Increased Prevalence of Congenital Heart Defects in Monozygotic and Dizygotic Twins

Running title: Herskind et al.; Congenital heart defects in twins

Anne Maria Herskind, MD, PhD1; Dorthe Almind Pedersen, MSc2; Kaare Christensen, MD, PhD2,3

1Dept of Pediatrics, Hans Christian Andersen Children’s Hospital at Odense University Hospital;
2The Danish Twin Registry, University of Southern Denmark; 3Dept of Clinical Genetics, Odense University Hospital & Dept of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark

Address for Correspondence:
Kaare Christensen, MD, PhD
The Danish Twin Registry, Epidemiology, Institute of Public Health
University of Southern Denmark
J. B. Winsløws Vej 9B
DK-5000 Odense C, Denmark
Tel: +45 6550 3049
Fax: +45 65503682
E-mail: kchristensen@health.sdu.dk

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Abstract:

Background—Smaller studies and many case series reports indicate that congenital heart defects (CHD) may be more common in monochorionic twins than in dichorionic twins and singletons.

Methods and Results—We investigated CHD occurrence in all twins and 5% of all singletons born in Denmark in the period 1977-2001 and followed through 2006, by linking the Danish Twin Registry and Statistics Denmark registers including the National Birth Register and the Danish National Patient Register. Among 41,525 twin individuals, a total of 584 twins (1.4%) had a CHD registered in the Danish National Patient Register, while the corresponding numbers for singletons were 648/74,473 (0.87%) (p<0.001), i.e. a 63% (95% CI: 45-82%) increased risk for CHD for twins. Patent ductus arteriosus and coarctation of aorta occurred more than three times as often in twins as in singletons: 3.9 (95% CI: 2.6-5.8) and 3.1(95% CI:1.5-6.4), respectively. The increased occurrence in twins was also found in sensitivity analyses including only inpatients or only surgically treated cases regardless of whether preterm patent ductus arteriosus was included or not. We were not able to demonstrate a higher risk for CHD among monozygotic twins compared to dizygotic twins and the CHD occurrence was also increased in dizygotic twins, which are all dichorionic.

Conclusions—CHD is more common in twins than in singletons, and the increased occurrence is not restricted to monochorionic twins. Intrauterine surveillance and a postnatal comprehensive cardiac assessment for both twins may be considered regardless of chorionicity and zygosity.

Key words: congenital heart disease, epidemiology, Chorionicity, Twins, Zygosity
Smaller studies and many case series reports indicate that congenital heart defects (CHD) are more common in monochorionic twins than in dichorionic twins and singletons.\textsuperscript{1-5} All monochorionic twins are monozygotic and they comprises about two third of all monozygotic twins, while all dizygotic twins and the remaining one third of the monozygotic twins are dichorionic.\textsuperscript{3,6} Some CHDs are clearly expected to be more frequent in twins: The acardiac twin (together with a “pump” or “donor” twin) represents a CHD unique for monozygotic twins, albeit rare with a frequency of about 1 in 50-70,000 deliveries and 1 in 200-280 monochorionic twins.\textsuperscript{7} It is also to be expected that patent ductus arteriosus (PDA) occurs more often among twins than singletons because prematurity is more common among twins.\textsuperscript{6}

For non-PDA congenital heart diseases, several explanations for a potentially increased occurrence of CHD among twins have been proposed.\textsuperscript{8} The previous literature, which is based on small samples, suggests that monochorionicity is the central mechanism for CHD in twins and in particular twin-to-twin transfusion syndrome (TTTS)-affected monochorionic twins.\textsuperscript{1-3} A register-based study in the Northwest of England during 1998-2002 found that twins, and particularly monochorionic twins, had higher risk of congenital anomalies overall. A total of 51 cases of CHD among twins were identified, corresponding to a 47% (95% CI:11-95%) higher risk compared to singletons and no difference according to chorionicity.\textsuperscript{9}

Among the strengths of the previous studies of CHD in twins have been the detailed information on chorionicity, but a limitation has been the very small sample size of each of the studies. Systematic reviews based on a series of small observational studies are known to be very vulnerable to publication bias (smaller negative studies are rarely published, whereas positive studies are). We therefore investigated whether congenital heart diseases are diagnosed more commonly in twins than in singleton by linking Danish nationwide registers covering all twins.
and a 5% random sample of all singletons born in Denmark 1977-2001 with the Danish National Patient Registry. The Discharge Register has previously been shown to be a reliable and valid source for identifying congenital heart disease malformations,\textsuperscript{10-13} and it has been used extensively in clinical epidemiological studies of CHD.\textsuperscript{14-18}

In the present study, we identified more than 500 CHD cases among both twins and singletons, which allowed for sub-analyses of specific CHDs and restricting to e.g. operated CHD cases. Information from the Danish Twin Registry\textsuperscript{19-21} provided information about zygosity of the twins, hereby enabling us to make inference also about the impact of chorionicity.

Methods

The Danish civil registration system identifies all live born individuals by unique social security numbers that can be linked through Statistics Denmark to several thematically organized population databases or registers. We used information from four registers to study the occurrence of CHD in twins and singletons born in Denmark in the period 1977-2001:

*The Danish Twin Registry* is a population-based register comprising more than 80,000 twin pairs born in Denmark since 1870 including all twins born since 1973 as identified through the Medical Birth Registry\textsuperscript{19-21} Zygosity of same-sexed twin pairs has been classified by means of four standard questions, a method with less than 5% misclassification.\textsuperscript{22} Information on all twins born in Denmark in the period 1977-2001 was drawn from this register for the study.

*The Medical Birth Registry* comprises information about births in Denmark since 1973\textsuperscript{23} including gestational age, which was obtained from this registry in order to enable the exclusion of preterm patent ductus arteriosus in a sub-analysis.

*The Central Population Registry* was established in 1968. It includes all persons who
have had a permanent residence in Denmark since April 2, 1968. Each person registered receives a unique identification number that enables accurate linkage between that individual and all other national registers. Information contained in this registry includes name, sex, date of birth, and vital status (alive, dead, or emigrated). A random 5% sample of all singletons born in Denmark in the period 1977-2001 was drawn from this register for the study.

*The Danish National Patient Registry* contains information on essentially all discharges from Danish hospitals since 1977. Recorded data include the dates of admission and discharge, up to 20 discharge diagnoses, hospital and department codes, and the surgical procedures performed. Discharge diagnoses were classified according to the Danish version of the ICD-8 (international classification of diseases, 8th revision) from 1977 to 1993 and the ICD-10 (10th revision) hereafter (version 9 was never used in Denmark).

The Danish National Patient Registry has been used extensively to study CHD and it has previously been shown to have high predictive power and completeness for identifying CHD cases. We used the same methodology to identify CHD cases as Olsen et al. did in a series of previous, influential CHD studies using the Danish National Patient Registry: the ICD-8 codes used to identify CHD patients were 746-747 (except for 746.7 and 747.5-747.9, which are not specific to CHD) and ICD-10 codes Q20-Q26 (except for Q26.5-Q26.6 which are not specific to CHD). We used the same grouping across ICD-8 and ICD-10 as in these previous studies, and, similarly, we excluded patients in which CHD was only coded as a secondary diagnosis. We then assigned each patient one CHD diagnostic code, namely the first primary discharge diagnosis of CHD. Again following the methods in Olsen et al., diagnoses of extracardiac defects and chromosomal abnormalities were identified in the Danish National Patient Registry through the codes: ICD-8: 310.40-310.41, 310.5, 311.40-311.41, 311.5, 312.40-
and ICD-10: DQ00.0-DQ99.9 other than CHD.

Finally, patients who had undergone either heart surgery or catheter-based therapeutic interventions were identified in the Danish National Patient Registry through the ICD-8 codes: 30000-32442, 32900-32990 and ICD-10 codes: KFAAOO-KFNW98, KFQAOO-KFXPOO, KTFCOO-KTFCIOB.

Statistical methods

The prevalence of CHD in twins and singletons were first compared using chi-square statistic and odds-ratio and then using multivariable logistic regression to take year of birth and sex into account. In particular, year of birth could act as an important confounder because both diagnostic possibilities and the twinning rate changed markedly in the period 1977-2001. The analyses were performed for CHD overall, as well as for the subtypes specified in Table 1, and finally for CHD excluding preterm patent ductus arteriosus to avoid confounding from the higher rate of prematurity among twin births.

Sensitivity analyses

To further test the robustness of the overall findings, a number of sensitivity analyses were performed by restricting the cases to: (i) Inpatients (hereby excluding outpatients who are likely to have the least severe CHD); (ii) Patients undergoing either heart surgery or catheter-based therapeutic interventions; (iii) Diagnosed in first year of life; (iv) Inpatients diagnosed in the first year of life; (v) Patients undergoing either heart surgery or catheter-based therapeutic interventions in the first year of life, and (vi) Patients with no extra-cardiac defects or chromosomal anomalies.

Using the same methodology to identify CHD cases as Olsen et al. 15-18, we excluded
patients with CHD coded only as a secondary diagnosis. The reason for this exclusion is to avoid
detection bias. In our study this is highly relevant as twins tend to be premature and therefore
more frequently hospitalized for longer periods after birth, which could potentially lead to
diagnosis of minor CHD of no clinical importance such as patent foramen ovale/small ASD. We,
however, performed a sensitivity analysis to assess the importance of excluding secondary
diagnosis.

Results

In the period 1977-2001, a total of 41,525 twin individuals were born alive, and of those
individuals 584 (1.4%) had a CHD registered in the Danish National Patient Register, while the
corresponding numbers for singletons in the random 5% sample were 648/74,473 (0.87%)
(p<0.001), i.e. there was a 63% (95% CI: 45-82%) increase in the odds for CHD for twins.

As illustrated in Figure 1, the overall yearly number of live births in Denmark in the
study period varied between 50,000 and 70,000 corresponding to the 5% sample for which the
number of live births varied between 2500 and 3500 per year. In contrast, the yearly number of
newborn twin individuals more than doubled in Denmark in the study period with approximately
1000 liveborn twin individuals per year in the beginning of the period and more than 2500 at the
end of the period, mainly due to infertility treatment.27 This increase in twin births, combined
with better diagnostic methods in the study period, makes birth year a possible confounder.
However, taking sex and birth year into account in a logistic regression only changed the
estimate of the increased CHD odds in twins from 1.63 in the raw analyses to 1.59 (95% CI:
1.42-1.79).

Another potential confounder is maternal age which is positively associated with twin
birth, but there was no significant association between maternal age and CHD, and including maternal age in the multivariable analyses only changed the risk estimate marginally from 59% to 62%, and if fathers’ age was also included in the analysis, the estimate was unchanged (62%). Excluding patients with extra-cardiac defects or chromosomal anomalies from the analyses also yield similar results with an odds ratio of 1.62 (95% CI: 1.42-1.84).

Table 1 with the raw data and Table 2 with the sex and birth year adjusted estimates show that the increased risk for CHD was very consistent over the CHD subtypes used previously by Olsen et al.15-18: In all the 10 subtypes with more than 10 twin cases, twins showed higher prevalence than singletons (the subgroups “malformation of great veins” and “common arterial trunk” are not included in the table as the total number of these in the studies was 8 cases). The highest risk estimate was as expected for PDA (3.9 (2.6-5.8)), whereas the second highest risk estimate was for coarctation of aorta which occurred 3.1 times (95% CI: 1.5-6.4) more often in twins than in singletons.

The increased risk for CHD in twins was consistent in all the sensitivity analyses as illustrated in Figure 2. Table 3 shows that the excess CHD risk in twins was found to be statistically significant in all the raw and the adjusted analyses with an effect size in the range of 1.3-2.0 in all the sub-analyses, restricting the cases to: (i) Inpatients; (ii) Patients undergoing either heart surgery or catheter-based therapeutic interventions (iii); Diagnosed in first year of life; (iv) Inpatients diagnosed in the first year of life, and (v) Patients undergoing either heart surgery or catheter-based therapeutic interventions in the first year of life.

Table 3 shows that the twin-singleton differences are still statistically significant after exclusion of preterm PDAs. This is a conservative estimate as half the twins are preterm (median gestational length was 37 weeks versus 40 weeks in singletons) and by this exclusion is not able
to have PDA. To further investigate the influence of prematurity, we performed an analysis excluding all preterm twins and singletons (<37 weeks) and still found a significantly increased CHD risk for twins (21% (95% CI: 3-42%).

**Table 2** shows the results stratified for zygosity. The increased risk for CHD overall and for the specific subtypes were found across zygosity group, most pronounced for the about one quarter of the twins for whom zygosity was unknown. We were not able to demonstrate a higher risk for CHD among monozygotic twins compared to dizygotic twins 1.06 (95% CI: 0.77-1.45). The overall CHD occurrence was significantly increased in dizygotic twins compared to singletons: Odds 1.3 (95% CI: 1.13-1.51%) and as can be seen in table 4, also in the subgroup of opposite- sexed twins: 1.22 (95% CI:1.01-1.46), for whom there is no room for misclassification of zygosity or chorionicity (all are dizygotic and dichorionic).

The analyses were repeated including also patients with CHD only as secondary diagnosis adding 1207 CHD cases (2.91%) among twins and 901 (1.21%) among singletons compared to 584 twins and 648 singletons with a primary diagnosis. Including those with CHD as secondary diagnosis in the analyses further increased the odds for CHD among twins to an odds ratio of 2.46 (95% CI: 2.25-2.70) and confirmed that our approach was conservative also in this respect.

From the mid 1980’s, a more extensive use of echocardiography began in Denmark, which together with the longer hospitalizations of twins due to prematurity could lead to earlier and more frequent diagnosis of VSDs that would otherwise have closed spontaneously. However, by dividing the time period into two periods (1977-1984 and 1985-2001), we found an increased risk for twins compared to singletons in both time periods. Actually, the point estimate was higher for the 1977-1984 period compared to the 1985-2001 period: 1.98 (1.10-3.58) vs 1.25
(0.96-1.63). Restricting the analyses to cases with a VSD diagnosis after 1 year of age only changed the risk estimate marginally from 1.35 (1.06-1.72) to 1.29 (0.93-1.79). To further investigate whether the VSDs in twins were clinically less severe than in singletons, we calculated the proportion of operated VSDs and found that 34/118 = 29% of the twin VSDs were surgically treated versus 39/149 = 26% of the singleton VSDs, which also do not suggest less severe VSDs in twins.

Discussion

Based on an unprecedentedly large sample size, the present study showed an increased risk for CHD in live born twins compared to singletons. The increased risk was a remarkably consistent finding over CHD subtypes, zygosity, patient groups (in- and outpatients, operated and non-operated, first year of life and later) and calendar time. Generally, the increased risk was in the range of 30-100%. The results were generally very robust in a series of sensitivity analyses excluding e.g. preterm PDAs, all premature births, CHDs as secondary diagnosis, and VSD diagnosed before one year of age, suggesting that the increased risk for CHD among twins is not driven solely by prematurity, PDAs or detection bias.

The previous literature suggests that mono-chorionicity is the key risk factor for CHD in twins and TTTS-affected monochorionic in particular.¹⁻³ However, our study showed an increased CHD risk in both monozygotic twins (35% (95% CI: 1-81%)) and dizygotic twins (30% (95% CI: 13-51%)) compared to singletons. The highest risk was found for the about one quarter of the twins for whom zygosity was unknown. It is a consistent finding in twin studies that those with unknown zygosity have on average poorer health outcome and socioeconomic position than those with known zygosity.²⁸ The reason is that in order for zygosity assignment to
be made, the parents to the twins are required to participate by answering the similarity questions or by providing permission to collect biological material for DNA testing. If the parents have poorer health and/or socioeconomic position and/or one or two severely sick twins, the likelihood that they participate in zygosity assignment is small. Furthermore, early life mortality among the most severely affected CHD twins also hampers zygosity assignment.

In theory, the increased risk for dizygotic twins could be due to CHD monozygotic twins being misclassified as dizygotic twins as the frequency of zygosity misclassification based on similarity questions is about 5% in same-sexed twins. However, the finding that the subgroup of opposite-sexed twins for whom there is no misclassification of zygosity (all dizygotic) also had an increased CHD risk, rules out this hypothesis. Because dizygotic twins are all dichorionic, we conclude that CHD is more common in twins than in singletons, and the increased occurrence is not restricted to monochorionic twins as the previous literature suggests. Therefore, intrauterine surveillance and a postnatal, comprehensive cardiac assessment for both twins may be considered regardless of chorionicity and zygosity.

A systematic literature review by Bahtiyar et al. of the prevalence of CHD in monochorionic/diamniotic twin gestations based on 40 CHD affected fetuses concluded that CHD was significantly more prevalent in monochorionic/diamniotic twins in the presence of twin-to-twin transfusion syndrome (TTTS) which occurs in 10-30% of all monochorionic twin pregnancies. However, an increased CHD risk was also found in the non-TTTS monochorionic twins. Overall, ventricular septal defects were found to be the most frequent in this sample of monochorionic/diamniotic twin gestations, whereas pulmonary stenosis and atrial septal defects were significantly more prevalent in pregnancies complicated by TTTS. Bahtiyar et al. suggest based on their review that conditions that lead to abnormal placentation may also
increase the risk for CHD, and that fetal echocardiography may be considered for all monochorionic/diamniotic twin pregnancies.

Van den Boom et al.\textsuperscript{8} reported on four monochorionic twin pairs affected by TTTS and found a tendency for an increased risk of coarctation in the donor twin and pulmonary stenosis in the recipient. Similarly, these authors recommend intrauterine surveillance and a postnatal, comprehensive cardiac assessment for both twins, particularly in monochorionic twins complicated by TTTS.

In their systematic literature review, Bahtiyar et al.\textsuperscript{2} found ventricular septal defects to be the most frequent in the sample of monochorionic/diamniotic twin gestations, which is in accordance with our finding of an increased risk for ventricular septal defects in monozygotic twins (and twins overall). The most frequent CHD subtype in our sample (second to PDA as expected due to the high frequency of prematurity in twins) was coarctation of aorta. This is in line with the case series of four twin pairs by Van den Boom et al.,\textsuperscript{8} in whom the donor twin had coartation of the aorta or a hypoplastic arch.

Among the strengths of the present study are the large sample size, the zygosity information, the ability to analyze subtypes of CHD although with limited statistical power for the rarest CHD, and the subgroups of patients over time allowing for sensitivity analyses. Among the weaknesses of the study are the facts that only live born children are included and that no direct information on chorionicity is available, only the indirect information that all dizygotic twins are dichorionic, while the monozygotic group is a mixture of mono- and dichorionic twins. Another weakness is that we did not have any information on whether the twins were conceived using assisted reproductive technologies (ART). Twins are more often than singletons conceived using ART, as there is some evidence that ART children have increased occurrence of birth
defects, including CHD, although it is not clear whether this is due to ART per se or it is secondary to problems inherent in the infertile couples.\textsuperscript{5,32,33}

Large routine registers will always have a certain degree of misclassification, but it is unlikely that misclassification of CHD diagnoses should be different between singletons and twins and, furthermore, any random misclassification will tend to bias the difference toward nil, i.e. we may be underestimating the twin-singleton difference. Lynge et al.\textsuperscript{26} point out that although the Danish National Patient Registry overall is a sound data source, the validity of single variables need to be assessed. The present study benefitted from the previous validation work that has shown that the Danish National Patient Registry is a reliable and valid source for identifying CHDs.\textsuperscript{10-13} In accordance, the register has been used extensively in clinical epidemiological studies of CHD.\textsuperscript{14-18}

It is interesting that the increased risk of CHD among recent cohorts of Danish twins is not accompanied by an increased mortality risk after childhood.\textsuperscript{34} Even for cardiovascular mortality, we found no differences between twins and singletons in a very powerful study of 19,986 Danish twin individuals from the birth cohorts 1870-1930 followed from 1952 through 1993\textsuperscript{35}, a finding recently confirmed in a large Swedish study.\textsuperscript{36,37} There are several possible explanations for this apparent discrepancy between higher CHD occurrence in twins and no increased cardiovascular mortality. Firstly, increased diagnostic activity in twins due to prematurity and morbidity in the co-twin could contribute to higher detection rate in twins than singletons although this is unlikely for the severe forms of CHD. Secondly, our study includes only recent cohorts whereas the mortality studies are mainly based on cohorts born before WWII, in whom many of the severe CHD cases may have died in childhood. Thirdly, although a 30-100% increase in CHD occurrence is substantial on a relative scale, it is still only in the
absolute order of less than 2% of the twins, and the cardiovascular mortality affects around 50% in these older cohorts, so an effect of CHD on late life mortality may be difficult to detect.

The present study documents an increased CHD risk in twins compared to singleton, and the finding that it is not restricted to monozygotic twins (which comprises all monochorionic twins and virtually all cases of TTTS syndrome) suggests that TTTS is not the only factor responsible for the increased risk. This emphasizes that the search for etiological factors for an increased risk for CHD in twins should be expanded beyond abnormal placentation.

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Conflict of Interest Disclosures: None.

References:


Table 1. Congenital heart defect in twins and a 5% random sample of singletons born in Denmark in the period 1977-2001.

<table>
<thead>
<tr>
<th>Congenital heart defect (all)</th>
<th>Singletons (N = 74,473)</th>
<th>All Twins (N = 41,525)</th>
<th>Monozygotic (N = 5,538)</th>
<th>Dizygotic (N = 24,435)</th>
<th>Unknown zygosity (N = 11,552)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Congenital heart defect (all)</td>
<td>648 (0.87)</td>
<td>584 (1.41)</td>
<td>63 (1.14)</td>
<td>282 (1.15)</td>
<td>239 (2.07)</td>
</tr>
<tr>
<td>Common arterial trunk</td>
<td>2 (0.003)</td>
<td>2 (0.005)</td>
<td>1 (0.02)</td>
<td>1 (0.004)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Transposition of great vessels</td>
<td>16 (0.02)</td>
<td>16 (0.04)</td>
<td>2 (0.04)</td>
<td>7 (0.03)</td>
<td>7 (0.06)</td>
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<tr>
<td>Tetralogy of Fallot</td>
<td>21 (0.03)</td>
<td>15 (0.04)</td>
<td>0 (0.00)</td>
<td>6 (0.02)</td>
<td>9 (0.08)</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>149 (0.20)</td>
<td>118 (0.28)</td>
<td>18 (0.33)</td>
<td>53 (0.22)</td>
<td>47 (0.41)</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>80 (0.11)</td>
<td>91 (0.22)</td>
<td>6 (0.11)</td>
<td>49 (0.20)</td>
<td>36 (0.31)</td>
</tr>
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<td>Atrioventricular septal defect</td>
<td>13 (0.02)</td>
<td>18 (0.04)</td>
<td>1 (0.02)</td>
<td>11 (0.05)</td>
<td>6 (0.05)</td>
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<tr>
<td>Anomalies of heart valve</td>
<td>46 (0.06)</td>
<td>54 (0.13)</td>
<td>9 (0.16)</td>
<td>24 (0.10)</td>
<td>21 (0.18)</td>
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<tr>
<td>Other anomalies of heart</td>
<td>252 (0.34)</td>
<td>141 (0.34)</td>
<td>18 (0.33)</td>
<td>63 (0.26)</td>
<td>60 (0.52)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>36 (0.05)</td>
<td>83 (0.20)</td>
<td>6 (0.11)</td>
<td>47 (0.19)</td>
<td>30 (0.26)</td>
</tr>
<tr>
<td>Coarctation of aorta</td>
<td>12 (0.02)</td>
<td>23 (0.06)</td>
<td>1 (0.02)</td>
<td>10 (0.04)</td>
<td>12 (0.10)</td>
</tr>
<tr>
<td>Other malformations of great arteries</td>
<td>18 (0.02)</td>
<td>22 (0.05)</td>
<td>1 (0.02)</td>
<td>10 (0.04)</td>
<td>11 (0.10)</td>
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<tr>
<td>Malformation of great veins</td>
<td>3 (0.04)</td>
<td>1 (0.002)</td>
<td>0 (0.00)</td>
<td>1 (0.004)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>
Table 2. Odds Ratio for congenital heart defect in twins compared to a 5% random sample of singletons born in Denmark in the period 1977-2001

<table>
<thead>
<tr>
<th>Condition</th>
<th>All Twins</th>
<th>Monozygotc</th>
<th>Dizygotic</th>
<th>Unknown zygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (95% CI)</td>
<td>Adjusted* (95% CI)</td>
<td>Raw (95% CI)</td>
<td>Adjusted* (95% CI)</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>1.63 (1.45-1.82)</td>
<td>1.59 (1.42-1.79)</td>
<td>1.31 (0.99-1.70)</td>
<td>1.35 (1.01-1.81)</td>
</tr>
<tr>
<td>Transposition of great vessels</td>
<td>1.79 (0.84-3.83)</td>
<td>1.85 (0.93-3.68)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tetralogy of Fallot</td>
<td>1.28 (0.61-2.60)</td>
<td>1.23 (0.62-2.47)</td>
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<td>-</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>1.42 (1.11-1.82)</td>
<td>1.35 (1.06-1.72)</td>
<td>1.63 (0.94-2.66)</td>
<td>1.73 (1.03-2.89)</td>
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<td>2.04 (1.50-2.79)</td>
<td>1.81 (1.33-2.45)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Atrioventricular septal defect</td>
<td>2.48 (1.15-5.51)</td>
<td>2.51 (1.25-5.05)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anomalies of heart valve</td>
<td>2.11 (1.40-3.19)</td>
<td>1.94 (1.29-2.91)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other anomalies of heart</td>
<td>1.00 (0.81-1.24)</td>
<td>1.09 (0.88-1.36)</td>
<td>0.96 (0.56-1.55)</td>
<td>0.97 (0.57-1.63)</td>
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<tr>
<td>Patent ductus arteriosus</td>
<td>4.14 (2.77-6.31)</td>
<td>3.91 (2.62-5.83)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coarctation of aorta</td>
<td>3.44 (1.64-7.58)</td>
<td>3.09 (1.49-6.39)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other malformations of great arteries</td>
<td>2.19 (1.12-4.34)</td>
<td>2.03 (1.05-3.92)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Logistic regression adjusted for sex and birth cohort
Only subgroups with more than 10 CHD cases in both twins and singletons are reported separately
Table 3. Odds ratio for congenital heart defect in twins compared to a 5% random sample of singletons born in Denmark in the period 1977-2001

<table>
<thead>
<tr>
<th></th>
<th>Singletons (N = 74,473)</th>
<th>All Twins (N = 41,525)</th>
<th>Raw OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHD incl. preterm ductus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In- and outpatients N (%)</td>
<td>648 (0.87)</td>
<td>584 (1.41)</td>
<td>1.63 (1.45-1.82)</td>
<td>1.59 (1.42-1.79)</td>
</tr>
<tr>
<td>Inpatients N (%)</td>
<td>498 (0.67)</td>
<td>404 (0.97)</td>
<td>1.46 (1.28-1.67)</td>
<td>1.51 (1.31-1.73)</td>
</tr>
<tr>
<td>Surgical procedure N (%)</td>
<td>223 (0.30)</td>
<td>201 (0.48)</td>
<td>1.62 (1.33-1.97)</td>
<td>1.55 (1.27-1.89)</td>
</tr>
<tr>
<td>In- and outpatients in first year of life N (%)</td>
<td>399 (0.54)</td>
<td>436 (1.05)</td>
<td>1.97 (1.71-2.26)</td>
<td>1.85 (1.60-2.13)</td>
</tr>
<tr>
<td>Inpatients in first year of life N (%)</td>
<td>318 (0.43)</td>
<td>306 (0.74)</td>
<td>1.73 (1.47-2.03)</td>
<td>1.74 (1.48-2.06)</td>
</tr>
<tr>
<td>Surgical procedure in first year of life N (%)</td>
<td>100 (0.13)</td>
<td>112 (0.27)</td>
<td>2.01 (1.52-2.66)</td>
<td>1.78 (1.34-2.36)</td>
</tr>
<tr>
<td><strong>CHD excl. preterm ductus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In- and outpatients</td>
<td>635 (0.85)</td>
<td>516 (1.24)</td>
<td>1.46 (1.30-1.65)</td>
<td>1.44 (1.27-1.62)</td>
</tr>
<tr>
<td>Inpatients N (%)</td>
<td>486 (0.65)</td>
<td>343 (0.83)</td>
<td>1.27 (1.10-1.46)</td>
<td>1.32 (1.14-1.52)</td>
</tr>
<tr>
<td>Surgical procedure N (%)</td>
<td>214 (0.29)</td>
<td>163 (0.39)</td>
<td>1.37 (1.11-1.69)</td>
<td>1.30 (1.06-1.60)</td>
</tr>
<tr>
<td>In- and outpatients in first year of life N (%)</td>
<td>389 (0.52)</td>
<td>380 (0.92)</td>
<td>1.76 (1.52-2.03)</td>
<td>1.65 (1.42-1.91)</td>
</tr>
<tr>
<td>Inpatients in first year of life N (%)</td>
<td>310 (0.42)</td>
<td>265 (0.64)</td>
<td>1.54 (1.30-1.82)</td>
<td>1.55 (1.30-1.84)</td>
</tr>
<tr>
<td>Surgical procedure in first year of life N (%)</td>
<td>96 (0.13)</td>
<td>98 (0.24)</td>
<td>1.83 (1.37-2.45)</td>
<td>1.60 (1.20-2.14)</td>
</tr>
</tbody>
</table>

*Logistic regression adjusted for sex and birth cohort
Table 4. Odds Ratio for congenital heart defect in dizygotic twins compared to a 5% random sample of singletons born in Denmark in the period 1977-2001

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dizygotic - opposite sex (N = 14,021)</th>
<th>Dizygotic - same sex (N = 10,414)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (N) (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>152 (1.08)</td>
<td><strong>1.25 (1.04-1.50)</strong></td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>27 (0.19)</td>
<td>0.96 (0.61-1.46)</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>34 (0.24)</td>
<td><strong>2.26 (1.47-3.42)</strong></td>
</tr>
<tr>
<td>Anomalies of heart valve</td>
<td>10 (0.07)</td>
<td>1.15 (0.52-2.32)</td>
</tr>
<tr>
<td>Other anomalies of heart</td>
<td>34 (0.24)</td>
<td>0.72 (0.48-1.03)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>23 (0.16)</td>
<td><strong>3.40 (1.92-5.89)</strong></td>
</tr>
</tbody>
</table>

* Logistic regression adjusted for sex and birth cohort
Only subgroups with more than 10 CHD cases in both twins and singletons are reported separately
Figure Legends:

**Figure 1.** The number of live births for twins and a 5% random sample of singletons born in Denmark in the period 1977-2002.

**Figure 2.** The prevalence of congenital heart defect in twins and singletons born in Denmark in the period 1977-2002.
Figure 1