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Trends in the prenatal diagnosis of trisomy 21 show younger maternal age and shift in the distribution of congenital heart disease over a 20-year period

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Abstract

Prenatal testing has changed greatly over the past two decades, which may affect the diagnosis of congenital heart disease (CHD) in Down syndrome. The present study aimed to analyze changes in the prevalence and distribution of CHD diagnosed via ultrasonography and fetopathology in 462 fetuses with trisomy 21 between two consecutive 10-year periods (1999–2018), as well as the associations between CHDs, ultrasound markers, and extracardiac malformations. Overall, the frequency of cardiovascular malformations in trisomy 21 was 27.7 and 26.5%, and ultrasound identified 70 and 62% of CHDs during these periods. A profound increase in first-trimester ultrasound findings and associated anomalies with CHDs (ventricular septal defect, Tetralogy of Fallot) since 2009 were observed. Second-trimester non-structural heart abnormalities were associated with ultrasound anomalies (74%) and major extracardiac malformations (42.9%). During both study periods, mothers carrying fetuses with CHD were significantly younger than those without CHD ($p = 0.038$, $p = 0.009$, respectively). Comparing the two 10-year periods, there were no changes in the prevalence and detection of CHDs. Trend analysis revealed that, although the frequency of CHD remained stable, the diagnostic spectrum had shifted between the study periods. Detection of nonstructural heart abnormalities necessitates detailed follow-up for cardiac/extracardiac malformations and chromosomal disorders.

KEYWORDS
congenital heart disease, Down syndrome, fetopathology, prenatal ultrasound, septal defects

1 | INTRODUCTION

Trisomy 21 (T21) is by far the most frequent type of autosomal chromosomal aneuploidy in liveborns and the most common genetic cause of intellectual disability (Bull, 2020). In addition to developmental delay, distinctive facial appearance, and internal malformations, congenital heart defects (CHDs) occur in 45–50% of children with T21 (Stoll et al., 2015). The frequency of CHDs in Down syndrome (DS) in descending order include atrioventricular septal defects (AVSDs), ventricular and atrial septal defects (VSDs, ASDs) followed by CHDs accompanied by complex anatomical defects such as tetralogy of Fallot (TOF) (Versacci et al., 2018). Because of their contribution to morbidity and mortality, early diagnosis in fetal life and postnatally is essential.

Overall, CHDs complicate approximately 0.8–1% of live births (Hoffman & Kaplan, 2002); however, postnatal studies have reported that approximately half of all newborns with T21 are diagnosed with CHD (Bergström et al., 2016; Freeman et al., 2008; Kallen et al., 1996; Morris et al., 2014; Santoro et al., 2018; Stoll et al., 1998; Stoll et al., 2015; Torfs & Christianson, 1998). Nonetheless, prenatal

studies have reported various rates of CHD prevalence (Hyett et al., 1995; Mogra et al., 2011; Morris et al., 2014; Paladini et al., 2000; Respondek-Liberska et al., 1999), but most have only included small numbers of patients with DS (Table 1). Moreover, a recent study has highlighted a phenotypic shift in the spectrum of CHDs, noting that fewer children are born with T21 and complex anatomical defects such as AVSD, presumably due to improvements in the prenatal diagnosis of CHDs and an increase in selective abortions in complex CHD cases (Bergström et al., 2016).

Advanced maternal age (AMA) has been associated with an increased risk of DS in the fetus. Although more cases of DS have been reported among those of AMA, this change has been balanced by an increase in the termination of pregnancy (TOP). The co-occurrence of T21 and a complex heart disease may add weight to the parents' decision to terminate the pregnancy. However, regional differences are of great importance, TOP rates following a prenatal diagnosis of DS have decreased in certain countries (The United States, Scandinavia) (Natoli et al., 2012). Indeed, a German study reported a growing number of newborns with DS, possibly due to improved medical management and altered social attitudes (Pfitzer et al., 2018). It is also important to emphasize that CHD in patients with DS is significantly influenced by fetal sex and by regional, geographical, environmental, and ethnic factors (Freeman et al., 2008; Santoro et al., 2018).

Despite the abovementioned findings, only one previous study examined ultrasonography (US) and fetopathology (FPATH) findings in an attempt to establish the profile of CHD in patients with DS (Table 1). Therefore, in the present study, we aimed to investigate the spectrum of CHDs in fetuses with DS detected via US and FPATH, as well as to examine the associations between CHDs, US findings and other cardiac/extracardiac anomalies at a single tertiary referral center over a 20-year period. To further analyze trends in prenatal characteristics, we compared the findings between the two consecutive 10-year periods.

2 | MATERIALS AND METHODS

This single-institution, retrospective, descriptive study was performed in the First Department of Obstetrics and Gynecology at Semmelweis University in Budapest. The department serves as a major, tertiary referral center for prenatal medicine in Hungary. The study was approved by the Ethics Committee of our institution.

From the fetal diagnostic database, we extracted sonographic reports, genetic and detailed FPATH results, and fetal echocardiogram findings (when applicable) between 1999 and 2018. The registry included karyotypically documented T21 cases for which prenatal ultrasound and cytogenetic examinations were performed at our institution. The data were compared between two consecutive 10-year periods (1999–2008 vs. 2009–2018). These intervals were chosen due to the introduction of standardized nuchal translucency (NT) assessments during routine first-trimester screening in

TABLE 1 Prenatal and population-based postnatal studies of congenital heart disease in Down syndrome (the frequency of congenital heart disease is indicated in % rounded)

Study	Study period	No. of pts with DS	CHD %
Population-based postnatal study			
1996	1976–1993	3694	32a (23b)
1998	1979–1996	398	46
1998	1983–1993	2894	56
2008	2000–2004	1469	44
2014	2000–2010	7044	44
2016	1992–2012	2588	54
2018	2003–2015	230	44
Prenatal study			
1995		36	56
1999	1994–1997	30	43
2000	1994–1997	41	56
2011	2002–2010	487	34 (24c)
2014	2000–20102000–2010	70651217	8 (US)18 (US + FPATH)
Present study, Hungary	1999–2018	462	20 (US)27 (US + FPATH)

Notes: EUROCAT is a network of population-based registries for the epidemiologic surveillance of congenital anomalies collecting data from 20 European countries.

Abbreviations: CHD, congenital heart disease; DS, Down syndrome; FPATH, fetopathology; pts, patients; US, ultrasonography.

[a]In Sweden.

[b]In France.

[c]After removal of cases when the indication for referral was suspected CHD.

Hungary in 2009, based on the Fetal Medicine Foundation (FMF) protocol.

Fetal sonographic examinations were performed by three sonographers and were further evaluated by obstetricians experienced in maternal–fetal medicine. From 1999–2008, Ultramark 9 HDI 300 (Philips Medical Systems, Bothell, Washington) and Voluson 730 systems (GE Healthcare, Milwaukee, Wisconsin) were used for US. From 2009–2018, a Voluson 730 US system (GE Healthcare) was used. Sonographic examinations were performed in accordance with the guidelines issued by the Hungarian Society of Ultrasound in Obstetrics and Gynecology. When fetal echocardiography was performed in cases of T21, the operator was an experienced pediatric cardiologist. Fetal echocardiography assessments were performed in accordance with International Society of Ultrasound in Obstetrics and Gynecology guidelines (Lee et al., 2008). Notably, fetal echocardiography was only performed in cases of T21 when anomaly scans suggested CHD, rather than routinely. In some cases, the pediatric echocardiologist recommended karyotyping based on certain findings during fetal echocardiography examinations performed for other reasons (e.g., AMA). In some cases, parents opted for fetal echocardiography examinations to aid in the decision to terminate the pregnancy when DS had been diagnosed. Neonatal heart assessments (i.e., postpartum echocardiography) were not routinely performed in liveborn cases of T21, and data related to neonatal/postnatal outcomes were unavailable.

The indications for referral to our tertiary institution included AMA at the time of conception (>35 years), abnormal levels of maternal serum biochemical factors, and any major or minor anomalies observed during sonographic examinations or the result of noninvasive prenatal testing (NIPT). Suspected CHD rarely constituted the primary indication for referral. From 1999 to 2009, the only serum maternal biochemical parameter used to screen for DS in Hungary was alpha-fetoprotein, while combined screening for T21 has been available in our country since 2009 and next-generation sequencing-based NIPT since 2014. Combined testing and NIPT are not covered by national health insurance and have been individually financed by patients thus far. In addition, first-trimester assessments of ductus venosus flow, tricuspid regurgitation (TR), and the presence of the nasal bone were not integral components of routine prenatal screenings in Hungary during the study period. Since 2009, the FMF protocol has mandated measurement of NT thickness during the first-trimester screening in Hungary. Prior to 2009, NT assessment was not necessarily performed in accordance with such strict criteria.

During sonographic examinations, abnormality was classified either as a minor ("soft") sonographic marker (nuchal fold thickness [>6 mm], choroid plexus cyst, mild ventriculomegaly [10–15 mm], short femur/humerus [<10th percentile], pyelectasis [>4 mm, anteroposterior], echogenic intracardiac focus [EIF], echogenic bowel, and single umbilical artery) in the second trimester or a major structural anomaly (cystic hygroma; hydrops; central nervous system anomalies; and cardiac, abdominal, urogenital, and limb defects). The presence of an aberrant right subclavian artery was not considered a marker during the study period. Among the major anomalies, we paid special attention to CHD and any US findings related to the fetal heart in the second trimester between 16 and 22 weeks of pregnancy. For the present study, heart anomalies were classified into two main categories. Structural heart defects (CHD), which were further categorized as (a) shunt defects (VSDs, ASDs), (b) complex defects (AVSDs, TOF, aortic arch abnormalities, conotruncal anomalies), and (c) other defects. Anomalies were classified as nonstructural/functional when an abnormal four-chamber view or function was detected without any evidence of CHD in US, fetal echocardiography, or FPATH examinations. Such anomalies were further categorized as follows: (a) isolated ventricular disproportion (right ventricle [RV] > left ventricle [LV]), (b) an isolated heart ultrasound axis deviation (left/right rotation or deviation of the heart axis or apex from the normal orientation [i.e., around 40°–45° anterior and to the left of the midline]), (c) pericardial effusion (PE, <2 mm in diameter), and (d) mild-to-moderate insufficiency/regurgitation in the mitral or tricuspid valve in the second trimester.

Cytogenetic fetal evaluations were performed via amniocentesis (Amnio), chorionic villous sampling (CVS) followed by amniotic cell culturing, or via a direct analysis method (CVS). A minimum of 10 metaphases from at least two cultures were analyzed. In cases where invasive diagnostic tests were performed at more than 20 weeks of gestation, rapid diagnoses were made based on quantitative fluorescent polymerase chain reaction analyses, in addition to conventional G-band or R-band karyotyping.

TOP was offered to parents after proper genetic counseling. Pregnancies were terminated via dilatation and curettage until 13 weeks of gestation and via dilatation, intrauterine prostaglandin, and intravenous oxytocin infusion between 14 and 23 weeks of gestation. In cases of early termination, fragmented specimens rather than intact fetuses were examined during autopsy. In Hungary, the legal deadline for TOP in cases of T21 is 23 weeks and 6 days of pregnancy. Autopsies were performed in all cases of TOP, intrauterine fetal death, and stillbirth after the parents had given their consent. All FPATH examinations were performed in accordance with the standard fetal autopsy protocol by pathologists trained in perinatal pathology (Keeling, 1993).

Statistical analysis was performed using SAS 9.4 software. For binary categorical data, Pearson chi-square tests and Fisher's exact tests were used to assess the independence of column and row variables in 2×2 tables, or the homogeneity of proportions in multiway tables. Maternal age was analyzed using a one-factor and two-factor mixed analyses of variance. Categorical data are presented as ratios and percentages, while continuous variables are presented as means and 95% confidence intervals. The p-values less than 0.05 were considered statistically significant.

3 | RESULTS

A total of 462 fetuses were diagnosed with DS via karyotyping (202 cases between 1999 and 2008, 260 cases between 2009 and 2018). The study population was considered homogenous, as more

than 98% of the patients were Caucasian. Amniocentesis represented the invasive procedure in 91.6% of cases during the first 10-year period, while the rate of CVS doubled to 16.9% during the second 10-year period ($p = 0.007$). Altogether, CVS was performed in 61 cases of DS during the 20-year study period (Table 2). CHD was identified via US and FPATH examinations in 19.8% of cases during the first period and 19.2% of cases during the second period. Similarly, nonstructural heart abnormalities were detected via US in 7.9 and 7.3% of cases during these periods, respectively (Table 2).

Between 1998 and 2008, the primary indications for referral were the presence of any fetal anomaly during US examination (47%) and AMA (39%), and US anomalies in the CHD group (65%). Between 2009 and 2018, the rate of referral solely due to AMA decreased by half (18%) relative to that reported in the previous period, while US-detected anomalies (40%) and maternal serum biochemistry results (33%) represented the main reasons for the tertiary visit. In the first study period, the vast majority of US anomalies were second-trimester minor malformations, but the number of increased NT thickness rose in the second study period. Significant differences in all indications for referral were noted between the two 10-year periods in the non-CHD group ($p = 0.001$). However, among patients with CHD, no significant difference was found in indications ($p = 0.054$) (Table 3).

TABLE 2 Cases diagnosed with Down syndrome and the distribution of the diagnostic methods between 1999–2008 and 2009–2018

	1999–2008n (%)	2009–2018n (%)
Total	202	260
Amnio	185/202 (91.6%)*	216/260 (83.1%)*
CVS	17/202 (8.4%)*	44/260 (16.9%)*
T21 without CHD	146/202 (72.3%)**	191/260 (73.5%)**
T21 with CHD	40/202 (19.8%)**	50/260 (19.2%)**
T21 with nonstructural heart anomaly	16/202 (7.9%)**	19/260 (7.3%)**

Notes: *$p = 0.007$, **NS.
Abbreviations: Amnio, amniocentesis; CHD, congenital heart disease; CVS, chorionic villous sampling; n, number; NS, not significant; T21, Down syndrome.

For fetuses diagnosed with T21 without CHD between 1999 and 2008, the average maternal age was 35.7 years, which was significantly lower than that in cases of CHD (33.8 years, $p = 0.038$). The female–male ratio was 0.8 in the non-CHD group and 1.35 in the CHD group. Between 2009 and 2018, the average maternal age in cases of T21 without CHD increased by 1.6 years when compared with the previous period (37.3 years, $p = 0.01$). Male predominance was also observed during this period (3:2). Maternal age was significantly lower in the CHD group than in the non-CHD group (35.0 years, $p = 0.009$), and female sex was overrepresented (F/M: 1.38). No differences in the fetal sex ratios were statistically significant ($p = 0.11$–0.25) (Table 4).

Between 1999 and 2008, CHDs included 19 cases of VSD, 17 of AVSD, 3 of TOF, and 1 case of total anomalous pulmonary vein return (40/202, 19.8%). Nearly half of VSDs, all but one AVSD, and all three cases of TOF were identified via US (28/40, 70%). Between 2009 and 2018, CHDs included 28 cases of VSD, 4 of ASD, 15 of AVSD, 2 of TOF, and 1 case of pulmonary stenosis (50/260, 19.2%). Almost half of VSDs, almost all AVSDs, and all cases of TOF were diagnosed via US (31/50, 62%). In contrast, three of the four secundum type ASD cases were diagnosed via FPATH examination. Isolated RV > LV and heart axis US deviation represented the most frequent nonstructural anomalies between 1999 and 2008, while it was RV > LV from 2009 to 2018. Overall, US identified 44 and 50 structural and nonstructural heart abnormalities (21.8 and 19.2%) during the first and second study periods, respectively. Application of FPATH examinations increased the rates of detection to 27.7% (56 cases) and 26.5% (69 cases) during these periods, respectively (Table 5).

We also examined associations between CHDs/nonstructural heart abnormalities and US findings, other cardiac anomalies, and extracardiac malformations (Table 6). VSDs were associated with US findings in 52 and 64% of cases during the first and second study periods, respectively. Between 1999 and 2008, sonographic signs included second-trimester soft markers in 78% of cases (mainly EIF and pyelectasis), while increased NT thickness was associated with two of the autopsy-detected VSD cases (2/10). Between 2009 and 2018, increased NT thickness was detected in 46% of US-diagnosed cases of VSD, representing a significant increase when compared with the first-trimester sonographic findings ($p = 0.045$). Associated heart anomalies were noted in 29% of cases with VSD from 2009 to 2018

TABLE 3 Indication for referral in Down syndrome cases and in cases when it was associated with structural heart disease (CHD) (the frequency of each parameter is indicated in % [rounded])

Indication for referral	1999–2008 T21	1999–2008 T21 with CHD	2009–2018 T21	2009–2018 T21 with CHD
Total number	202	40	260	50
AMA	79 (39%)*	9 (22.5%)**	47 (18%)*	6 (12%)**
US anomaly	95 (47%)*	26 (65%)**	105 (40%)*	25 (50%)**
Se. biochemistry	29 (14%)*	5 (12.5%)**	85 (33%)*	16 (32%)**
NIPT	–	–	23(9%)*	3 (6%)**

Notes: *$p = 0.001$, comparison of the T21 cohorts. **NS ($p = 0.054$), comparison of the T21 with CHD cohorts.
Abbreviations: AMA, advanced maternal age; CHD, congenital heart disease; NIPT, noninvasive prenatal test; se, serum; T21, Down syndrome; US, ultrasonography.

TABLE 4 Characteristics of patients with Down syndrome without heart anomalies, with structural heart disease (CHD) diagnosed by ultrasonography and fetopathology examination, and with nonstructural heart anomalies diagnosed by sonography

	Maternal age (year) mean (95%CI)	Female fetusn (%)	Male fetusn (%)	Female/male ratio
1999–2008				
T21 without CHD	35.7 (34.7–36.7)*	65/146 (45%)	81/146 (56%)	0.80***
T21 with CHD (US + FPATH)	33.8 (31.8–35.7)*	23/40 (58%)	17/40 (43%)	1.35***
T21 with nonstructural heart anomaly	35.0 (32.6–37.4)	6/16 (38%)	10/16 (63%)	0.60***
2009–2018				
T21 without CHD	37.3 (36.7–37.8)**	76/191 (40%)	115/191 (60%)	0.66***
T21 with CHD (US+FPATH)	35.0 (33.2–36.8)**	29/50 (58%)	21/50 (42%)	1.38***
T21 with nonstructural heart anomaly	36.1 (33.6–38.7)	7/19 (37%)	12/19 (63%)	0.58***

Notes: Maternal age is indicated by average year, female and male fetuses by number, percentage and ratio. Percentage is indicated in % (rounded).
*p = 0.038, **p = 0.009, ***NS.
Abbreviations: CHD, congenital heart disease; CI, confidence interval; FPATH, fetopathology; NS, not significant; T21- Down syndrome; US, ultrasonography.

(two cases of RV > LV, severe TR, and PE; one case of hypoplastic left heart and ASD detected via US and FPATH, p = 0.011). Among the 47 VSDs diagnosed during the 20-year period, most were perimembranous defects, while only few were muscular defects (1/19 and 2/28, respectively). All VSDs missed during US were perimembranous defects. Muscular defects were not associated with any other US findings or CHDs.

AVSDs were associated with sonographic findings in 47 and 53% of cases during the first and second study periods, respectively. First trimester US findings were found in only two cases (increased NT), while mostly second trimester soft markers were associated with AVSDs during the whole study period. Although major extracardiac malformations were detected in 16% of AVSD cases, AVSDs were not associated with any other type of heart disease. The associated US findings with TOFs were second trimester soft markers from 1999 to 2008 and increased NT from 2009 to 2018. Autopsy-detected cases of ASD were associated with second-trimester soft markers (one case with unilateral cleft lip and palate, another case with RV > LV). The one case of ASD diagnosed in utero was not associated with any US or FPATH findings.

Among the 35 cases of nonstructural heart abnormalities, isolated RV > LV was greatly associated with sonographic signs (83 and 67%) and with other extracardiac malformations (50 and 56%) during the respective study periods. The main US findings in the 15 cases of RV > LV were increased NT thickness in the first trimester (5/15, 33%) and mild ventriculomegaly in the second trimester (5/15, 33%). Isolated heart US axis deviation, PE and mild-to-moderate second-trimester TR was also often associated with sonographic malformations, including abnormal NT and hydrops in the first trimester and multiplex soft markers in the second trimester (Table 6). Altogether, 15 major extracardiac malformations were observed among the 35 cases of nonstructural heart abnormalities, 74% of which (26/35) were associated with first-trimester or second-trimester sonographic findings.

Throughout the 20-year study period, we observed 25 extracardiac structural malformations associated with heart disease in fetuses with DS. The most prevalent noncardiac finding was hydrops, associated mainly with nonstructural heart findings, followed by hydrocephalus and double bubble. While RV > LV and PE were most likely to be associated with extracardiac structural anomalies (8/15 and 5/9), its occurrence was the least likely with CHDs (10/90, 11%) (Table 6).

4 | DISCUSSION

To date, a significant number of postnatal cohort studies, population-based studies, and many prenatal cohort studies have investigated the prevalence of CHD among patients with DS (Table 1). However, there are postnatal and prenatal study results that contradict each other concerning the number of patients with T21 that are diagnosed with CHD. A necropsy study by Hyett et al. (1995) reported a high CHD rate in fetuses with T21 in the first trimester when analyzing suction terminations using a special microdissection method. Their study included a high-risk cohort of fetuses with both T21 and increased NT thickness, and the authors noted cardiac septal defects in 22 of the 36 fetuses. Paladini et al. (2000) reported that, among 41 fetuses with known DS, fetal echocardiography identified CHD in 56% of cases (i.e., AVSD and VSD). Although their study reported the highest prenatal prevalence of CHD, the number of included cases was again small. In the only prenatal study including a large series of fetuses with T21, Mogra et al. (2011) performed fetal echocardiography in all cases at a specialized single fetal heart center, reporting a structural CHD rate of 34% (mainly AVSD). If those cases were removed from the analysis when the indication for referral was any suspected CHD, the rate of major CHDs fell to 24%. The same study reported that functional heart anomalies occurred in 36.8% of cases, most of which involved first-trimester TR. The only population-based study to report both prenatal and postnatal data was the EUROCAT study published in 2014 (Morris et al., 2014). The authors extracted data from the European Surveillance of Congenital Anomalies Central Register for 20 European countries between 2000 and 2010, resulting in a total of more than 14,000 patients with DS. Overall, 43.6% of neonates with

TABLE 5 Distribution and type of structural heart defects (CHD) diagnosed via ultrasonography and fetopathology examinations and second trimester nonstructural heart abnormalities detected via ultrasonography examination in down syndrome between 1999–2008 and 2009–2018

Type of heart disease	US detected	1999–2008 FPATH detected	US + FPATH detected	US detected	2009–2018 FPATH detected	US + FPATH detected
CHD						
Septal defects						
VSD	9	10	19 (9.4%)a	13	15	28 (10.8%)a
ASD	–	–	–	1	3	4 (1.6%)a
Complex defects						
AVSD	16	1	17 (8.4%)a	14	1	15 (5.8%)a
TOF	3	–	3 (1.5%)a	2	–	2 (0.78%)a
Others						
TAPVR	–	1	1 (0.5%)	–	–	–
Pulmonary stenosis	–	–	–	1	–	1 (0.39%)
Total	28 (13.9%)	12 (5.9%)	40/202 (19.8%)a	31 (12.0%)	19 (7.4%)	50/260 (19.2%)a
Nonstructural heart abnormality						
Ventricular disproportion (RV > LV)	6			9		
Heart axis deviation	6			3		
Pericardial effusion (PE)	4			5		
Tricuspid regurgitation (TR)	–			2		
Total	44 (21.8%)		56/202 (27.7%)a	50 (19.2%)		69/260 (26.5%)a

Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart disease; FPATH, fetopathology; NS, not significant; TAPVR, total anomalous venous return; TOF, Tetralogy of Fallot; VSD, ventricular septal defect; US, ultrasonography.
[a]NS (not significant).

T21 exhibited a cardiac anomaly, while the prenatal prevalence of CHD was 8.1%. When an autopsy was also performed, the rate of the detected fetal CHDs increased to 18.1%. In the present study, we diagnosed 90 cardiovascular malformations in 462 fetuses with DS (19.5%) using US alone, and the detection rate increased to 27.1% with the addition of FPATH examinations. US alone identified 70% (1999–2008) and 62% (2009–2018) of all CHDs, corresponding to 13.9 and 12% of the whole DS cohort.

Previous research has suggested that sex influences rates of CHD in the general population and in patients with DS (Sokal et al., 2014). Moreover, some authors have suggested that CHD is more common among female patients with DS than among their male counterparts. A population-based European study by Morris et al. (2014) reported that female infants with DS exhibited a higher prevalence of VSDs, AVSDs, and ASDs than male infants with DS, who exhibited a relatively higher prevalence of TOF. An Italian cohort study by Santoro et al. (2018) also noted a female preponderance among patients with DS diagnosed with CHD, indicating that sex may modify the association between DS and CHD. However, Mogra et al. (2011) identified no sex-based differences in rates of CHD among patients with DS, although AVSDs were more common among female fetuses. In our series, we observed remarkable sex differences in fetuses with DS, whereas DS without CHD and with nonstructural heart abnormalities was more prevalent in male fetuses, DS accompanied by CHD in female fetuses.

The exact etiology of the association between maternal age and CHD is unclear. Maternal obesity, a higher rate of assisted reproductive methods and the consequent multiple pregnancies are more common with AMA and are related to an increased risk of CHD (Best & Rankin, 2015). The child-bearing age of mothers has increased in recent years (Cocchi et al., 2010). It is a well-known fact that maternal age is associated with chromosomal abnormalities since age-related chromosome segregation errors occur during meiosis II in oocytes and are the dominant source of aneuploidy (Fragouli et al., 2013). Chromosomal anomalies increase the risk of CHD and might influence the effect of maternal age on the occurrence of CHD (Pierpont et al., 2018). Several studies have examined maternal age as a risk factor for CHD in the United States. After excluding cases with chromosomal anomalies, such studies have reported a higher prevalence of CHD when maternal age exceeds 35 years. AMA may increase the risk of milder CHDs such as VSD or ASD (Miller et al., 2011). However, a more recent study conducted in the United Kingdom using a population-based database, demonstrated that maternal age alone is not an independent risk factor for CHD (Best & Rankin, 2016). In accordance with the previously reported trends, we found that mothers carrying DS fetuses were approximately 1.5 years older during the 2009–2018 period than during the 1999–2008 period. During both study periods, mothers carrying fetuses with CHD were significantly younger (1.9 and 2.3 years, respectively) than those carrying fetuses without heart disease.

TABLE 6 Associations between the different types of structural heart defects (CHD) and nonstructural heart abnormalities with first and second trimester sonographic markers, extracardiac malformations and other cardiac anomalies diagnosed via ultrasound and fetopathology examinations

Associated anomaly CHD	Number	Ultrasound anomaly	1st trimester US finding	2nd trimester US finding	Associated extracardiac malformation	Associated cardiac anomaly
VSD (1999–2008)	19	10/19 (53%)	3/19 (16%)	8/19 (42%)	2/19 (11%)	0/19**
US detected	9	7/9 (78%)	1/9 (11%)*	7/9 (78%)	Unilateral renal agenesis	–
FPATH detected	10	3/10 (30%)	2/10 (20%)	1/10 (10%)	Polydactyly	–
VSD (2009–2018)	28	18/28 (64%)	7/28 (25%)	11/28 (39%)	2/28 (7%)	8/28 (29%)**
US detected	13	9/13 (69%)	6/13 (46%)*	3/13 (23%)	Bilateral club foot	2 TR, 2 RV>LV, 2 PE
FPATH detected	15	9/15 (60%)	1/15 (7%)	8/15 (53%)	Hydrocephalus	HLHS, ASD
AVSD (1999–2008)	17	8/17 (47%)	2/17 (12%)	4/17 (24%)	4/17 (24%)	–
US detected	16	8/16 (50%)	2/16 (13%)	4/16 (25%)	Hydrocephalus, double bubble, hydrops	–
FPATH detected	1	–	–	–	Intestinal malrotation	–
AVSD (2009–2018)	15	8/15 (53%)	–	8/15 (53%)	1/15 (7%)	–
US detected	14	8/14 (57%)	–	8/14 (57%)	–	–
FPATH detected	1	–	–	–	Bilobed lung	–
ASD (2009–2018)	4	3/4 (75%)	–	3/4 (75%)	1/4 (25%)	1/4 (25%)
US detected	1	–	–	–	–	–
FPATH detected	3	3/3 (100%)	–	2/3 (67%)	Cleft lip and palate	RV > LV
TOF (1999–2008)	3	3/3 (100%)	–	3/3 (100%)	–	–
US detected	3	3/3 (100%)	–	3/3 (100%)	–	–
FPATH detected	–	–	–	–	–	–
TOF (2009–2018)	2	2/2 (100%)	2/2 (100%)	2/2 (100%)	–	–
US detected	2	2/2 (100%)	2/2 (100%)	2/2 (100%)	–	–
FPATH detected	–	–	–	–	–	–
RV > LV (1999–2008)	6	5/6 (83%)	3/6 (50%)	5/6 (83%)	3/6 (50%): hydrops, double bubble, hydrocephalus	–
RV > LV (2009–2018)	9	6/9 (67%)	3/9 (33%)	3/9 (33%)	5/9 (56%): double bubble, hydrocephalus, club foot, bilobed lung, intestinal malrotation	–
Heart axis deviation (1999–2008)	6	3/6 (50%)	–	3/6 50%)	–	–
Heart axis deviation (2009–2018)	3	3/3 (100%)	2/3 (67%)	1/3 (33%)	2/3 (67%): hydrops, polydactyly	–
PE (1999–2008)	4	4/4 (100%)	1/4 (25%)	4/4 (100%)	2/4 (50%): hydrops, intestinal malrotation	–
PE (2009–2018)	5	3/5 (60%)	3/5 (60%)	3/5 (60%)	3/5 (60%): 2 hydrops, spina bifida	–
TR (2009–2018)	2	2/2 (100%)	2/2 (100%)	2/2 (100%)	–	–

Notes: Data is indicated in absolute numbers and percentages (in % rounded). *p = 0.045, **p = 0.011.
Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart disease; FPATH, fetopathology; HLHS, hypoplastic left heart syndrome; PE, pericardial effusion; RV > LV, ventricular disproportion; TOF, Tetralogy of Fallot; TR, tricuspid regurgitation; US, ultrasonography; VSD, ventricular septal defect.

This result suggests that maternal age is not an independent risk factor for CHD among patients with DS.

Indications for referral to tertiary centers have evolved over time. Although AMA has been the primary indication for prenatal genetic counseling for decades, advancements in ultrasound technology and the widespread application of early noninvasive screening methods have shifted the profile of referred patients. According to our data, the primary indications for referral in cases of DS due to AMA alone had decreased by approximately 50%, while abnormal results in serum maternal biochemical tests (e.g., combined testing) emerged as a major indication for referral (in addition to US-detected anomalies) from 2009. Among fetuses with CHD, the presence of any US-detected

anomaly remained the main indication for referral over time; however, we observed a profound increase in rates of abnormal NT thickness in the first trimester (mainly VSD, TOF) after 2009. The application of cell-free fetal DNA (cffDNA, NIPT) testing has revolutionized prenatal screening and diagnosis (Fan et al., 2008). Such advancements may have contributed to decreases in Amnio and a near doubling of CVS rates, although the number of such referrals was moderate in our cohort.

Data from large, population-based European cohort studies have indicated that, while the overall prevalence of CHD in newborns with DS has remained stable over time, the risk of complex heart malformations has dropped dramatically (~40%), and the incidence of AVSDs has decreased by approximately 14–16% (Bergström et al., 2016; Pfitzer et al., 2018). Although AVSD is considered the most common and specific US marker of DS, new results suggest that rates of AVSD and VSD are equal in infants born with DS. This phenomenon may be related to advancements in prenatal detection of fetal malformations and the consequent increase in the number of selective terminations in cases of complex fetal CHD. AVSD is particularly important in prenatal screening and diagnosis, as this septal defect is highly detectable and its presence strongly suggests a chromosomal abnormality, especially T21. Typically, AVSD occurs as an isolated defect with balanced ventricular morphology in patients with DS.

Langford et al. observed that the presence of an isolated AVSD with normal situs increases the risk of DS 107-fold (Langford et al., 2005). In a recent study investigating the association between AVSD, chromosomal abnormalities and the influence of prior combined screening on diagnosis, the prevalence of DS among 110 fetuses with an AVSD was 46%, as suggested by fetal echocardiography (Morlando et al., 2017). They found that the diagnosis of an AVSD in the second trimester, even in patients with a low risk result of the prior first trimester combined screening, was indicative to T21. They reported that the frequency of AVSDs remained unchanged in the second trimester, presumably due to age-related increases in rates of T21, which may have counterbalanced increases in rates of detection/termination.

Prenatal detection of VSDs is challenging, and the intrauterine detection rate is low. Among VSDs, perimembranous defects are most commonly associated with T21, although they are often hidden by the septal leaflets and do not usually result in an abnormal four-chamber view. While isolated VSDs are the most common cardiac defects, accounting for almost 30% of all pediatric cardiac defects, prenatal diagnosis of isolated VSDs remains difficult (Wren et al., 2000). Some prenatally diagnosed VSDs close spontaneously in utero, and most VSDs resolve within the first year of life (Gomez et al., 2014; Wren et al., 2000). Although isolated VSDs do not cause hemodynamic instability in the fetus, their potential association with chromosomal abnormalities is important. In a prenatal cohort at a single tertiary institution, 12.6% of all CHDs were isolated VSDs, and 6.2% of all fetuses with VSDs had T21 (Bahtiyar et al., 2008). In a nonselected prospective cohort including 279 VSDs, 62% were muscular VSDs. Isolated muscular defects were associated with aneuploidies in 1.2% of cases, while small perimembranous defects were observed in 23.5% of cases, many of which exhibited T21. None of the muscular VSDs and only 13% of the isolated perimembranous defects were detected prenatally, but the detection rate reached 52% when associated aneuploidies and extracardiac malformations were also present (Tegnander et al., 2006). Although AVSDs have traditionally represented the most common CHD in European and North American patients with DS, VSDs are the most frequent type of CHD in fetuses with DS carried by Asian and Hispanic mothers (Freeman et al., 2008).

In our study, nearly half of all CHDs were AVSDs, most of which were isolated and the diagnoses were confirmed primarily via sonography, while approximately half of all VSDs were missed during US examinations. After 2009, a significant number of diagnosed VSDs were associated with increased NT thickness, presumably due to the introduction of NT thickness measurements during the first-trimester screening in Hungary, in accordance with the FMF protocol. Interestingly, the introduction of standardized NT measurements alone did not increase rates of VSD detection, as the overall proportion of diagnosed VSDs remained unchanged. The increased NT thickness is the most effective early US marker in the screening of DS, and a NT thickness greater than 99th percentile (>3.5 mm) is largely associated with an increased risk of a major CHD (Mogra et al., 2012). However, recent data has demonstrated that increased NT thickness alone exerts only a moderate effect on the screening of major CHDs in unselected populations, and there appears to be no correlations between NT thickness and specific types of CHD (Jorgensen et al., 2015).

Nonstructural heart findings (e.g., TR, RV > LV, PE, and heart axis deviation) in the second trimester have been associated with chromosomal disorders (Concolino et al., 2005; DeVore, 2003; Geipel et al., 2010; Stressig et al., 2011). Moreover, the presence of functional heart abnormalities despite a normal cardiac structure may also be an early sign of later detectable structural CHDs. Although some of these abnormalities are benign, transient findings or functional consequences of cardiac malfunction/incipient heart failure, others are noteworthy US markers of aneuploidy in the second trimester. In such cases, comprehensive fetal medicine examinations are necessary to exclude any possible genetic or heart abnormalities.

In one review, the author noted that using RV > LV as an isolated second-trimester marker increased the rates of DS detection from 60 to 75%, while the addition of other markers such as septal defects, PE, and TR during echocardiography further improved the detection rate to 91% (DeVore, 2003). In some cases, isolated RV > LV in the second trimester is the earliest sign of aortic coarctation, which is most often diagnosed late in the third trimester or during the postpartum period (Kailin et al., 2017). Mogra et al. (2011) reported functional heart abnormalities in 179 of 487 fetuses with DS that had undergone detailed echocardiography examination. In their study, nonstructural heart abnormalities (isolated RV > LV, mitral valve regurgitation, and TR) were detected only in the first trimester and the incidence of TR was much higher in fetuses with increased NT thickness. Mild or moderate TR may be a transient/physiological finding in the second half of pregnancy and during postnatal life. However, TR has been associated with

an increased risk of T21. Indeed, TR is present in approximately 1.5% of euploid fetuses and in 27% of trisomic fetuses in the second trimester (Geipel et al., 2010). Other authors have reported that the prevalence of TR in the second trimester is 11% in fetuses with DS, with a likelihood ratio of 7.4 when TR is used as an isolated marker (Stressig et al., 2011). An Italian study further reported a high prevalence of isolated PE in fetuses with DS, after excluding cases of fetal infection (Concolino et al., 2005). Nonetheless, other authors have reported that most cases of isolated PE are not associated with aneuploidy and resolve spontaneously (Kyeong et al., 2014). In the present study, nonstructural heart abnormalities identified during the second trimester were strongly associated with major extracardiac malformations (43%), such as hydrops, hydrocephalus or double bubble, and we detected associated US findings in more than two-third of cases (e.g., increased NT thickness, mild brain ventriculomegaly, multiplex soft markers).

In summary, the present study analyzed a relatively large cohort of DS fetuses assessed at a leading tertiary referral center in the central, most populated region of Hungary. The primary strength of the study was the relatively large number of T21 fetuses examined over the 20-year study period. We observed a higher prevalence of CHD in T21 fetuses using US and FPATH than the authors of the EUROCAT study. In addition, our findings indicated that the frequency of CHD was higher in female fetuses than in male fetuses and maternal age was significantly younger in the CHD group than in the non-CHD group. According to our data maternal age is not an independent risk factor for CHD in fetuses with DS. Trend analyses revealed no significant difference in the prevalence of CHDs between the two 10-year periods, despite a change in distribution. Most notably, the diagnostic spectrum of CHDs (ASD, other anomalies) widened from the first to the second study period, while the frequency of associated cardiac anomalies in fetuses with VSDs increased. Our data suggest that the presence of first-trimester and second-trimester sonographic markers can facilitate the diagnosis of less detectable malformations such as VSD in fetuses with DS. After the introduction of the FMF protocol (i.e., second study period), increased NT thickness during the first trimester was mainly associated with VSD, TOF, and nonstructural heart abnormalities. However, despite the widespread application of standardized NT measurements, beginning in 2009, we observed no increases in the prenatal detection of CHDs in our selected cohort. Detection of nonstructural heart abnormalities in the second trimester necessitates detailed follow-up for cardiac and noncardiac malformations and testing for chromosomal abnormalities.

We hypothesized that advancements in US technology would increase rates of CHD detection from the first to second study period. However, no such increases were observed. This lack of change may have been due to the greater availability of early screening methods (NIPT, combined testing) and early diagnostic interventions (e.g., doubling of the CVS rate) after 2009. The consequent increase in early TOP may have also reduced the diagnostic accuracy of fetal heart US and autopsy (especially prior to week 14), which may have in turn counterbalanced the improvements in the detection rate observed since the 1999–2008 study period.

The present study possesses several limitations, including its retrospective, observational, nonpopulation-based design. One substantial limitation was that approximately 11% of pregnancies were terminated at a different institution, in these cases we were unable to obtain FPATH results. In the remaining cohort, the combined TOP and stillborn rate was 88%. Furthermore, we were unable to confirm findings in liveborn cases via postpartum echocardiography, and neonatal outcome data were unavailable. Therefore, complete US and FPATH data were available for approximately 77–78% of the study population. Fetal echocardiography was not performed in all cases of DS, although the application of routine fetal echocardiography in cases of T21 could have increased rates of CHD detection (Gardiner, 2018). Notably, NIPT has been available in Hungary since 2014, allowing for early diagnosis and TOP in cases of DS, which may have decreased rates of CHD detection in fetuses with DS during the last 5 years of the study period. Together, these factors may have resulted in an underestimation of CHD prevalence in our DS cohort.

Our study provides data regarding cardiovascular malformations detected in fetuses with DS over the last 20 years, as well as their associations with US markers and major noncardiac findings. The detection of associated CHD or extracardiac malformations in fetuses with DS necessitates interdisciplinary consultation, as this can certainly influence the parents' decision to continue or terminate the pregnancy as well as peripartum management.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

Zsolt Tidrenczel: Conceived the study design, collected, analyzed and interpreted the data, and wrote the manuscript. Julia Hajdu: Performed the fetal echocardiography's, revised data interpretation, and wrote the manuscript. Aténé Simonyi: Collected data and reviewed the literature. István Szabó: Performed the sonography examinations and revised the manuscript. Nándor Ács and János Demeter: Revised the manuscript. Artúr Beke: Conceived the study design, revised data interpretation, and wrote the manuscript. All authors reviewed and approved the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

Bahtiyar, M. O., Dulay, A. T., Weeks, B. P., Friedman, A. H., & Copel, J. A. (2008). Prenatal course of isolated muscular ventricular septal defects

diagnosed only by color Doppler sonography: Single-institution experience. *Journal of Ultrasound Medicine*, *27*, 715–720. https://doi.org/10.7863/jum.2008.27.5.715

Bergström, S., Carr, H., Petersson, G., Stephansson, O., Bonamy, A. K., Dahlström, A., & Johansson, S. (2016). Trends in congenital heart defects in infants with down syndrome. *Pediatrics*, *138*, e2016012. https://doi.org/10.1542/peds.2016-0123

Best, K. E., & Rankin, J. (2015). Increased risk of congenital heart disease in twins in the north of England between 1998 and 2010. *Heart*, *101*, 1807–1812. https://doi.org/10.1136/heartjnl-2015-307826

Best, K. E., & Rankin, J. (2016). Is advanced maternal age a risk factor for congenital heart disease? *Birth Defects Research Part A: Clinical and Molecular Teratology*, *106*, 461–467. https://doi.org/10.1002/bdra.23507

Bull, M. J. (2020). Down syndrome. *The New England Journal of Medicine*, *382*, 2344–2352. https://doi.org/10.1056/NEJMra1706537

Cocchi, G., Gualdi, S., Bower, C., Halliday, J., Jonsson, B., Myrelid, A., & Annerén, G. (2010). International trends of down syndrome 1993–2004: Births in relation to maternal age and terminations of pregnancies. *Birth Defects Research*, *88*, 474–479. https://doi.org/10.1002/bdra.20666

Concolino, D., Pascuzzi, A., Pietragalla, E., Lia, R., Canepa, S., & Strisciuglio, P. (2005). High prevalence of isolated pericardial effusion in down syndrome. *American Journal of Medical Genetics Part A*, *132*, 331–332. https://doi.org/10.1002/ajmg.a.30399

DeVore, G. R. (2003). The role of fetal echocardiography in genetic sonography. *Seminars in Perinatology*, *27*, 160–172. https://doi.org/10.1053/sper.2003.50015

Fan, H. C., Blumenfeld, Y. J., Chitkara, U., Hudgins, L., & Quake, S. R. (2008). Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 16266–16271. https://doi.org/10.1073/pnas.0808319105

Fragouli, E., Alfarawati, S., Spath, K., Jaroudi, S., Sarasa, J., Enciso, M., & Wells, D. (2013). The origin and impact of embryonic aneuploidy. *Human Genetics*, *132*, 1001–1013. https://doi.org/10.1007/s00439-013-1309-0

Freeman, S. B., Bean, L. H., Allen, E. G., Tinker, S. W., Locke, A. E., Druchel, C., & Sherman, S. L. (2008). Ethnicity, sex, and the incidence of congenital heart defects: A report from the National Down Syndrome Project. *Genetics in Medicine*, *10*, 173–180. https://doi.org/10.1097/GIM.0b013e3181634867

Gardiner, H. M. (2018). Advances in fetal echocardiography. *Seminars in Fetal and Neonatal Medicine*, *23*, 112–118. https://doi.org/10.1016/j.siny.2017.11.006

Geipel, A., Willruth, A., Vieten, J., Gembruch, U., & Berg, C. (2010). Nuchal fold thickness, nasal bone absence or hypoplasia, ductus venosus reversed flow and tricuspid valve regurgitation in screening for trisomies 21, 18 and 13 in the early second trimester. *Ultrasound in Obstetrics and Gynecology*, *35*, 535–539. https://doi.org/10.1002/uog.7597

Gomez, O., Martinez, J. M., Olivella, A., Bennasar, M., Crispi, F., Masoller, N., & Gratacós, E. (2014). Isolated ventricular septal defects in the era of advanced fetal echocardiography: Risk of chromosomal anomalies and spontaneous closure rate from diagnosis to age of 1 year. *Ultrasound in Obstetrics and Gynecology*, *43*, 65–71. https://doi.org/10.1002/uog.12527

Hoffman, J. I., & Kaplan, S. (2002). The incidence of congenital heart disease. *Journal of the American College of Cardiology*, *39*, 1890–1900. https://doi.org/10.1016/S0735-1097(02)01886-7

Hyett, J. A., Moscoso, G., & Nicolaides, K. H. (1995). First-trimester nuchal translucency and cardiac septal defects in fetuses with trisomy 21. *American Journal of Obstetrics and Gynecology*, *172*, 1411–1413. https://doi.org/10.1016/0002-9378(95)90470-0

Jorgensen, D. E., Vejlstrup, N., Jørgensen, C., Maroun, L. L., Steensberg, J., Hessellund, A., & Tabor, A. (2015). Prenatal detection of congenital heart disease in a low risk population undergoing first and second trimester screening. *Prenatal Diagnosis*, *35*, 325–330. https://doi.org/10.1002/pd.4525

Kailin, J. A., Santos, A. B., Yilmaz, F. B., Tejtel, S. K., & Lantin-Hermoso, R. (2017). Isolated coarctation of the aorta in the fetus: A diagnostic challenge. *Echocardiography*, *34*, 1768–1775. https://doi.org/10.1111/echo.13578

Kallen, B., Mastroiacovo, P., & Robert, E. (1996). Major congenital malformations in down syndrome. *American Journal of Medical Genetics*, *65*, 160–166. https://doi.org/10.1002/(SICI)1096-8628(19961016)65:2<160::AID-AJMG16>3.0.CO;2-O

Keeling, J. W. (1993). The perinatal autopsy. In J. W. Keeling (Ed.), *Fetal and neonatal pathology* (pp. 465–469). London, England: Springer-Verlag.

Kyeong, K. S., Won, H. S., Lee, M. Y., Shim, J. Y., Lee, P. R., & Kim, A. (2014). Clinical outcomes of prenatally diagnosed cases of isolated and nonisolated pericardial effusion. *Fetal Diagnosis and Therapy*, *36*, 320–325. https://doi.org/10.1159/000358591

Langford, K., Sharland, G., & Simpson, J. (2005). Relative risk of abnormal karyotype in fetuses found to have an atrioventricular septal defect (AVSD) on fetal echocardiography. *Prenatal Diagnosis*, *25*, 137–139. https://doi.org/10.1002/pd.1037

Lee, W., Allan, L., Carvalho, J. S., Chaoui, R., Copel, J., Devore, G., & Yagel, S. (2008). ISUOG consensus statement: What constitutes a fetal echocardiogram? *Ultrasound in Obstetrics and Gynecology*., *32*, 239–242. https://doi.org/10.1002/uog.6115

Miller, A., Riehle-Colarusso, T., Siffel, C., Frias, J. L., & Correa, A. (2011). Maternal age and prevalence of isolated congenital heart defects in an urban area of the United States. *American Journal of Medical Genetics Part A*, *155*, 2137–2145. https://doi.org/10.1002/ajmg.a.34130

Mogra, R., Zidere, V., & Allan, L. D. (2011). Prenatally detectable congenital heart defects in fetuses with Down syndrome. *Ultrasound in Obstetrics and Gynecology*, *38*, 320–324. https://doi.org/10.1002/uog.8977

Mogra, R., Alabbad, N., & Hyett, J. (2012). Increased nuchal translucency and congenital heart disease. *Early Human Development*, *88*, 261–267. https://doi.org/10.1016/j.earlhumdev.2012.02.009

Morlando, M., Bhide, A., Familiari, A., Khalil, A., Morales-Rosello, J., Papageorghiou, A. T., & Carvalho, J. S. (2017). The association between prenatal atrioventricular septal defects and chromosomal abnormalities. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, *208*, 31–35. https://doi.org/10.1016/j.ejogrb.2016.10.039

Morris, J. K., Garne, E., Wellesley, D., Addor, M. C., Arriola, L., Barisic, I., & Dolk, H. (2014). Major congenital anomalies in babies born with down syndrome: A EUROCAT population-based registry study. *American Journal of Medical Genetics Part A*, *164*, 2979–2986. https://doi.org/10.1002/ajmg.a.36780

Natoli, J. L., Ackerman, D. L., McDermott, S., & Edwards, J. G. (2012). Prenatal diagnosis of down syndrome: A systematic review of termination rates (1995-2011). *Prenatal Diagnosis*, *32*, 142–153. https://doi.org/10.1002/pd.2910

Paladini, D., Tartaglione, A., Agangi, A., Teodoro, A., Forleo, F., Borghese, A., & Martinelli, P. (2000). The association between congenital heart disease and down syndrome in prenatal life. *Ultrasound in Obstetrics and Gynecology*, *15*, 104–108. https://doi.org/10.1046/j.1469-0705.2000.00027.x

Pfitzer, C., Helm, P. C., Rosenthal, L. M., Berger, F., Bauer, U. M. M., & Schmitt, K. R. (2018). Dynamics in prevalence of down syndrome in children with congenital heart disease. *European Journal of Pediatrics*, *177*, 107–115. https://doi.org/10.1007/s00431-017-3041-6

Pierpont, M. E., Brueckner, M., Chung, W. K., Garg, V., Lacro, R. V., McGuire, A. L., & Russell, M. W. (2018). Genetic basis for congenital heart disease: Revisited: A scientific statement from the American Heart Association. *Circulation*, *138*, 653–711. https://doi.org/10.1161/CIR.0000000000000606

Respondek-Liberska, M., Nowicki, G., Krasoń, A., Kaczmarek, P., Kociszewska, I., & Danuta, B. (1999). Can we suspect fetal down syndrome by heart evaluation during the second half of pregnancy? *Fetal Diagnosis and Therapy*, *14*, 143–148. https://doi.org/10.1159/000020908

Santoro, M., Coi, A., Spadoni, I., Bianchi, F., & Pierini, A. (2018). Sex differences for major congenital heart defects in Down syndrome: A population based study. *European Journal of Medical Genetics*, *61*, 546–550. https://doi.org/10.1016/j.ejmg.2018.05.013

Sokal, R., Tata, L. J., & Fleming, K. M. (2014). Sex prevalence of major congenital anomalies in the United Kingdom: A national population-based study and international comparison meta-analysis. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *100*, 79–91. https://doi.org/10.1002/bdra.23218

Stoll, C., Alembik, Y., Dott, B., & Roth, M. P. (1998). Study of Down syndrome in 238,942 consecutive births. *Annals of Genetics*, *41*, 44–51.

Stoll, C., Dott, B., Alembik, Y., & Roth, M. P. (2015). Associated congenital anomalies among cases with Down syndrome. *European Journal of Medical Genetics*, *58*, 674–680. https://doi.org/10.1016/j.ejmg.2015.11.003

Stressig, R., Kozlowski, P., Froehlich, S., Siegmann, H. J., Hammer, R., Blumenstock, G., & Kagan, K. O. (2011). Assessment of the ductus venosus, tricuspid blood flow and the nasal bone in second-trimester screening for trisomy 21. *Ultrasound in Obstetrics and Gynecology*, *37*, 444–449. https://doi.org/10.1002/uog.7749

Tegnander, E., Williams, W., Johansen, O. J., Blaas, H. G., & Eik-Nes, S. H. (2006). Prenatal detection of heart defects in a nonselected population of 30,149 fetuses—Detection rates and outcome. *Ultrasound in Obstetrics and Gynecology*, *27*, 252–265. https://doi.org/10.1002/uog.2710

Torfs, C. P., & Christianson, R. E. (1998). Anomalies in Down syndrome individuals in a large population-based registry. *American Journal of Medical Genetics*, *77*, 431–438. https://doi.org/10.1002/(SICI)1096-8628(19980605)77:5<431::AID-AJMG15>3.0.CO;2-J

Versacci, P., Di Carlo, D., Digilio, M., & Marino, B. (2018). Cardiovascular disease in Down syndrome. *Current Opinion in Pediatrics*, *30*, 616–622. https://doi.org/10.1097/MOP.0000000000000661

Wren, C., Richmond, S., & Donaldson, S. (2000). Temporal variability in birth prevalence of cardiovascular malformations. *Heart*, *83*, 414–419. https://doi.org/10.1136/heart.83.4.414

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