOP), intrauterine fetal demise, neonatal mortality and 1-year-survival rate. We reviewed medical files from 381 fetuses, presenting at our center between 2018 and 2021 with CHD for which prenatal advice by a pediatric cardiologist was sought. 341 fetuses met the inclusion criteria for the study. Twin pregnancies (7.62%; OR 4.76 ($p < 0.001$)) and pregnancies resulting from assisted reproductive technology (7.33%; OR 2.44 ($p < 0.001$)) were more prevalent compared to the general population. CHD categories and CHD severity scores, ranging from A (extremely high risk based on CHD or ECA type) to D (low risk), were assigned to each fetus. Prenatal or postnatal chromosomal microarray results were available for 232 fetuses (68%) and were abnormal in 30 (12.9%). Logistic regression analysis was used to determine significant predictors for the primary outcomes 'TOP', 'postnatal demise before the age of 1 month' and 'survival at the age of 1 year'. TOP was carried out significantly more with: prenatal genetic diagnosis, severity score A and severity score B. Interestingly, a prenatal genetic diagnosis was negatively correlated with pregnancy continuation, but it was not a significant predictor for postnatal mortality, while a postnatal diagnosis of a genetic disorder impacted early but not late postnatal mortality. In addition, postnatal mortality both before the age of 1 month or before the age of 1 year was significantly associated with lower postmenstrual age at birth, CHD severity score B and major ECA at birth. These results underscore the importance of genotyping and of accurate cardiac and extracardiac phenotyping for prognostication in fetuses with CHD.

Keywords Congenital heart defects, Chromosomal, Prenatal, Outcome

Congenital heart disease (CHD) is the most common type of birth defect, occurring in about 1% of pregnancies and in 0.8% of live births^{1,2}. Although surgical advances have improved the pooled survival rate of CHD to adulthood to about 90%³, CHD remains the leading cause of mortality from birth defects and imposes a heavy disease burden. Neonatal outcome of prenatally-diagnosed CHD primarily depends on the type and severity of the defect. CHD types are often grouped together in CHD categories based on shared morphology or embryological mechanisms. The most common prenatally diagnosed CHD categories are septal defects (16–48%), conotruncal heart defects (20%), left ventricle outflow tract obstruction (LVOTO, 7–21%), right-sided anomalies (5–7%), univentricular hearts (UVH) (5%) and heterotaxy (6%)^{4–7}. Postnatal mortality ranges from 38% in UVH to 2.5% in ventricular septal defects^{8,9}. However, outcome varies within each CHD category and even between individuals with the same CHD type. For example individuals with isolated d-transposition of the great arteries (d-TGA) have a better outcome compared to those with complex d-TGA¹⁰. Neonatal CHD outcome is further determined by perinatal CHD management, by pregnancy complications, such as prematurity, dysmaturity

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or multiple pregnancies, and by demographic parameters^{5,11,12}. In addition, worse cardiac (and extra-cardiac) prognosis is observed when extra-cardiac anomalies (ECA) and/or genetic pathogenic variants are present^{5,7}.

The diagnostic yield of genetic testing highly depends on CHD type, family history and co-occurrence of ECA. The majority of CHD are non-syndromic (NS-CHD or isolated CHD) (80%) and have a complex background with an interplay of hitherto unknown genetic and environmental factors. Chromosomal or genetic pathogenic variants are found in less than 5% of individuals with sporadic NS-CHD, increasing up to 10–30% for familial CHD, which represents 3–5% of the NS-CHD cohort^{13–15}. Syndromic CHD (S-CHD or non-isolated CHD), defined as CHD in association with additional congenital defects and/or abnormal growth (>2SD or <-2SD), development and/or behavior, represents 20% of the CHD cohort. Aneuploidy, including monosomy X and trisomy 21, 13 or 18, is observed in about 14% of individuals with S-CHD¹⁶, while submicroscopic copy number variants (CNVs) are identified by chromosomal microarray (CMA) or CNV sequencing (CNVseq) in 15–20%⁴. The spectrum of CHD-related pathogenic CNVs ranges from recurrent clinically recognizable CNV syndromes, like 22q11.2 deletion syndrome or Williams-Beuren syndrome, to partially overlapping CNVs with unique breakpoints comprising dosage-sensitive genes or regulatory elements. Pathogenic or likely pathogenic single nucleotide variants (SNVs), diagnosed by Sanger sequencing, by targeted gene panels or by exome or genome sequencing, are found in about 35–40% of individuals with S-CHD with normal CMA results^{17–19}.

When CHD is diagnosed prenatally, S-CHD is difficult to differentiate from NS-CHD, as assessment of facial features and development is impossible or limited, and some ECA, such as coloboma, minor limb defects or cleft palate, may be missed. Therefore, prenatal genetic testing is gaining importance in the prognostication of fetuses with complex CHD. Non-invasive prenatal testing (NIPT) is increasingly applied to screen for fetal aneuploidies, leading to diagnosis of trisomy 21, 18 or 13, even before CHD is diagnosed on prenatal ultrasound²⁰. Although sensitivity to detect submicroscopic CNVs by NIPT is increasing, prenatal invasive CNV analysis by CMA or CNVseq is considered the gold standard to identify pathogenic CNV when complex CHD or S-CHD is diagnosed prenatally, with a diagnostic yield of about 10–15%^{5–7,20}. Salzer-Sheelo et al. showed that the yield from CMA analysis in a prenatal and postnatal CHD cohort was not significantly different (10.8% versus 14.7%), and confirmed that in both groups the detection rate was significantly higher for non-isolated versus isolated CHD (22.4% versus 6.4%)²⁰. In fetuses with CHD and normal CNV results, a pathogenic genetic variant is found by prenatal trio exome sequencing (ES) in 5–21%, varying according to presence of ECA and/or to CHD type^{5–7,21–23}. In a systematic review, comprising 18 studies, the yield of prenatal ES was 21%, 11% and 37% respectively in all fetuses with CHD, in fetuses with apparently isolated CHD and in fetuses with CHD associated with ECA²³.

This single-center retrospective chart review is aimed at finding markers for short-term outcome prediction of prenatally-diagnosed CHD, focusing in particular on the impact of CHD category, of CHD severity score and of prenatal or postnatal diagnosis of ECA or submicroscopic pathogenic CNVs on 4 primary outcomes: termination of pregnancy (TOP), intrauterine fetal demise (IUM), neonatal mortality and 1-year-survival rate.

Patients and methods

Study approval

Ethical approval to study these files was obtained from the ethical commission of KU and UZ Leuven (MP020190). Due to the retrospective nature of the study, the ethical commission of KU and UZ Leuven waived the need of obtaining informed consent. The study was conducted in accordance with the ethical standards of the Helsinki Declaration.

Inclusion criteria

Medical charts were reviewed from all fetuses or children who were diagnosed and/or followed prenatally with CHD at the obstetrics and pediatric cardiology department at University Hospitals Leuven (UZL) between January 1, 2018 and November 22, 2021. Inclusion was restricted to fetuses or children with CHD requiring prenatal counseling by a pediatric cardiologist at UZL, regardless of ethnic background, type of CHD on prenatal ultrasound, course of the pregnancy, neonatal outcome, and regardless of the eventual cardiac diagnosis made postnatally or post-mortem. Fetuses appearing to have a normal heart on first trimester ultrasound and diagnosed with fetal aneuploidy (trisomy 21, 18 or 13, or monosomy X) by non-invasive prenatal screening (NIPS) were excluded. Additional exclusion criteria were: (1) fetuses with prenatal diagnosis of arrhythmia, cardiomyopathy, left-sided superior caval vein or cardiac tumors in the absence of CHD, (2) children with postnatal diagnosis of CHD, (3) fetuses referred to UZL for a second opinion after CHD diagnosis in a different university hospital, (4) fetuses with CHD for whom no prenatal advice by a pediatric cardiologist was sought either because of a low risk CHD (not requiring neonatal intervention), or because of a suspected poor prognosis due to severe ECA (for which termination of pregnancy (TOP) was requested regardless of the cardiac prognosis).

Data collection

Medical information, demographic data and results from diagnostic genetic testing were retrieved from fetal, pediatric and maternal medical files, and were pseudonymized. Familial history of CHD (up to 3rd degree relatives) and/or familial occurrence of known pathogenic CNVs or SNVs for congenital or developmental disorders was recorded. Pregnancy information included: maternal age at the start of pregnancy, gravidity, spontaneous pregnancy versus assisted reproduction, singleton versus multiple pregnancy, and postmenstrual age (PMA) at the time of fetal CHD diagnosis. Pre- and postnatal CHD types were recorded based on the recordings by the pediatric cardiologist. ECA diagnosed either prenatally, at birth or at post-mortem investigation were documented. ECA included additional congenital anomalies, intrauterine growth restriction (IUGR), micro/macrocephaly, increased nuchal translucency and congenital anomalies with little or no functional impact (e.g. facial dysmorphic features, hyperechogenic bowel, single umbilical artery...). Major ECA were defined as

structural extracardiac anomalies that are potentially life-threatening (e.g. diaphragmatic hernia, esophageal atresia, right or left isomerism, fetal hydrops...) or result in severe disability (e.g. structural brain anomalies). Minor anomalies include structural anomalies on prenatal ultrasound that are deemed to have little or no effect on body functionality (e.g. dysmorphic features, polydactyly, hydronephrosis, club feet, small thymus, IUGR...). Acquired anomalies (e.g. ischemic or infectious brain damage, post-surgical diaphragm paralysis, feeding difficulties...) were not considered as ECA. Primary pregnancy outcomes were (1) termination of pregnancy, (2) intrauterine fetal demise, (3) live birth. Postnatal outcome was documented as (1) 1-year survival, (2) early postnatal demise (< 1 month) (3) demise during infancy (> 1 month < 1 year).

CHD categories and CHD severity scores

CHD types were grouped together in CHD categories based on shared morphology or embryological mechanisms in accordance with the classification that was used by Gowda et al.²⁴ (Table 1). Only one CHD category was ascribed to each fetus. If several CHD types were diagnosed, CHD classification was based on the most important prognostic and anatomical cardiac defect (e.g. isolated pulmonary valve atresia (PA), tetralogy of Fallot with PA and univentricular heart with PA were classified respectively as a right sided heart defect, conotruncal heart defect and univentricular heart).

In addition, a severity score was assigned to each fetus based on the cardiac phenotype or the co-occurrence of additional major congenital anomalies and/or chromosomal aneuploidy. A 4-class scoring system (A, B, C or D) was applied as described by Gowda et al.²⁴ (Supplementary Table 1). In summary, severity score A was assigned to CHD associated with severe potentially lethal ECA (e.g. congenital diaphragmatic hernia or hydrops fetalis), with aneuploidy or with genetic disorders associated with severe intellectual impairment (extremely high risk). Score B corresponds to isolated CHD requiring multiple surgeries and associated with high mortality after surgery (high risk). Score C (moderate risk) and score D (low risk) relate to isolated CHD with respectively variable and good prognosis after surgery. For severity scores A and B, the option of TOP was considered whenever legally permissible. For scores C and D continued monitoring of pregnancy with postnatal surgery was emphasized if applicable. Prenatal genetic work-up by CMA was recommended for fetuses with scores A or B, and CMA was offered, either prenatally or postnatally, for all other fetuses²⁵.

Isolated versus non-isolated CHD

Fetuses were classified as having non-isolated CHD when associated with at least one of the following prenatal ultrasound findings: (1) additional major congenital anomaly, (2) microcephaly and/or IUGR (<-2 SD), (3) facial dysmorphic features, (4) increased nuchal translucency (> 3.5 mm at 12 weeks of gestation), cystic hygroma or hydrops fetalis, (5) isomerism/situs anomalies. If prenatal phenotyping was restricted to the cardiac phenotype, fetuses were considered to have CHD with undetermined extracardiac status.

Postnatal classification of non-isolated CHD was defined as CHD in association with at least one of the following criteria: (1) additional major congenital anomaly, (2) microcephaly and/or abnormal growth (<-2.5 SD or > + 2.5 SD) taking gestational age at birth into consideration, (3) facial dysmorphic features (defined as the presence of at least 3 minor facial anomalies), (4) severe unexplained hypotonia or motor delay, (5) pathogenic or likely pathogenic CNVs or SNVs associated with developmental disorders. If postnatal development or growth could not be assessed due to intrauterine demise, termination of pregnancy or postnatal loss of follow-up, patients were considered to have CHD with undetermined extracardiac status.

CHD categories and types	Number	%	Hureau et al.	Qiao et al.	van Nesselrooij et al.	Gowda et al.	Stallings et al.	Average literature (%)
Conotruncal ToF, d-TGA, truncus art	115	33.72	25%	25%	19.77%	32.85%	39.54%	28.43
LVOTO AS, CoAo, IAA	64	18.77	21%	7.22%	15.82%	25.55%	33.76%	20.67
UVH HLHS	39	11.44	21%	NA	9.46%	5.84%	18.28%	13.65
Septal VSD, ASD, AVSD	36	10.56	16%	48.34%	20.34%	32.85%	NA	29.38
Right-sided PS, TA, DORV	31	9.09	5%	6.94%	16.65%	32.85%	25.02%	17.29
Situs anomaly heterotaxy, left/right isomerism	16	4.69	NA	5.83%	1.69%	4.37%	NA	3.96
AVR TAPVR, PAPVR	9	2.64	NA	NA	2.54%	8.03%	6.64%	5.74
Other RAA	31	9.09	12%	6.67%	13.42%	1.46%	NA	8.39
Total	341	100						

Table 1. CHD categories (and common CHD types per category). Distribution of CHD categories within this study was compared to that of previously reported cohorts (4–7,27). Abbreviations: art: arteriosus; AS: aortic stenosis; AVR: anomalous venous return; CoAo: aortic coarctation; CHD: congenital heart defect; DORV: double outlet right ventricle; d-TGA: dextro-transposition of great arteries; IAA: interrupted aortic arch; LVOTO: left ventricle outflow tract obstruction; PS: pulmonary valve stenosis; RAA: right aortic arch; TA: tricuspid atresia; TOF: tetralogy of Fallot; UVH: univentricular heart.

Genetic data

The retrieval of genetic data was restricted to documented pathogenic or likely pathogenic CNV or SNV (in accordance to the ACMG guidelines for CNV or SNV classification)^{26,27} which were identified by prenatal or postnatal CMA or sequencing. Prenatal CMA by OGT 60k array, postnatal CMA by OGT 180k array and NGS by clinical exome sequencing were performed as described^{28–30}. NIPS was done as described³¹. Raw genetic data were not re-analyzed nor were newly generated for this retrospective chart review.

Statistical analyses

One sample t-test was used to compare mean values of continuous variables (e.g. maternal age, gestational age) and chi-square test (or Fisher exact test) to compare categorical variables (e.g. IVF versus spontaneous pregnancy, singleton versus twins) between the patient population and the Belgian reference population^{32–34}.

A logistic regression analysis was used to determine significant predictors for three primary outcomes: (1) TOP versus non-TOP in the entire prenatal CHD cohort, (2) early postnatal demise: comparison of liveborn patients with prenatally diagnosed CHD that survived > 1 month to those who did not, and (3) survival: comparing liveborn patients with prenatally diagnosed CHD that were alive at the age of 1 year to those who were not. Fetuses or live births with missing outcome data were excluded.

We took into account multiple parameters that potentially impact pre- and postnatal outcome. For the outcome ‘TOP’, the following parameters were considered: CHD category, CHD severity score (A-D), prenatal genetic diagnosis, prenatal diagnosis of minor ECA and prenatal diagnosis of major ECA. For the outcomes ‘<1 month demise’ and ‘survival at 1 year’ we selected: CHD category, CHD severity score (A-D), prenatal genetic diagnosis, postnatal genetic diagnosis, postmenstrual age at birth (continuous variable), birth weight (continuous variable), postnatal diagnosis of minor ECA or postnatal diagnosis of major ECA. Parameter selection for each logistic regression model was done by stepwise removal of the parameter with the highest p-value until lowest AIC (Akaike Information Criterion) values were reached. We used Variance Inflation Factor (VIF) to detect multicollinearity between the selected parameters per outcome. Parameters with high collinearity (> 5) were removed from the model. CHD severity scores (A-D) and the 8 different CHD categories were one hot encoded. P-values of < 0.05 were judged as significant. To capture the size of the effect, we provided forest plots with OR values and their confidence intervals for the selected parameters (Fig. 1 and supplementary Table 5).

Results

Fetal cohort

From January 2018 until November 2021 prenatal advice by the pediatric cardiologist was sought for 384 fetuses with prenatally diagnosed CHD. After exclusion of 43 fetuses (25 referrals from other university hospitals for second opinion; 5 without prenatal CHD; 5 with isolated arrhythmia; 4 with isolated cardiomyopathy; 3 with cardiac tumors and 1 with isolated left VCS), maternal and fetal data from 341 fetuses with CHD were reviewed (supplementary Fig. 1). The average maternal age at the time of CHD diagnosis was 30.93 years (range 18–44), which is equal to the average maternal age of all pregnancies in Belgium in 2020³² (supplementary Fig. 2). The majority of pregnancies involved G1 (N=96) and G2 (N=98) pregnancies (supplementary Fig. 3). Twin pregnancies represented 7.62% of the fetal CHD cohort (26/341), including 10 DCDA, 14 MCDA and 2 MCMA twins (including one MCDA twin with CHD in both fetuses). At least 25 pregnancies (7.33%) were documented to have occurred after assisted reproduction by in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Familial history was positive for CHD (regardless the CHD type) in 40 out of 341 fetuses (11.73%), with respectively 4.99%, 2.34% and 4.4% having at least one first, second or third-degree relative with CHD.

Prenatal CHD diagnosis

The median postmenstrual age (PMA) at CHD diagnosis at our center was 23 weeks: most diagnoses were made between PMA week 20–23, at week 30–31 or at week 36 (supplementary Fig. 4). CHD categories and CHD severity scores were assigned to each fetus²⁴. Conotruncal heart defects (33.72%) and LVOTO (18.77%) were the most common CHD categories (Table 1). Severity scores A, B, C and D were assigned respectively to 7%, 40%, 34% and 19% of the fetuses (Table 2).

Non-isolated CHD

Non-isolated CHD was diagnosed prenatally in 86 out of 341 (25%) fetuses. At birth or after TOP, 14 fetuses with ECA on prenatal ultrasound (16%) were considered to have isolated CHD, while 61 fetuses (71%) were confirmed to have non-isolated CHD, including 54 patients with various syndromes and 7 patients presenting with left or right isomerism. Eleven fetuses (13%) of the prenatal ECA cohort could not be assessed due to insufficient phenotypic information at birth or post-TOP. Among 206 fetuses with apparently isolated CHD on prenatal ultrasound, nineteen (19/206 (9%)) were diagnosed with syndromic CHD at birth or after TOP (supplementary Table 4).

NIPS results

NIPS results were available for 154 pregnancies (45%), retrieving 7 abnormal results (trisomy 7 (2x), 18, 20, 21 (2x) and 22q11 deletion), 4 of which (T7 (2x), T20 and T21 (1x)) could not be confirmed by prenatal CMA on amniotic fluid due to false positive results or to confined placental mosaicism. The fetuses with confirmed T18, T21 and 22q11DS had a diagnosis of CHD on prenatal ultrasound prior to performing NIPS. NIPS was not performed or results were not available for the other 187 pregnancies.

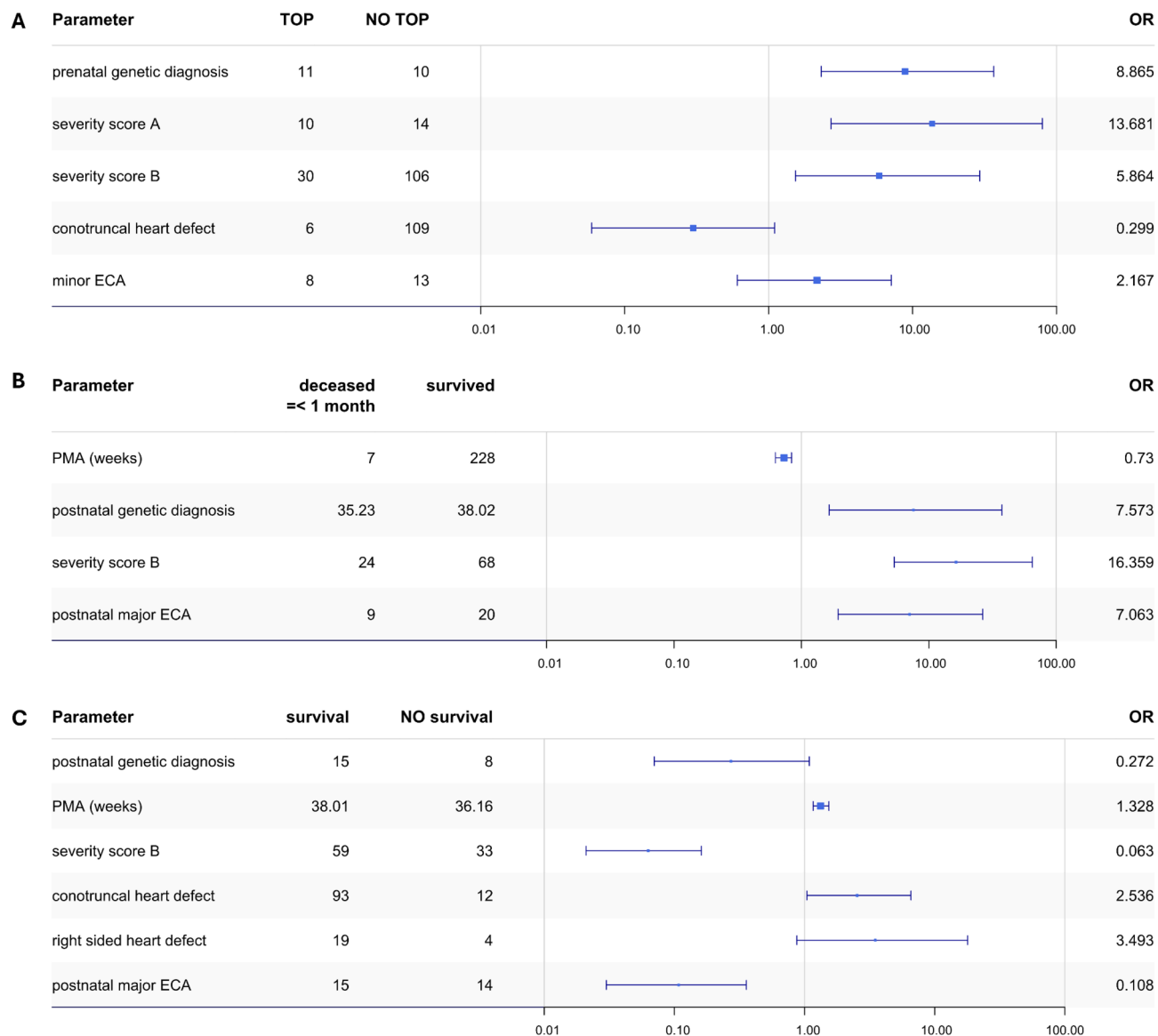


Fig. 1. Forest plots displaying odds ratios for the following three outcomes: (A) pregnancies which resulted in a termination (B) liveborns who died within the first month after birth. (C) Liveborns who survived past 1 year of age. X-axis shows the odds ratio. ECA: extracardiac anomalies; PMA: postmenstrual age; TOP: termination of pregnancy.

CHD severity score	Score A	Score B	Score C	Score D	Total
Number	24	136	116	65	341
%	7%	40%	34%	19%	100%
Gowda et al.	10%	45.5%	20.5%	24%	

Table 2. CHD severity scores (from score A to D) based on the scoring system reported by Gowda et al.²⁷ and available in supplementary table 1.

CMA results

After excluding fetuses with an abnormal NIPS, CMA results were available for 232 fetuses (68%, 232/341), either performed prenatally ($N=126$) or after birth/TOP ($N=106$). Pathogenic or likely pathogenic chromosomal variants were identified for 30 patients either prenatally ($N=18$; 18/126 (14%)) or postnatally ($N=12$; 12/106 (11%)) (Table 3; supplementary Table 2). The most common chromosomal disorders were 22q11.2 deletion syndrome ($N=10$) and trisomy 21 ($N=4$) (supplementary Table 3). For 27 fetuses with normal prenatal CMA

Prenatal ECA status	CMA	Total	Positive	Negative	Diagnostic yield (%)
Isolated-CHD N=206	PREN CMA	60	5	55	
	POST CMA	75	5	70	
		135	10	125	7.4%
Non-isolated CHD N=86	PREN CMA	43	13	30	
	POST CMA	25	6	19	
		68	19	49	27.9%
CHD of unknown ECA status N=49	PREN CMA	23	0	23	
	POST CMA	6	1	5	
		29	1	28	3.4%
Total CHD cohort N=341	PREN CMA	126	18	108	
	POST CMA	106	12	94	
		232	30	202	12.9%

Table 3. Diagnostic yield by prenatal (PREN) or postnatal (POST) chromosomal microarray analysis (CMA) in fetuses with apparently isolated versus non-isolated CHD on prenatal ultrasound. Chromosomal pathogenic variants identified by NIPS were excluded.

results, CMA was repeated postnatally by 180k OGT array, but did not yield additional pathogenic variants. In accordance with the Belgian guidelines for variant reporting of prenatal genetic testing²⁸, CNVs of unknown significance (VUS) identified by prenatal CMA were not communicated. These regulations did not apply for postnatal CMA results, reporting back VUS for 21 liveborn children who only had postnatal CMA analysis ($N=107$) or who had both prenatal and postnatal CMA analysis ($N=27$) (15.5%; 21/134). None of these VUS were reclassified as pathogenic or likely pathogenic after parental segregation analysis and clinical assessment.

We also compared CMA results between fetuses with and without prenatal diagnosis of ECA. Pre- or postnatal CMA results were available for 135 fetuses with prenatal apparently isolated CHD, for 68 fetuses with non-isolated CHD and for 29 fetuses with undetermined extracardiac status, and identified pathogenic CNVs in respectively 7.4% (10/135), 27.9% (19/68) and 3.4% (1/29) fetuses. Diagnostic yield of CMA was not significantly different between fetuses with prenatally diagnosed minor ECA versus major ECA.

NGS results

NGS-based targeted gene panel testing or clinical exome sequencing (ES) was not systematically conducted, but was restricted to 16 individuals with diagnosis of non-isolated CHD and normal CMA results. Pathogenic or likely pathogenic SNVs were retrieved by prenatal NGS in 1 patient (*RIT1*) and by postnatal (or post-TOP) NGS in 11 patients (*KMT2D* (2x), *PTPN11*, *RAF1*, *SON*, *DYNC2H1*, *PBX1*, *FOXF1*, *SMARCA4*, *KAT6A*, *PAH*). Variants of unknown significance, inherited from an unaffected parent, were reported in 2 patients (*FLT4*, *SMAD6*).

Outcome

Prenatal outcome was not documented for 16 out of 341 pregnancies. The pregnancy in the remaining 325 fetuses ended either in TOP, an intrauterine demise or a live birth in respectively 44 (13.5%), 4 (1.2%) and 277 (85.3%) pregnancies. Early (<1 month) and late (>1 month and <1 year) postnatal demise were documented for respectively 33 (11.9%) and 15 (5.5%) liveborn children ($N=277$). Full-term and premature birth (<36 weeks PMA) was recorded for respectively 232 and 31 liveborn children. Extreme prematurity (≤ 28 weeks PMA) was documented in 7 children. PMA at birth was not available for 14 liveborn children. Neonatal palliative care was proposed when counseling parents of fetuses with high risk CHD, but no comfort care was documented for any of these patients. Prenatal and postnatal outcome according to CMA results, CHD category, CHD severity score and multiple pregnancies are summarized in Table 4.

Markers for outcome

Logistic regression was performed to find parameters predisposing to 'TOP', to 'early postnatal demise' (<1 month demise) and to 'survival' beyond the age of 1 year.

1. Logistic regression analysis for 'TOP': within the cohort of 341 fetuses with prenatally diagnosed CHD, TOP was documented for 44 fetuses. We excluded 16 fetuses with undetermined pregnancy outcome. Parameters with the highest p values were removed until the lowest AIC value (111.4) for the logistic regression model was obtained. The parameter 'prenatal diagnosis of major ECA' was removed due to high collinearity (>5) by VIF. By logistic regression we showed that TOP was significantly associated with prenatal genetic diagnosis ($p=0.001$), severity score A ($p=0.001$) and severity score B ($p=0.015$). No statistical significance was reached for the presence of prenatal minor ECA ($p=0.211$). The CHD category 'conotruncal CHD' showed a trend towards higher continuation of the pregnancy, but this finding was not statistically significant either ($p=0.096$). Although not significant, removal of these two parameters would cause a major increase in AIC. Therefore, these two parameters were kept in the model.
2. The logistic regression analysis for '<1 month demise': within the cohort of 277 liveborn children with prenatally diagnosed CHD, 33 died within the first month after birth. Statistically significant predictors for early

		Numbers	TOP	IUM	Unknown	Live birth	Mortality < 1 month	Mortality 1-12 months	Survival to 12 months
Pregnancy	singleton	315	43 (14%)	4 (1%)	15 (5%)	253 (80%)	29 (11.5%)	14 (5.5%)	210 (83%)
	multiple	26	1 (4%)	0	1 (4%)	24 (92%)	4 (17%)	1 (4%)	19 (79%)
ECA	no	206	7 (3.5%)	2 (1%)	1 (0.5%)	196 (95%)	8 (4%)	8 (4%)	180 (92%)
	unknown	49	23 (47%)	1 (2%)	11 (22.5%)	14 (28.5%)	11 (78.5%)	0	3 (21.5%)
	yes	86	14 (16.5%)	1 (1%)	4 (4.5%)	67 (78%)	14 (21%)	7 (10.5%)	46 (68.5%)
	minor ECA	50	8 (16%)	0	1 (2%)	41 (82%)	8 (19.5%)	2 (5%)	31 (75.5%)
	major ECA	36	6 (17%)	1 (3%)	3 (8%)	26 (72%)	6 (23%)	5 (19%)	15 (58%)
Genetic diagnosis	yes	45	12 (27%)	0	1 (2%)	32 (71%)	8 (25%)	1 (3%)	23 (72%)
	no	296	32 (11%)	4 (1%)	15 (5%)	245 (83%)	25 (10%)	14 (6%)	206 (84%)
Severity score	A	24	9 (37.5%)	0	2 (8.5%)	13 (54%)	3 (23%)	3 (23%)	7 (54%)
	B	136	30 (22%)	4 (3%)	10 (7%)	92 (68%)	24 (26%)	9 (10%)	59 (64%)
	C	116	4 (3%)	0	2 (2%)	110 (95%)	6 (5.5%)	2 (2%)	102 (92.5%)
	D	65	1 (1.5%)	0	2 (3%)	62 (95.5%)	0	1 (1.5%)	61 (98.5%)
CHD category	conotruncal	115	6 (5%)	1 (1%)	3 (3%)	105 (91%)	7 (6.5%)	5 (5%)	93 (88.5%)
	LVOTO	64	5 (8%)	1 (1.5%)	3 (4.5%)	55 (86%)	7 (12.5%)	3 (5.5%)	45 (82%)
	UVH	39	18 (46%)	0	3 (8%)	18 (46%)	10 (55.5%)	3 (16.5%)	5 (28%)
	septal	36	6 (16.5%)	0	2 (5.5%)	28 (78%)	2 (7%)	0	26 (93%)
	right-sided	31	5 (16.25%)	1 (3.25%)	2 (6.5%)	23 (74%)	4 (17.5%)	0	19 (82.5%)
	situs anomaly	16	2 (12.5%)	1 (6%)	3 (19%)	10 (62.5%)	0	4 (40%)	6 (60%)
	AVR	9	0	0	0	9 (100%)	3 (33.5%)	0	6 (66.5%)
	other	31	2 (6.5%)	0	0	29 (93.5%)	0	0	29 (100%)
Total	all	341	44 (13%)	4 (1%)	16 (5%)	277 (81%)	33 (12%)	15 (5.5%)	229 (82.5%)

Table 4. Pregnancy outcomes in accordance to severity score, CHD category, genetic diagnosis and singleton versus multiple pregnancy. The percentages for mortality (< 1 month and 1–12 months) and for survival are based on the number of live births. Abbreviations: AVR: anomalous venous return; IUM: intra-uterine demise; LVOTO: left ventricle outflow tract obstruction; TOP: termination of pregnancy; UVH: univentricular heart.

postnatal mortality were: lower postmenstrual age at birth ($p < 0.001$), postnatal genetic diagnosis ($p = 0.009$), severity score B ($p < 0.001$) and the presence of major ECA ($p < 0.001$).

- The logistic regression model for '>1 year survival': after stepwise removal of parameters with high p-values or with high collinearity, as described above, we retained 4 parameters that significantly impacted one-year survival: PMA at birth ($p < 0.0001$) and conotruncal heart defects ($p = 0.042$) had a positive effect, while CHD severity score B ($p < 0.0001$) and major ECA at birth ($p < 0.0001$) had a negative effect on survival. No significant association between right-sided heart defects ($p = 0.1$), or postnatal genetic diagnosis ($p = 0.06$) and survival was detected, but removing these variables from the regression model caused a significant increase in AIC.

Odds ratios for these associations are provided in Fig. 1 and in supplementary Table 5.

Discussion

Early and accurate prenatal diagnosis of CHD is crucial to optimize perinatal care to reduce CHD-related mortality and to improve quality of survival. However, risk stratification after prenatal diagnosis of CHD remains challenging³⁵. Heart anatomy and coexisting congestive heart failure, reflected by ultrasound markers such as cardiomegaly, hydrops, abnormal myocardial function or abnormal venous Doppler, play the most important role in fetal and postnatal survival, underlying the importance of longitudinal prenatal echocardiographic examination for individualized outcome prediction of fetuses with CHD^{36,38}. In this single-center retrospective chart review of 341 fetuses with CHD, the pregnancy was terminated in 13% and was complicated by fetal death in 1%. Pregnancy outcome was not documented in 4.5%. The overall postnatal mortality in our cohort was 17% (<4wk in 33/277 and >4wk in 15/277 liveborn children), which is similar to previous studies²⁴ (Table 4). We showed that in addition to high CHD severity scores (severity score B) other factors such as prenatal diagnosis of pathogenic CNVs and prenatal co-occurrence of potentially life-threatening extracardiac anomalies (severity score A) were significantly associated with a decision to terminate pregnancy as well. This is in line with the study of Qiu et al. showing that more complex CHD and presence of ECA were inversely correlated with continuation of the pregnancy³⁶. In addition, the presence of high complexity CHD and of major ECA, as well as prematurity, were negatively correlated with postnatal survival beyond the age of 1 month and of 1 year. Interestingly, a prenatal genetic diagnosis was negatively correlated with pregnancy continuation, but it was not a significant predictor for postnatal mortality, while a postnatal genetic diagnosis impacted early (< 1 month) but not late (at 1 year) mortality. In addition to potentially life-threatening congenital anomalies, genetic syndromes are usually associated with long-term morbidity due to developmental, behavioral and physical constraints, impacting quality of life. Therefore, when a genetic diagnosis is made prenatally, the decision to terminate pregnancy will

be more likely when a genetic disorder is predicted with higher morbidity (e.g. severe intellectual disability) or when it is associated with more severe cardiac or extracardiac anomalies on prenatal ultrasound. Fetuses with a prenatally diagnosed genetic disorder who are brought to the point of birth, may represent a subgroup of genetic syndromes predicted with a better outcome, which may explain why prenatal genetic diagnosis in this study was not associated with higher postnatal mortality. The effect of a postnatal genetic diagnosis on early postnatal mortality may be related to an increased risk of peri-operative complications, as was described previously for genetic syndromes such as 22q11 deletion syndrome³⁷, to a higher occurrence of life-threatening ECA, or to a higher likelihood to opt for redirection of care in children with genetic syndromes with high morbidity. The subgroup of children with genetic syndromes that survived the first weeks of life at the intensive care unit, may represent genetic syndromes with lower morbidity, explaining the lower impact on later mortality. Follow-up studies are required to inquire about the long-term effects of prenatal or postnatal genetic diagnoses on morbidity and mortality in children born with CHD. Finally, survival was significantly higher in fetuses with conotruncal CHD. Larger sample sizes per CHD category are required to attain sufficient statistical power to accurately associate CHD categories to TOP decisions or to short- or long-term survival. Ultrasound markers for outcome prediction, as described by Wieczorek et al.³⁸ and Qiu et al.³⁶, were not available and therefore the use of ultrasound parameters, such as the cardiovascular profile score, could not be applied.

The distribution of CHD categories in this study aligns well with the average distribution based on previously reported prenatal CHD cohorts⁴⁻⁷. (Table 1). Differences between individual reports is likely due to differences in classification of CHD categories. In this study, each fetus was attributed to only one CHD category based on the CHD type that was anatomically or prognostically most prominent, explaining the relatively low prevalence of septal defects in our cohort. Prenatal counseling by pediatric cardiologists in our center is less frequently requested in fetuses with CHD of lower complexity (severity score D) or in fetuses with chromosomal aneuploidy and/or life-threatening ECA (severity score A). Therefore, a shift towards CHD severity score C was observed, in comparison to the CHD severity distribution reported by Gowda et al.²⁴. (Table 2).

As expected, TOP and postnatal mortality were significantly higher among fetuses with severity scores A and B compared to severity score D. We used the same parameters to score CHD severity as those introduced by Gowda et al.²⁴. Although the distribution of CHD severity scores was comparable between both cohorts (except for score C which was more represented in our study population), we observed in our CHD cohort a higher survival rate beyond 6 months: overall (67% versus 40%) and across the subgroups with scores A (29% versus 7%), B (44% versus 9.7%) and C (88% versus 55.6%). These differences were related to a lower fetal death rate (1% versus 11.7%) and lower neonatal mortality (9.6% versus 24.1%), which may be due to differences in TOP policy as a more restrictive TOP policy may add to higher mortality rates. Large sample size follow-up studies are required to confirm these findings.

A significantly higher rate of multiple pregnancies (7.62%; $p < 0.001$) and of IVF/ICSI pregnancies (7.33%; $p < 0.001$) was observed in this CHD cohort compared to the general population (twinning rate of 1.6% and IVF/ICSI rate of 5.1% according to the 2019 report of the Flemish Center for Study of Perinatal Epidemiology). Compared to singletons, multiple pregnancies are known to be at increased risk of CHD, particularly among monozygotic twins due to altered intra-uterine hemodynamics³⁹⁻⁴². In addition, we confirm the findings of Giorgione et al. showing that assisted human reproduction by IVF or ICSI was associated with a higher CHD incidence as well (odds ratio 1.45)^{41,43}. CHD (of any type) in first-degree relatives was documented in 4.99% fetuses with CHD, which is similar to previous reports on familial CHD⁴⁴.

A pathogenic variant was identified by pre- or postnatal CMA in 27.9% of fetuses presenting prenatally with non-isolated CHD. Nine additional fetuses from this subgroup were diagnosed by NGS with a pathogenic SNV. The diagnostic yield of chromosomal testing in fetuses with apparently isolated CHD ($N = 206$) or with CHD of undetermined extracardiac status ($N = 49$) was significantly lower (5.8%; 1 by NIPS, 5 by prenatal CMA, 6 by postnatal or post-TOP CMA, 3 by WES). All these patients presented with ECA at birth or post-mortem, despite having an isolated CHD on prenatal ultrasound (supplementary Table 3). Our results are in line with those by van Nisselrooij et al. reporting chromosomal or genetic pathogenic variants in 15%: in 28.7% of fetuses with non-isolated CHD and in 11% of fetuses with apparently isolated CHD on prenatal ultrasound⁷. The most common chromosomal and genetic syndromes in fetuses with CHD identified by us and others⁷ were 22q11 deletion syndrome and Noonan syndrome. We also confirmed that CHD categories 'septal defects' (AVSD and VSD) and 'LVOTO' (interrupted aortic arch and CoAo) were associated with the highest yield of genetic testing, respectively in 19.4% and 20.3% (supplementary Table 2). Results from NIPS were available for 45% of fetuses (154/341), which is lower compared to the uptake of NIPS in the general population of pregnant women in Belgium (78.7%), where NIPS is available as a publicly funded nationwide first-tier screening as of July 2017⁴⁵. Prenatal invasive testing is preferred over NIPS when first trimester ultrasound is abnormal, which explains the lower uptake in the CHD cohort. Since fetal aneuploidy (T21, T18 or T13) weighs heavily on the decision to terminate pregnancy regardless of the presence of CHD⁴⁶ potentially causing a bias with respect to outcome prediction in this study, we excluded fetuses with abnormal non-invasive aneuploidy screening prior to the diagnosis of CHD on prenatal ultrasound. NGS was performed in only a small subset of patients, either presenting with prenatal features of a rasopathy as described⁴⁷, explaining the high incidence of Noonan syndrome in our cohort, or presenting postnatally or post-mortem with unexplained non-isolated CHD.

The outcome measures of this retrospective study were restricted to pregnancy continuation, and postnatal survival at 1 month and at 1 year. Long-term follow-up data on growth, development, behavior or survival into infancy were not available. 'CHD category', 'extracardiac anomalies' and 'CHD severity score' were included in the logistic regression outcome prediction model. However, as CHD severity scores were based on CHD type and on the presence of major ECA, these variables were not independent. We applied VIF to correct for parameters with high collinearity. As previously mentioned, CMA analysis was offered, either prenatally or postnatally, for all fetuses, but CMA results were available for only 68%, failing us to provide insight into chromosomal

anomalies in the full cohort. NGS was not offered systematically, and was restricted to fetuses or live born children with non-isolated CHD and normal CMA results. This NGS policy is a limitation of this study. As NGS technology becomes broadly available and sequencing costs are dropping, ultrarapid trio exome or genome sequencing emerges as the standard-of-care prenatal genetic test when non-isolated CHD is diagnosed, and could be considered in fetuses with apparently isolated complex CHD despite the low diagnostic yield. However, when moving from postnatal to prenatal genetic testing, some challenges need to be taken into consideration, including technology availability, access to health care, health insurance issues and sociocultural differences. Moreover, short turn-around-times should be guaranteed and adequate pre-test counseling is required, dealing with diagnostic yield, risks of invasive prenatal procedures and the reporting of incidental findings. Expectations and preferences of the parents should be prioritized when offering prenatal genomic testing.

In conclusion, we showed that cardiac and extracardiac phenotyping by prenatal ultrasound and elaborate prenatal genetic testing are crucial to provide adequate counseling to parents of fetuses with CHD with respect to postnatal survival rates.

Data availability

The dataset generated and analysed during this retrospective study is not publicly available due to the sensitive nature of the data. However, inquiries regarding the dataset and its use can be directed to the corresponding author upon reasonable request.

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References

- van der Linde, D. et al. Birth prevalence of congenital heart disease worldwide. *J. Am. Coll. Cardiol.* **58**, 2241–2247 (2011).
- Wu, W., He, J. & Shao, X. Incidence and mortality trend of congenital heart disease at the global, regional, and national level, 1990–2017. *Medicine* **99**, (2020).
- Best, K. E. & Rankin, J. Long-term survival of individuals born with congenital heart disease: A systematic review and Meta-analysis. *J. Am. Heart Association: Cardiovasc. Cerebrovasc. Disease* **5**, (2016).
- Stallings, E. B. et al. Prevalence of critical congenital heart defects and selected co-occurring congenital anomalies, 2014–2018: A U.S. population-based study. *Birth Defects Res.* **114**, 45–56 (2022).
- Qiao, F. et al. Comprehensive evaluation of genetic variants using chromosomal microarray analysis and exome sequencing in fetuses with congenital heart defect. *Ultrasound Obstet. Gynecol.* **58**, 377–387 (2021).
- Hureauux, M. et al. Chromosomal microarray analysis in fetuses with an isolated congenital heart defect: A retrospective, nationwide, multicenter study in France. *Prenat. Diagn.* **39**, 464–470 (2019).
- van Nisselrooij, A. E. L. et al. The prevalence of genetic diagnoses in fetuses with severe congenital heart defects. *Genet. Sci.* **22**, 1206–1214 (2020).
- Ozturk, A. G. et al. Long-term survival in patients with univentricular heart: A nationwide, register-based cohort study. *Eur. Heart J.* **43**, (2022).
- Eckerström, F., Nyboe, C., Maagaard, M., Redington, A. & Hjortdal, V. E. Survival of patients with congenital ventricular septal defect. *Eur. Heart J.* **44**, 54–61 (2023).
- Kiener, A. et al. Long-term survival after arterial Versus Atrial switch in d-Transposition of the great arteries. *Ann. Thorac. Surg.* **106**, 1827–1833 (2018).
- Lopes, S. A. V. et al. Mortality for critical congenital heart diseases and associated risk factors in newborns. A cohort study. *Arq. Bras. Cardiol.* <https://doi.org/10.5935/abc.20180175> (2018).
- Lopez, K. N., Morris, S. A., Sexson Tejtel, S. K., Espaillat, A. & Salemi, J. L. US mortality due to congenital heart disease across the lifespan from 1999–2017 exposes persistent racial/ethnic disparities. *Circulation* **142**, 1132 (2020).
- Jia, Y. et al. The diagnostic value of next generation sequencing in familial nonsyndromic congenital heart defects. *Am. J. Med. Genet. Part. A.* **167**, 1822–1829 (2015).
- Blue, G. M. et al. Targeted next-generation sequencing identifies pathogenic variants in familial congenital heart disease. *J. Am. Coll. Cardiol.* **64**, 2498–2506 (2014).
- Alankarage, D. et al. Identification of clinically actionable variants from genome sequencing of families with congenital heart disease. *Genet. Sci.* **21**, 1111–1120 (2019).
- Russell, M. W., Chung, W. K., Kaltman, J. R. & Miller, T. A. advances in the understanding of the genetic determinants of congenital heart disease and their impact on clinical outcomes. *J. Am. Heart Association* **7**, (2018).
- Jin, S. C. et al. Contribution of rare inherited and de novo variants in 2871 congenital heart disease probands. *Nat. Genet.* **49**, 1593 (2017).
- Homsy, J. et al. De novo mutations in congenital heart disease with neurodevelopmental and other birth defects. *Sci. (New York N Y)*. **350**, 1262 (2015).
- Sifrim, A. et al. Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing. *Nat. Genet.* **48**, 1060–1065 (2016).
- Salzer-Sheelo, L. et al. Prenatal and postnatal chromosomal microarray analysis in 885 cases of various congenital heart defects. *Arch. Gynecol. Obstet.* **306**, 1007–1013 (2022).
- Lord, J. et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): A cohort study. *Lancet (London England)*. **393**, 747 (2019).
- Li, R. et al. Prenatal exome sequencing in fetuses with congenital heart defects. *Clin. Genet.* **98**, 215–230 (2020).
- Mone, F. et al. COngenital heart disease and the diagnostic yield with exome sequencing (CODE) study: Prospective cohort study and systematic review. *Ultrasound Obstet. Gynecol.* **57**, 43–51 (2021).
- Gowda, M. et al. Prenatal grading of fetal congenital heart disease and its influence on decision making during pregnancy and postnatal period: A prospective study. *J. Maternal-Fetal Neonatal Med.* **35**, 3158–3166 (2022).
- Wilde, A. A. M. et al. European heart rhythm association (EHRA)/Heart rhythm society (HRS)/Asia Pacific heart rhythm society (APHRS)/Latin American heart rhythm society (LAHRS) expert consensus statement on the state of genetic testing for cardiac diseases. *Heart Rhythm.* **19**, e1–e60 (2022).
- Riggs, E. R. et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American college of medical genetics and genomics (ACMG) and the clinical genome resource (ClinGen). *Genet. Medicine: Official J. Am. Coll. Med. Genet.* **22**, 245–257 (2020).
- Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet. Sci.* **17**, 405–424 (2015).

28. Vanakker, O. et al. Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges. *Eur. J. Med. Genet.* **57**, 151–156 (2014).
29. Breckpot, J. et al. Copy number variation analysis in adults with catatonia confirms haploinsufficiency of SHANK3 as a predisposing factor. *Eur. J. Med. Genet.* **59**, 436–443 (2016).
30. Winters, L. et al. Massive parallel sequencing identifies RAPSN and PDHA1 mutations causing fetal akinesia deformation sequence. *Eur. J. Pediatr. Neurol.* **21**, 745–753 (2017).
31. Lannoo, L. et al. Rare autosomal trisomies detected by non-invasive prenatal testing: An overview of current knowledge. *Eur. J. Hum. Genet.* **30**, 1323 (2022).
32. STATBEL. Daling van het geboortecijfer in (2020). [https://statbel.fgov.be/nl/nieuws/daling-van-het-geboortecijfer-2020#:~:text=Mama op 29%2C33 jaar,\(34%2C1 jaar\) \(2022\).](https://statbel.fgov.be/nl/nieuws/daling-van-het-geboortecijfer-2020#:~:text=Mama op 29%2C33 jaar,(34%2C1 jaar) (2022).)
33. Goemaes, R. F. E., L. M., D. C. K. R. K. & B. A. -vzw S. voor P. E. (SPE). Belangrijkste trends in geboorte en bevalling: Jaarrapport Studiecendrum voor Perinatale Epidemiologie 2020. (2020). <https://www.zorg-en-gezondheid.be/belangrijkste-trends-in-geboorte-en-bevalling>
34. BELRAP. Assisted Reproductive Technology 2019 National Summary Report. (2019). <https://www.belrap.be/Public/Reports.aspx>
35. Bonnet, D. Impacts of prenatal diagnosis of congenital heart diseases on outcomes. *Translational Pediatr.* **10**, 2241 (2021).
36. Qiu, X. et al. Prenatal diagnosis and pregnancy outcomes of 1492 fetuses with congenital heart disease: Role of multidisciplinary joint consultation in prenatal diagnosis. *Sci. Rep.* **10**, (2020).
37. AlAshgar, T. M. et al. The outcomes of cardiac surgery in children with DiGeorge syndrome in a single center experience: A retrospective cohort study. *Cureus* **16**(2), e55186. <https://doi.org/10.7759/cureus.55186> (2024).
38. Wiczorek, A. et al. Prediction of outcome of fetal congenital heart disease using a cardiovascular profile score. *Ultrasound Obstet. Gynecol.* **31**, 284–288 (2008).
39. Bahtiyar, M. O., Dulay, A. T., Weeks, B. P., Friedman, A. H. & Copel, J. A. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations. *J. Ultrasound Med.* **26**, 1491–1498 (2007).
40. Herskind, A. M., Pedersen, A., Christensen, K. & D. & Increased prevalence of congenital heart defects in monozygotic and dizygotic twins. *Circulation* **128**, 1182–1188 (2013).
41. Panagiotopoulou, O. et al. Congenital heart disease in twins: The contribution of type of conception and chorionicity. *Int. J. Cardiol.* **218**, 144–149 (2016).
42. Best, K. E. & Rankin, J. Original article: increased risk of congenital heart disease in twins in the North of England between 1998 and 2010. *Heart* **101**, 1807 (2015).
43. Giorgione, V. et al. Congenital heart defects in IVF/ICSI pregnancy: Systematic review and meta-analysis. *Ultrasound Obstet. Gynecol.* **51**, 33–42 (2018).
44. Calcagni, G., Digilio, M. C., Sarkozy, A., Dallapiccola, B. & Marino, B. Familial recurrence of congenital heart disease: An overview and review of the literature. *Eur. J. Pediatrics.* **166**, 111–116 (2006).
45. Van Den Bogaert, K. et al. Outcome of publicly funded nationwide first-tier noninvasive prenatal screening. *Genet. Sci.* **23**, 1137–1142 (2021).
46. Shaffer, B. L., Caughey, A. B. & Norton, M. E. Variation in the decision to terminate pregnancy in the setting of fetal aneuploidy. *Prenat. Diagn.* **26**, 667–671 (2006).
47. Stuurman, K. E. et al. Prenatal ultrasound findings of rasopathies in a cohort of 424 fetuses: Update on genetic testing in the NGS era. *J. Med. Genet.* **56**, 654–661 (2019).

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Author contributions

J.B. and M.V. conceptualized the study, J.B., K.D., K.V.d.B, B.C., L.D.C. and M.G. retrospectively included patients. M.V. reviewed medical files and collected relevant data. J.B., M.V. and L.H. analyzed the data and wrote the main text of the manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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