Prevalence of chromosomal disorders in cases with congenital heart defect: registry-based study from Denmark between 2008 and 2018

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CONTRIBUTION

What are the novel findings of this work?
This study presents chromosomal analysis findings from the largest, nationwide cohort of cases with a major congenital heart defect (CHD), all of which completed first- and second-trimester screening. The prevalence of chromosomal disorders in cases with a major CHD was 12.9%. The prevalence varied considerably according to CHD diagnosis and presence of associated extracardiac malformations. Moreover, we provide information on specific chromosomal disorders for each CHD type.

What are the clinical implications of this work?
The knowledge from the findings of our study should be important for prenatal counseling and useful for countries in which whole exome or whole genome sequencing is still not used routinely.

ABSTRACT

Objective To estimate the prevalence of chromosomal conditions in all fetuses and children with major congenital heart defect (CHD) in Denmark between 2008 and 2018.

Methods This was a national registry-based study including all singleton pregnancies with a prenatally or postnatally diagnosed major CHD usually requiring surgery within the first year after birth and a due date between July 2008 and December 2018 in Denmark. Data were retrieved from the Danish Fetal Medicine Database (DFMD) and the Danish Cytogenetic Central Register (DCCR) in October 2020. The DCCR contains information on all prenatal and postnatal genetic analyses, including karyotyping, chromosomal microarray, polymerase chain reaction, multiplex ligation-dependent probe amplification and fluorescence in-situ hybridization. All cases were reviewed by a clinical geneticist, and genetic changes were classified as pathogenic, likely pathogenic, variant of uncertain significance, likely benign or benign. Pathogenic and likely pathogenic variants were considered to be abnormal. Cases with CHD without any registered chromosomal analysis reported were considered genetically normal. Isolated CHD was defined as a case with major CHD without any other structural malformations detected prenatally or postnatally. Results are given as n (%). Comparisons between isolated and non-isolated cases were performed using logistic regression analysis, and data are presented as odds ratios (ORs) with 95% CIs.

Results A total of 8482 cases with any cardiovascular diagnosis were retrieved from the DFMD. Twins (n = 112) and minor CHD cases (n = 6921) were excluded, resulting in 1449 cases with major CHD. Of the included cases, 918 (63.4%) underwent chromosomal analysis. An abnormal test result was found in 187 cases, giving a prevalence of a chromosomal condition of 12.9% (95% CI, 11.2–14.7%) among all cases with major CHD. The highest prevalence of a chromosomal condition was found in cases with pulmonary atresia with intact ventricular septum and those with truncus arteriosus (both 28.6%), while the lowest prevalence was found in cases with transposition of the great arteries (2.2%)

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ORIGINAL PAPER

and congenitally corrected transposition of the great arteries (0%). In isolated cases of transposition of the great arteries, the prevalence of a chromosomal condition was 0.6%. The overall OR for a chromosomal condition in non-isolated cases compared with isolated cases was 2.72 (95% CI, 1.90–3.88).

Conclusions *We found an overall prevalence of a chromosomal condition of 12.9% among cases with major CHD in a national cohort with a high participation rate in first- and second-trimester screening, without employing whole genome and whole exome sequencing. The prevalence of a chromosomal condition varied considerably according to CHD diagnosis and presence of associated extracardiac malformations. These findings are important for prenatal counseling. © 2022 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Congenital heart defect (CHD) is one of the most common congenital malformations, accounting for approximately 1% of cases, with major CHD having a prevalence of 0.2%[1–3]. The etiology of CHD is not clear, but genetic changes have been reported to account for up to 35% of cases[4].

CHD is associated with an increased risk of neonatal morbidity and mortality, although the prognosis has improved over the years with better treatments and early diagnosis. It is also known that when CHD is part of a genetic disorder, the outcome deteriorates[5,6]. This information is important for expectant parents, as an isolated diagnosis of CHD differs from a CHD diagnosis combined with a genetic disorder, which may be accompanied by other malformations and/or neurodevelopmental delay. It has been shown that parents are more likely to choose termination of pregnancy in cases with CHD and pathogenic genetic abnormalities[4,7]. In Denmark and many other countries, termination of pregnancy is a reproductive choice when a major CHD is diagnosed prenatally. In Denmark, termination because of a major CHD can be performed before $22 + 6$ weeks' gestation. The decision to terminate should be made following optimal counseling that includes information from genetic testing of the fetus. Therefore, genetic testing should be offered, where possible, to all pregnant women expecting a baby with a prenatally diagnosed CHD.

Types of prenatal genetic analysis include karyotyping, chromosomal microarray (CMA) and whole exome sequencing (WES), of which CMA is still the standard of care in many countries, including Denmark. Many studies have found CHD as part of genetic syndromes and investigated smaller cohorts of CHD using both CMA and WES, but fewer have investigated larger cohorts of CHD[8–16]. A large study based on the data from The Society of Thoracic Surgeons Congenital Heart Surgery Database included more than 15 000 cases with CHD and found a prevalence of chromosomal aberrations of 17.3%; however, the study included only cases that had surgery performed within the first month postpartum, thus not accounting for the terminated pregnancies[17]. A recent Dutch study included 708 fetuses with severe CHD and found a prevalence of genetic disorders of about 35%[4]. However, the number of cases in the study was limited for some diagnoses. The publication was followed up by a correspondence by Thibodeau and Langlois requesting details on the specific subtypes of CHD associated with genetic diagnoses, demonstrating the need for more thorough information on CHD cohorts with respect to genetics[18].

This study aimed to estimate the prevalence of chromosomal disorders in all fetuses and children with major CHD in Denmark between 2008 and 2018, overall and according to specific type of CHD.

METHODS

This was a national registry-based study of data prospectively collected over 11 years including all singleton fetuses and children with a prenatally or postnatally diagnosed CHD and a due date between July 2008 and December 2018 in Denmark.

Data were retrieved from the Danish Fetal Medicine Database (DFMD) and the Danish Cytogenetic Central Register (DCCR) in October 2020. The DFMD contains information on prenatal findings in all pregnancies with first- and/or second-trimester ultrasound screening as well as pregnancy and perinatal outcomes, including prenatal and postnatal diagnoses according to the International Classification of Diseases 10th Revision. Postnatal data are captured from the National Patient Registry and include data 1 year after birth[19]. The DCCR contains information on all prenatal and postnatal genetic analyses, including karyotyping, CMA, polymerase chain reaction, multiplex ligation-dependent probe amplification and fluorescence *in-situ* hybridization. Data from the DCCR are updated in the DFMD regularly until 10 years after birth. All patients in Denmark are identified using a personal registration number, and the mother's identification number is linked to the child's identification number in the DFMD and DCCR.

Inclusion criteria were a singleton pregnancy, a due date between July 2008 and December 2018, prenatal ultrasound screening performed in the first and/or second trimester and a prenatal or postnatal diagnosis of CHD at any time during the pregnancy or up to 1 year postpartum. Validation of CHD diagnoses in the DFMD has been published previously[3]. The study was approved by The Danish Data Protection Agency (P-2020-739).

All pregnant women in Denmark are offered two prenatal ultrasound screening examinations free of charge: combined first-trimester screening (cFTS), including a risk assessment for trisomies 21, 18 and 13, and second-trimester screening with an anomaly scan at $18 + 0$ to $21 + 6$ weeks' gestation. All screening scans are performed by specially trained midwives or nurses

who are certified by the Fetal Medicine Foundation or supervised by a certified individual. In Denmark, more than 95% of pregnant women attend the offered screening program[20]. The Danish prenatal detection rate of trisomy 21 is approximately 92.4% before 14 weeks and 94.9% before 22 full weeks[20]. During the study period, the fetal heart was screened during the second-trimester scan. According to the national guideline, women are referred for an ultrasound scan by a fetal medicine specialist and/or a fetal cardiologist when a CHD is suspected at all times during pregnancy. If CHD is confirmed, the parents are offered genetic testing, and invasive testing is recommended in cases of structural anomaly, including CHD. During the study period, the prenatal genetic test of choice changed from karyotyping to CMA at different timepoints across Denmark. All Danish children are offered a postnatal screening exam with their family doctor at 5 weeks and 5 months as well as every year until they reach 5 years of age.

In this study, we included only cases with major CHD, defined as a lesion usually requiring surgery within the first year of age. The results on isolated ventricular septal defects were not included in this study, as we have already published these data[21]. Moreover, pulmonary valve and aortic valve stenosis in cases with a biventricular heart were not included as individual diagnoses owing to the wide range of severity of these conditions. Interrupted aortic arch was also not included, as it is poorly registered in the DFMD owing to the lack of an independent code for antenatal diagnosis. Thus, the included diagnoses were as listed in Table 1. Each patient was included only once even if they had more than one diagnosis. In the case of more than one CHD diagnosis, the case with the most severe diagnosis was included. Women with more than one affected pregnancy were also included.

Results from chromosomal analysis were obtained from the DCCR, which is updated regularly. Any genetic change found in a subject was included for interpretation. Interpretation and classification of all chromosomal disorders was done according to the guideline for interpretation of copy number variants (CNVs) published by The American College of Medical Genetics and Genomics[22]. This classification allocates abnormal results into five categories according to their expected clinical relevance: pathogenic, likely pathogenic, variant of uncertain significance (VOUS), likely benign or benign. Pathogenic and likely pathogenic results were pooled and considered to be 'abnormal genetic findings' regardless of whether the chromosomal disorder was known to be associated with a CHD. Each abnormal karyotype was evaluated and interpreted based on current knowledge. If breakpoints or reference frames were not available but were essential for interpretation, the result was classified as a VOUS. All pathogenic and likely pathogenic cases were included in the analysis of the prevalence of chromosomal disorders.

In this study, cases with CHD without any registered chromosomal analysis reported were considered genetically normal. Consequently, all numbers presented in this study are conservative estimates of the prevalence of chromosomal disorders. However, the prevalence is also presented as the number of cases with a chromosomal disorder out of the total number of genetically tested subjects. All cases resulting in intrauterine death or termination of pregnancy were also included in the analysis, as exclusion would have increased the risk of selection bias, probably leading to fewer cases of chromosomal disorders.

Isolated CHD was defined as a case with a major CHD without any other structural malformation detected prenatally or postnatally. Soft markers at the second-trimester scan (echogenic bowel, short femur, echogenic intracardiac focus, choroid plexus cyst and mild pyelectasis (5–10 mm)) were not considered structural malformations.

Table 1 Number of singleton cases with congenital heart defect (CHD), those that underwent genetic testing and those with abnormal genetic test result*, according to type of CHD, in Denmark between 2008 and 2018

			Abnormal genetic test result	
CHD	*Total* (n = 1449)	*Genetic test performed*	*Among all cases*	*Among cases with genetic test performed*
Atrioventricular septal defect	337 (23.3)	183/337 (54.3)	83/337 (24.6)	83/183 (45.4)
Coarctation of aorta	277 (19.1)	128/277 (46.2)	16/277 (5.8)	16/128 (12.5)
Hypoplastic left ventricle	208 (14.4)	161/208 (77.4)	27/208 (13.0)	27/161 (16.8)
Transposition of great arteries	180 (12.4)	118/180 (65.6)	4/180 (2.2)	4/118 (3.4)
Tetralogy of Fallot	164 (11.3)	134/164 (81.7)	26/164 (15.9)	26/134 (19.4)
Hypoplastic right ventricle	81 (5.6)	67/81 (82.7)	6/81 (7.4)	6/67 (9.0)
Double outlet right ventricle	79 (5.5)	54/79 (68.4)	8/79 (10.1)	8/54 (14.8)
Truncus arteriosus	35 (2.4)	31/35 (88.6)	10/35 (28.6)	10/31 (32.3)
Ebstein's anomaly	26 (1.8)	13/26 (50.0)	2/26 (7.7)	2/13 (15.4)
TAPVR	26 (1.8)	9/26 (34.6)	1/26 (3.8)	1/9 (11.1)
ccTGA	20 (1.4)	11/20 (55.0)	0/20 (0)	0/11 (0)
PA-VSD	9 (0.6)	7/9 (77.8)	2/9 (22.2)	2/7 (28.6)
PA-IVS	7 (0.5)	2/7 (28.6)	2/7 (28.6)	2/2 (100)
Total	1449 (100)	918/1449 (63.4)	187/1449 (12.9)	187/918 (20.4)

Data are given as *n* (%) or *n*/*N* (%). *Only pathogenic and likely pathogenic variants were considered abnormal. ccTGA, congenitally corrected transposition of great arteries; IVS, intact ventricular septum; PA, pulmonary atresia; TAPVR, total anomalous pulmonary venous return; VSD, ventricular septal defect.

Data were analyzed using Stata version 13.1 (StataCorp., College Station, TX, USA). Results are given as *n* (%). Comparison between isolated and non-isolated cases was performed using logistic regression analysis and data are presented as odds ratios (ORs) with 95% CIs.

RESULTS

A total of 8482 cases with any cardiovascular diagnosis were retrieved from the DFMD (Figure 1). We excluded twins (*n* = 112) and minor CHD (*n* = 6921), resulting in 1449 cases with major CHD. A total of 1058 children
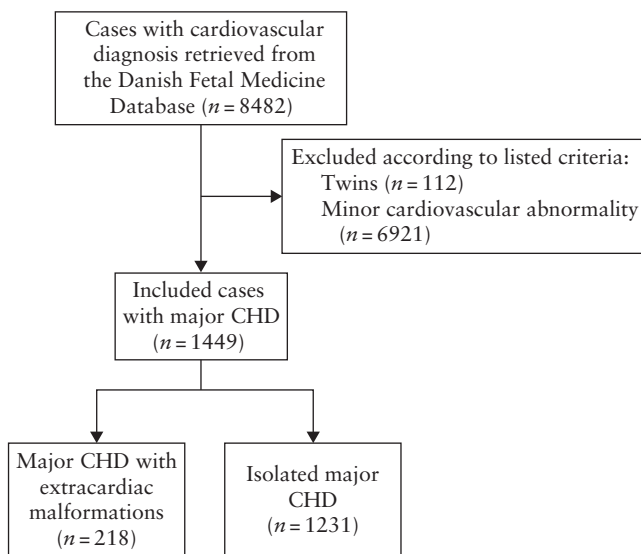

Figure 1 Flowchart summarizing inclusion of cases with major congenital heart defect (CHD).

were liveborn and 391 cases resulted in miscarriage, termination of pregnancy, intrauterine death or perinatal death. Of the 391 cases of perinatal loss, 335 (85.7%) underwent chromosomal analysis. Between the second half of 2008 and the end of 2018, approximately 609 000 singletons were liveborn in Denmark, resulting in a prevalence of the included major CHD of 0.17% among liveborns. Fifteen women had two pregnancies complicated by CHD and were thus included twice. Of these 30 pregnancies, a chromosomal disorder was found in two cases, which were not from the same mother.

Table 1 shows the type and number of each major CHD diagnosed during the study period along with the information on chromosomal analysis for each diagnosis. Of the 1449 cases with major CHD, 918 (63.4%) underwent chromosomal analysis, of which 187 had an abnormal test result. Hence, the conservative prevalence of a chromosomal disorder in the included cases with major CHD was 12.9%. The prevalence of a chromosomal disorder among the tested subjects was 20.4%. The prevalence varied significantly among the specific diagnoses from 0% (0/20) for congenitally corrected transposition of the great arteries (ccTGA) to 28.6% (10/35) for truncus arteriosus (*P* < 0.01). The rate of chromosomal analysis also varied significantly by CHD diagnosis, ranging from 28.6% (2/7) for pulmonary atresia with intact ventricular septum to 88.6% (31/35) for truncus arteriosus (*P* < 0.01).

Table 2 presents CHD diagnoses along with the information on chromosomal analysis according to the presence of extracardiac malformations. A total of 1231 cases had an isolated major CHD and 218 (15.0%) CHD cases had extracardiac malformations. Of the cases with an isolated major CHD, 133/1231 (10.8% (95% CI, 9.1–12.7%)) had an abnormal test

Table 2 Number of singleton cases with congenital heart defect (CHD) and proportion of CHD cases with abnormal genetic test result*, according to presence (non-isolated) or absence (isolated) of extracardiac congenital malformations, in Denmark between 2008 and 2018

	Isolated CHD		Non-isolated CHD		
CHD	*Total*	*Proportion with abnormal genetic test (%) (95% CI)*	*Total*	*Proportion with abnormal genetic test (%) (95% CI)*	*OR (95% CI)†*
AVSD (*n* = 337)	261 (77.4)	20.3 (15.6–25.7)	76 (22.6)	39.5 (28.4–51.4)	2.56 (1.48–4.44)
Coarctation of aorta (*n* = 277)	247 (89.2)	6.1 (3.4–9.8)	30 (10.8)	3.3 (0.1–17.2)	0.53 (0.07–4.19)
Hypoplastic left ventricle (*n* = 208)	185 (88.9)	10.8 (6.7–16.2)	23 (11.1)	30.4 (13.2–52.9)	3.61 (1.33–9.83)
TGA (*n* = 180)	160 (88.9)	0.6 (0.0–3.4)	20 (11.1)	15.0 (3.2–37.9)	28.06 (2.76–284.88)
Tetralogy of Fallot (*n* = 164)	137 (83.5)	16.8 (11.0–24.1)	27 (16.5)	11.1 (2.4–29.2)	0.62 (0.17–2.23)
Hypoplastic right ventricle (*n* = 81)	67 (82.7)	4.5 (0.9–12.5)	14 (17.3)	21.4 (4.7–50.8)	5.82 (1.04–32.60)
Double outlet right ventricle (*n* = 79)	71 (89.9)	8.5 (3.2–17.5)	8 (10.1)	25.0 (3.2–65.1)	3.61 (0.59–21.97)
Truncus arteriosus (*n* = 35)	29 (82.9)	24.1 (10.3–43.5)	6 (17.1)	50.0 (11.8–88.2)	3.14 (0.51–19.25)
Ebstein's anomaly (*n* = 26)	23 (88.5)	4.3 (0.1–22.0)	3 (11.5)	33.3 (0.8–90.6)	11.00 (0.48–250.87)
TAPVR (*n* = 26)	23 (88.5)	4.3 (0.1–22.0)	3 (11.5)	0	—
ccTGA (*n* = 20)	16 (80.0)	0	4 (20.0)	0	—
PA-VSD (*n* = 9)	7 (77.8)	28.6 (3.7–71.0)	2 (22.2)	0	—
PA-IVS (*n* = 7)	5 (71.4)	20.0 (0.5–71.6)	2 (28.6)	50.0 (1.3–98.7)	4.00 (0.12–136.96)
Total (*n* = 1449)	1231 (85.0)	10.8 (9.1–12.7)	218 (15.0)	24.8 (19.2–31.1)	2.72 (1.90–3.88)

Data are given as *n* (%), unless stated otherwise. *Only pathogenic and likely pathogenic variants were considered abnormal. †Odds ratio (OR) of abnormal genetic test in non-isolated *vs* isolated CHD group. AVSD, atrioventricular septal defect; ccTGA, congenitally corrected transposition of great arteries; IVS, intact ventricular septum; PA, pulmonary atresia; TAPVR, total anomalous pulmonary venous return; TGA, transposition of great arteries; VSD, ventricular septal defect.

Table 3 Congenital heart defect (CHD) and associated chromosomal findings (CF), including pathogenic, benign and likely benign variants and variants of uncertain significance (VOUS)

CHD type/CF	Cases with CF (n)	Significance	Association with CHD	Clinical outcome
Atrioventricular septal defect	86			
47,+21	62	Pathogenic	Known	TOP/IUD ($n = 21$), live birth ($n = 38$), postnatal death ($n = 3$)
47,+18	10	Pathogenic	Known	TOP/IUD ($n = 8$), postnatal death ($n = 2$)
47,+22	1	Pathogenic	Known	TOP
47,+9	1	Pathogenic	Known	TOP
47,+13	2	Pathogenic	Known	TOP ($n = 1$), postnatal death ($n = 1$)
XXY/XY, del(7)(q11.23), Klinefelter mosaicism and Williams syndrome	1	Pathogenic	Known	TOP
del(7)(q11.23), Williams syndrome	1	Pathogenic	Known	Live birth
del(4)(p16.3p16.1), del(X)(p22.33), Wolf–Hirschhorn syndrome and SHOX deletion (incidental finding)	1	Pathogenic	Known	Live birth
del(14)(q11.2)	1	VOUS	Unknown	Live birth
del(17)(p13.1)	1	VOUS	Unknown	Live birth
del(18)(q21.2)	1	Pathogenic	Possible due to size of deletion	Live birth
del(Y)(p11.31q11.221)	1	Pathogenic	Unknown	Live birth
dup(4)(p12q12)	1	VOUS/benign	Unlikely	TOP
dup(16)(p11.2)	1	Pathogenic (susceptibility variant)	Abnormal aorta and/or aortic valve	Live birth
45,X, Turner syndrome	1	Pathogenic	Known	TOP
Coarctation of aorta	22			
47,+21	3	Pathogenic	Known	Live birth
47,+18	1	Pathogenic	Known	Postnatal death
45,X, Turner syndrome	4	Pathogenic	Known	Live birth
del(22)(q11)	4	Pathogenic (susceptibility variant)	Known	Live birth ($n = 3$), postnatal death ($n = 1$)
del(1)(p33)	1	VOUS	Unknown but possible	Live birth
del(8)(q22.3)	1	VOUS	Unknown	Postnatal death
del(8)(q24.13), del(9)(q31.1)	1	Pathogenic	Unknown	Live birth
del(14)(q32.2q32.31), Gabriele–de Vries syndrome	1	Pathogenic	Known	Live birth
del(14)(q32.31q31.33)	1	Pathogenic	Unknown but possible	Live birth
dup(2)(p23.1p22.3)	1	VOUS	Unknown	Live birth
dup(22)(q11.21)	2	VOUS	Unknown	Postnatal death
dup(X)(p21.2p21.1)	1	Pathogenic	Unknown	Live birth
Xp22.31*1~2	1	Likely benign	Unknown	Live birth
Hypoplastic left ventricle	31			
45,X, Turner syndrome	8	Pathogenic	Known	TOP/IUD
47,+18	4	Pathogenic	Known	TOP
47,+21	2	Pathogenic	Known	IUD ($n = 1$), postnatal death ($n = 1$)
47,+13	4	Pathogenic	Known	TOP/IUD
47,XY,+18 [24]/46,XY [6]	1	Pathogenic	Known	TOP
del(2)(q13), del(5)(p15.2p14.3), dup(X)(p22.32)	1	Pathogenic	Likely	Live birth
del(2)(p16.3)	1	Pathogenic (susceptibility variant)	Unknown	Live birth
del(4)(p15.3)	1	Pathogenic	Likely	Lost to follow-up (not live birth)
del(5)(p15.33p15.2), dup(18)(q12.1q23), Cri-du-chat syndrome	1	Pathogenic	Known	TOP
del(6)(q27), dup(8)(p23.3p11.21)	1	Pathogenic	Known	TOP
del(15)(q11.1–q11.2)	1	Benign	Unknown	TOP
del(16)(p13.3), Rubinstein–Taybi syndrome	1	Pathogenic	Known	TOP

Continued over.

Table 3 *Continued*

CHD type/CF	Cases with CF (n)	Significance	Association with CHD	Clinical outcome
del(22)(q12.3)	1	Pathogenic	Likely	TOP
dup(1)(p36.32)	1	VOUS	Unknown	Postnatal death
dup(1)(q42.13q43), del(1)(q43q44)	1	Pathogenic	Likely	Postnatal death
dup(2)(q12.3q13)	1	VOUS	Unknown	TOP
dup(6)(p22.3), dup(7)(p15.2)	1	VOUS	No	Postnatal death
Transposition of great arteries	9			
47,+22	1	Pathogenic	Likely	TOP
69,XXX	1	Pathogenic	Known	TOP
del(7)(q22.1)	1	VOUS (candidate gene)	Unknown (candidate gene)	Live birth
del(7)(q33)	1	Pathogenic	Likely	Live birth
del(10)(p12.31)	1	VOUS	Unknown	Live birth
del(22)(q11.1q11.21), Cat eye syndrome area	1	Benign	Unknown	TOP
dup(16)(q24.3)	1	VOUS	Unknown	IUD
dup(17)(p13.2)	1	VOUS	Unknown	Live birth
9*2/9*3, trisomy 9 mosaicism	1	Pathogenic	Known	TOP
Tetralogy of Fallot	29			
47,+21	4	Pathogenic	Known	Live birth (n = 3), TOP (n = 1)
47,+18	1	Pathogenic	Known	Postnatal death
47,XXY	1	Pathogenic	Unknown, incidental finding	TOP
del(22)(q11)	12	Pathogenic	Known	TOP/IUD (n = 9), live birth (n = 2), postnatal death (n = 1)
del(1)(q43q44), dup(8)(q24.21q24.3)	1	Pathogenic	Likely	TOP
del(3)(p13p11.1)	1	Pathogenic	Likely	TOP
del(4)(pter+, qter−)	1	VOUS	Unknown	Postnatal death
del(6)(q16q22)	1	Pathogenic	Likely	IUD
del(9)(q34.3), Kleefstra syndrome	1	Pathogenic	Known	Live birth
del(16)(p13.12p13.11)	1	Pathogenic (susceptibility variant)	Known	Live birth
del(17)(p13.3), dup(11)(p15.5), Miller–Dieker syndrome	1	Pathogenic	Likely	TOP
dup(4)(q31.22q35.2) [5]/46,XX [5]	1	Pathogenic	Unknown	TOP
dup(11)(p15.5p15.4)	1	Likely benign	Unknown	Live birth
dup(17)(p12)	1	Pathogenic	Unknown, incidental finding	Postnatal death
t(1;10)(q43;q21)	1	Likely benign	Unknown	Live birth
Hypoplastic right ventricle	7			
47,+18	1	Pathogenic	Known	TOP
47,+13	1	Pathogenic	Known	TOP
del(4)(qter)	1	Pathogenic	Likely	TOP
del(8)(p21)	1	Pathogenic	Likely	TOP
del(17)(p12)	1	Pathogenic	Unknown	TOP
dup(2)(q33.1)	1	VOUS	Unknown	Live birth
4q24q25*2 ∼ 3, 4q25q31.21*1 ∼ 2	1	Pathogenic	Likely	TOP
Double outlet right ventricle	10			
47,+21	2	Pathogenic	Known	Live birth
47,+18	1	Pathogenic	Known	TOP
47,XXX	1	Pathogenic	Known	TOP
del(1)(p21.3)	1	Pathogenic	Likely	TOP
del(7)(p22.1)	1	VOUS	Unknown	Postnatal death
del(22)(q11)	2	Pathogenic	Known	Live birth
dup(5)(qter)	1	VOUS	Unknown	Live birth
dup(17)(q12)	1	Pathogenic (susceptibility variant)	Known	Live birth
Truncus arteriosus	12			
47,+13	3	Pathogenic	Known	TOP
47,+21	1	Pathogenic	Known	Live birth
del(6)(p25), dup(11)(q24.1q25)	1	Pathogenic	Likely	TOP

Continued over.

Table 3 *Continued*

CHD type/CF	Cases with CF (n)	Significance	Association with CHD	Clinical outcome
del(22)(q11)	5	Pathogenic	Known	TOP
dup(6)(p25.3)	1	VOUS	Unknown	Postnatal death
dup(X)(p11.23)	1	VOUS	Unknown	Live birth
Ebstein's anomaly	2			
46,+12, der(12;15)(p10;q10)	1	Pathogenic	Likely	Live birth
del(1)(p36.32p36.33)	1	Pathogenic	Known	Live birth
Total anomalous pulmonary venous return	2			
der(2)(q11.2q21?)	1	Pathogenic	Likely	Live birth
ish der(5)t(4;5)	1	VOUS	Unknown	Live birth
Pulmonary atresia with ventricular septal defect	2			
47,+13	1	Pathogenic	Known	Postnatal death
del(22)(q11)	1	Pathogenic	Known	Live birth
Pulmonary atresia with intact ventricular septum	2			
del(18)(p11.32), del(18)(q21.2q23)	1	Pathogenic	Likely	Live birth
dup(22)(q11)	1	Pathogenic (susceptibility variant)	Known	Live birth

del, deletion; der, derivative chromosome; dup, duplication; ish, *in-situ* hybridization; IUD, intrauterine death (including miscarriage); TOP, termination of pregnancy; VOUS, variant of uncertain significance.

as opposed to cases with extracardiac malformations, of which 54/218 (24.8% (95% CI, 19.2–31.1%)) had an abnormal genetic test result (OR, 2.72 (95% CI, 1.90–3.88)). Besides ccTGA, in which no chromosomal disorders were found, only 0.6% (95% CI, 0.0–3.4%) of isolated cases with TGA had a chromosomal disorder. Conversely, 20.3% (95% CI, 15.6–25.7%) of isolated cases with atrioventricular septal defect (AVSD) and 24.1% (95% CI, 10.3–43.5%) of cases with isolated truncus arteriosus had chromosomal disorders.

In Table 3, different types of CHD are listed along with the corresponding chromosomal disorders detected. The most common chromosomal disorder was trisomy in cases with AVSD (76/83 abnormal results) and 22q11 deletion syndrome in cases with tetralogy of Fallot (12/26 abnormal results). The observed chromosomal disorders in all other CHD subgroups varied and consisted of both aneuploidies and CNVs. The likely association between chromosomal disorders and CHD is also listed in Table 3.

The overall proportion of cases with chromosomal analysis performed increased from 2008 to 2018 (47.6% to 75.2%; $P < 0.05$). However, the total number and proportion of abnormal results remained similar (Table S1). The proportion of cases undergoing CMA increased over time, as the proportion of those undergoing karyotyping decreased simultaneously.

DISCUSSION

To our knowledge, this study presents chromosomal analysis results from the largest, nationwide cohort of CHD cases, all of which completed first- and second-trimester screening. Chromosomal disorders were present in 12.9% of all cases with major CHD. As we classified all the non-tested cases as genetically normal, this is likely to be a conservative estimate. The odds of having genetic changes increased by 2.7 times when CHD

was complicated by extracardiac malformations. There was great variability in the prevalence of chromosomal disorders depending on the type of CHD. These results are essential for prenatal counseling.

The total prevalence of chromosomal disorders found in our study was generally lower than that in previous studies. Two large cohort studies, including a study based on the Dutch PRECOR registry and the PAGE study from the UK, found a genetic change in 33% (both isolated and non-isolated CHD) and 29.4% (isolated CHD) of cases, respectively[4,16].

There are several possible explanations for these differences. Firstly, the lower prevalence in our study may be explained partly by differences in the prenatal screening program offered as well as the uptake, as these vary significantly between countries. In Denmark, all pregnant women are offered prenatal screening free of charge, including the cFTS risk assessment and second-trimester screening for malformations. More than 90% of Danish women attend cFTS. Hence, our study population may include fewer pregnancies with trisomy compared with other populations, in which cFTS or non-invasive prenatal testing (NIPT) is not widely available. In 2019, 70% of cases that were positive for trisomy 21 at cFTS in Denmark underwent chorionic villus sampling and 23% underwent NIPT (unpubl. data). Consequently, most of the severely affected pregnancies with CHD, other malformations and genetic changes had been terminated before they could be diagnosed with CHD, as up to 95% of the Danish pregnancies with prenatally detected trisomy 21 are terminated (comparable with other countries)[23–25]. In the Dutch study, almost 20% had trisomy 21, 18 or 13[4], as compared with only 7% in our cohort.

Secondly, WES and whole-genome sequencing (WGS) were not included in our study, as WES/WGS was not widely offered during the study period. As the additional diagnostic yield of WES and WGS compared with CMA

in CHD is approximately 4.5–7.5%, the prevalence of all types of genetic aberration found in our population could have been higher if this technology had been included[4,8,16]. Moreover, the Dutch study also reported a conservative estimate; however, approximately 80% of their population underwent genetic testing as compared with 63% in the current study. Lastly, the definition of major CHD used is another factor to take into consideration when comparing our results with those of other studies, as some studies included only critical CHD requiring surgery within the first month postpartum[17]. Moreover, some studies reported results from single referral centers only[8–13,15]. The high termination rate of fetuses with CHD before genetic testing could be a possible explanation for the lower prevalence of chromosomal disorders found; however, 86% of the non-viable cases underwent genetic testing. Finally, our results represent a conservative estimate, as fetuses or children that had not undergone genetic testing were included as genetically normal, thus there was a risk of underestimating the prevalence. However, this risk is small, as children with signs of abnormal development or disability without any obvious cause would have been offered genetic testing. Nevertheless, as our follow-up period for the children born in 2018 was only 2 years, there is a risk that some children have not yet shown signs of delay or disability and hence have not been tested, thus underestimating the prevalence of chromosomal disorders.

We found that 15% of cases with CHD also had extracardiac malformations. This finding is in line with a study including 4005 CHD cases, which reported non-syndromic malformations in 15% of cases[26], as well as with another study that found malformations in 15.6% of cases with CHD[13].

The prevalence of major CHD among liveborns was 0.17%, which is comparable with the prevalence reported in previous studies, albeit slightly lower[1–3]. This could be explained by the high first-trimester screening uptake and a high prenatal detection rate, both of which could increase the prenatal termination rate. Moreover, the Danish pregnant population may be healthier than those of other countries, hence the lower prevalence of CHD.

This study has several strengths. The results represent a national cohort over 11 years with nationwide, standardized prenatal care with a very high participation rate. Our databases allowed access to thorough data on prenatal and postnatal CHD diagnoses and extensive information on genetic analyses, in which each case and genetic test result had been evaluated by a clinical geneticist. Moreover, the study had one of the largest cohorts evaluated with respect to this topic. The study also has several limitations. We did not have genetic data available for all cases, as some parents opted out of prenatal genetic testing. Hence, the prevalence is a conservative estimate and is possibly underestimated. Moreover, we know from a previous validation study that the prenatal CHD diagnoses registered in the DFMD are not complete and are sometimes missing[3]. Cases with a terminated pregnancy and a missing CHD diagnosis cannot be found in the DFMD, which could lead to both underestimation and overestimation of the prevalence of chromosomal disorders. Moreover, some of the CNVs from the early study period were missing both reference frames and breakpoints, which made them impossible to classify, and were therefore assigned as VOUS. We also excluded multiple pregnancies, which may also be a factor. Lastly, we were not able to include cases with interrupted aortic arch, which is often associated with chromosomal disorders, owing to the lack of a proper diagnostic code in our registries. This may have contributed to the overall lower prevalence of chromosomal disorders in our cohort compared with previous studies.

In conclusion, we found an overall conservative prevalence of chromosomal disorders in cases with a major CHD of 12.9% in a large, national cohort with a high participation rate on first- and second-trimester screening, without including WES and WGS. The prevalence of chromosomal disorders varied considerably depending on type of CHD and presence of associated extracardiac malformations. The results of our study show that chromosomal disorders in CHD are often not common trisomies; hence, NIPT in its current form cannot be used as a substitute for amniocentesis when a CHD is detected. This knowledge will be important for prenatal counseling and would be useful in many countries in which WES or WGS is still not routinely used.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Number of genetic tests per year and number of genetic changes per year