SUPPLEMENTAL MATERIAL

Risk of Congenital Heart Defects
in Offspring of Affected Mothers and Fathers

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Since 1968, the Danish Civil Registration System has registered demographics, vital status, and kinship information on all persons alive in 1968 or persons born thereafter in Denmark, using the unique personal identification number assigned to each Danish resident. The personal identification number permits accurate linkage of individual-level information from Denmark’s nationwide registers, including the National Patient Register (inpatient diagnoses from 1977, outpatient diagnoses from 1995), the Medical Birth Register, the Causes of Death Register, and the Danish Cytogenetic Central Register (postnatal diagnoses since 1968, prenatal diagnoses since 1978).

Study cohort

Our study cohort included all persons born in Denmark between 1977 and 2011 with at least one identifiable parent, excluding all individuals, regardless of congenital heart defect status, with chromosomal aberrations (Down syndrome, trisomy 13, trisomy 18, Turner syndrome, other sex chromosome aneuploidy, deletions, and other chromosome abnormalities), as ascertained from the Danish Cytogenetic Central Register. Within this design, parents with congenital heart defects were considered as the exposure, and congenital heart defects in their offspring (cohort members) were measured as the outcome.

Identification of parents with congenital heart defects (exposure)

For each cohort member, we identified parents using the Civil Registration System. We then identified any congenital heart defect in parents using the National Patient Register and the Causes of Death Register, applying the same definitions used for congenital heart defects in
cohort members (the offspring) themselves (see Identification and classification of congenital heart defects).

**Identification and classification of congenital heart defects (outcome)**

We identified persons with congenital heart defects (International Classification of Diseases, 8th version [ICD-8] codes 746-747, 10th version [ICD-10] codes Q20-Q26) using the National Patient Register and the Causes of Death Register. The registration of a congenital heart defect diagnosis in the National Patient Register could occur at any point over the life course since each affected individual may have a history of successive ICD-8 codes (before 1994) or ICD-10 codes (1994 or thereafter) for cardiac defects. We prioritized ICD-10 codes before ICD-8 codes, and codes registered at university hospital before codes registered in local/regional hospitals. Lesions were classified and prioritized using the algorithm we developed previously for use with Danish and Norwegian register data. For each individual, we grouped registered heart defect codes into embryologically-related defect phenotypes, as initially suggested by Botto and colleagues. The algorithm classified cardiac phenotypes hierarchically, as follows: Heterotaxia with or without any other heart defect; Conotruncal defect [truncus arteriosus, transposition of the great arteries, Tetralogy of Fallot, double outlet right ventricle, other (aortic arterial atresia/stenosis/hypoplasia)]; Atrioventricular septum defect (AVSD) with or without anomalous pulmonary venous return; Anomalous pulmonary venous return (APVR) including total or partial APVR; Left ventricular outflow tract obstruction (LVOTO) [coarctation of the aorta, valvular aortic stenosis with or without ventricular septal defect (VSD), other (hypoplastic left heart syndrome, mitral stenosis/atresia)]; Right ventricular outflow tract obstruction (RVOTO) [valvular pulmonary stenosis, other (hypoplastic right heart syndrome, tricuspid atresia, Ebstein anomaly,
pulmonary valve atresia, and pulmonary artery atresia)]; Septal defects [VSD only, atrial septal defect (ASD) only and recorded from postnatal age 6 weeks, VSD and ASD only]; Other complex defect [single ventricle, congenital corrected transposition of the great arteries]; Valve defect [isolated valve defects not included in LVOTO or RVOTO]; Persistent ductus arteriosus (PDA) at postnatal age >6 weeks or with surgical correction in live births with gestational age ≥37 weeks, or with gestational age <37 weeks (preterm PDA); Other specified heart defect; and Unspecified heart defect.

Isolated physiological heart conditions (persistent foramen ovale (PFO), pulmonary valve stenosis) present at birth but not mentioned >6 weeks postnatal age, and isolated ASDs registered at birth but neither re-registered >6 weeks after birth nor surgically corrected (likely PFOs) were not considered. Parent and offspring were considered to have similar congenital heart defects (referred to hereafter as the “same cardiac phenotype”) if both parent and offspring defects were classified by the algorithm in the same phenotypic group (e.g., conotruncal defects) and were therefore presumed to result from problems at the same stage of embryological development.

**Statistical analysis**

In the analyses of recurrence of congenital heart defects, we restricted the study cohort to those who were born as singletons. Familial clustering of congenital heart defects was evaluated using recurrence risk ratios that compared the risk of congenital heart defects in the offspring of parents with congenital heart defects with the risk in the offspring of unaffected parents, estimated using log-linear binominal regression. All risk ratios were adjusted for offspring year of birth (5-year intervals) and congenital heart defects in other
family members (first-, second-, and third-degree relatives with CHD (Yes/No) for each degree of relationship)). The latter was determined by identifying siblings, half-siblings, and other second- and third-degree family members using the Danish Family Relations Database, a family database constructed using information from the Danish Civil Registration System, and then determining which relatives, if any, had congenital heart defect diagnoses registered in the National Patient Register and the Causes of Death Register.

To compare maternal and paternal effects, we estimated the ratio of the mother-child and father-child recurrence risk ratios using logistic regression with adjustment for offspring year of birth (5-year intervals). In the analyses of same cardiac phenotypes, some of the exposed case numbers was small, therefore, the inherent approximation may in these situations be less precise. All inference was based on Wald statistics.

Sensitivity Analysis
In a sensitivity analysis, we assigned parents whose congenital heart defect was diagnosed within a year of the cohort member’s birth as “unaffected”, since pregnant women with a previously unregistered diagnosis would be more likely to be registered with congenital heart defect than men with an unregistered congenital heart defect.

Sex-specific birth rates in women and men with or without congenital heart defects
We estimated sex-specific birth rates among all women and men (singleton and multiples) born from 1970 onwards, and calculated birth rate ratios to compare birth rates in persons with congenital heart defects with birth rates in persons without congenital heart defects. We followed women and men from birth until the first of the following events: birth of a first
child, death, or end of 2011. We estimated these birth rate ratios by sex and parent congenital heart defect status (included in the model as an interaction term) using log-linear Poisson regression, with parental age (1-year intervals) and calendar period (5-year intervals) as adjustment variables.

**Mother-offspring recurrence risk ratios after adjustment for cardiovascular medication use**

Because women with a congenital heart defect are more likely than women without a congenital heart defect to use medication to control hypertension, heart failure, and arrhythmias, we investigated the impact of adjusting the overall RR for mother-child recurrence of congenital heart defects for maternal use of these cardiovascular medications. The main study data set did not contain information on cardiovascular medication use. However, we performed identical maternal-offspring analyses in an external dataset based on the same registers with information on 1,135,186 pregnancies in the period 1995 to 2012, that also included information from the National Prescription Register on maternal cardiovascular medication use (Anatomical Therapeutic Chemical (ATC) codes C02 [antihypertensives], C03 [diuretics], C07 [beta blocking agents], C08 [calcium channel blockers], C09 [agents acting on the renin-angiotensin system]) from 6 months before conception to 20 weeks after conception.

All statistical analyses were carried out using SAS (SAS Institute, Inc., Cary, North Carolina).
Table I. Number of births with congenital heart defects* with the corresponding prevalence of congenital heart defects, by year of birth, among 2,377,504 births in Denmark, 1977-2011.

| Heart Defect Phenotype | 1977-1981 | 1982-1986 | 1987-1991 | 1992-1996 | 1997-2001 | 2002-2006 | 2007-2011 | Total |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------|
| Any heart defect       | 2,440     | 2,257     | 2,841     | 4,044     | 3,682     | 3,498     | 2,834     | 21,596|
|                       | 75.7      | 75.4      | 83.0      | 107.0     | 102.3     | 101.0     | 85.9      | 90.8  |
| Heterotaxia           | 29        | 27        | 31        | 57        | 59        | 40        | 27        | 270   |
|                       | 0.90      | 0.90      | 0.91      | 1.51      | 1.64      | 1.16      | 0.82      | 1.14  |
| Conotruncal           | 233       | 214       | 270       | 342       | 345       | 342       | 244       | 1,990 |
|                       | 7.23      | 7.15      | 7.89      | 9.05      | 9.59      | 9.88      | 7.39      | 8.37  |
| AVSD                  | 73        | 54        | 98        | 87        | 95        | 102       | 105       | 614   |
|                       | 2.27      | 1.80      | 2.86      | 2.30      | 2.64      | 2.95      | 3.18      | 2.58  |
| APVR                  | 7         | 6         | 19        | 29        | 31        | 39        | 39        | 170   |
|                       | 0.22      | 0.20      | 0.56      | 0.77      | 0.86      | 1.13      | 1.18      | 0.72  |
| LVOTO                 | 256       | 210       | 245       | 327       | 302       | 285       | 203       | 1,828 |
|                       | 7.95      | 7.02      | 7.16      | 8.66      | 8.39      | 8.23      | 6.15      | 7.69  |
| RVOTO                 | 73        | 112       | 163       | 251       | 260       | 233       | 160       | 1,252 |
|                       | 2.27      | 3.74      | 4.76      | 6.64      | 7.23      | 6.73      | 4.85      | 5.27  |
| Septal                | 794       | 694       | 916       | 1,534     | 1,593     | 1,626     | 1,350     | 8,507 |
|                       | 24.65     | 23.18     | 26.77     | 40.61     | 44.27     | 46.96     | 40.90     | 35.78 |
| ASD                   | 273       | 215       | 277       | 403       | 445       | 562       | 487       | 2,662 |
|                       | 8.47      | 7.18      | 8.10      | 10.67     | 12.37     | 16.23     | 14.76     | 11.20 |
| VSD                   | 481       | 456       | 601       | 1,000     | 1,009     | 951       | 785       | 5,283 |
|                       | 14.93     | 15.23     | 17.57     | 26.47     | 28.04     | 27.47     | 23.79     | 22.22 |
| ASD & VSD             | 24        | 19        | 29        | 98        | 109       | 94        | 66        | 439   |
|                       | 0.75      | 0.63      | 0.85      | 2.59      | 3.03      | 2.72      | 2.00      | 1.85  |
| Unspecified           | 16        | <5        | 9         | 33        | 30        | 19        | 12        | 123   |
|                       | 0.50      | 0.13      | 0.26      | 0.87      | 0.83      | 0.55      | 0.36      | 0.52  |
| Complex               | <5        | 5         | 5         | 11        | 8         | 7         | 7         | 44    |
|                       | 0.03      | 0.17      | 0.15      | 0.29      | 0.22      | 0.20      | 0.21      | 0.19  |
| Valve defects         | 123       | 97        | 162       | 183       | 129       | 131       | 39        | 864   |
|                       | 3.82      | 3.24      | 4.74      | 4.84      | 3.58      | 3.78      | 1.18      | 3.63  |
| Other specified       | 196       | 190       | 206       | 226       | 90        | 86        | 87        | 1,081 |
|                       | 6.08      | 6.35      | 6.02      | 5.98      | 2.50      | 2.48      | 2.64      | 4.55  |
| Unspecified           | 507       | 478       | 513       | 697       | 472       | 275       | 183       | 3,125 |
|                       | 15.74     | 15.97     | 14.99     | 18.45     | 13.12     | 7.94      | 5.54      | 13.14 |
| PDA                   | 148       | 170       | 213       | 300       | 298       | 332       | 390       | 1,851 |
|                       | 4.59      | 5.68      | 6.23      | 7.94      | 8.28      | 9.59      | 11.82     | 7.79  |

*Births with chromosomal aberrations were not included.

Atrioventricular septum defect (AVSD); anomalous pulmonary venous return (APVR), left ventricular outflow tract obstruction (LVOTO); right ventricular outflow tract obstruction (RVOTO); atrial septum defect (ASD); ventricular septum defect (VSD); persistent ductus arteriosus (PDA)
Supplemental Table II. Risk ratios (RR) for congenital heart defect different from the parent, in offspring of mothers and fathers by parental cardiac phenotype, among 2,341,061 singleton births* in Denmark, 1977-2011.

| Cardiac phenotype in the parent | Offspring of mothers with congenital heart defect | Offspring of fathers with congenital heart defect | Ratio of mother-offspring and father-offspring RRs |
|--------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                                | Total no. of offspring | No. with any other heart defects | Any other heart defects per 10,000 | RR† | 95% CI | Total no. of offspring | No. with any other heart defects | Any other heart defects per 10,000 | RR† | 95% CI | Ratio of RRs‡ | 95% CI | p-value |
|--------------------------------|------------------------|---------------------------------|--------------------------------|------|--------|------------------------|---------------------------------|--------------------------------|------|--------|-------------|--------|---------|
| Heterotaxia                   | 77                     | <5                              | 260                           | 2.91 | 0.74-11.4 | 103                    | <5                              | 291                           | 3.44 | 1.13-10.5 | 0.83       | 0.14-5.12 | 0.84    |
| Conotruncal defects           | 365                    | 9                               | 247                           | 3.00 | 1.57-5.72 | 351                    | <5                              | 85                            | 1.05 | 0.34-3.23 | 2.90       | 0.78-10.8 | 0.11    |
| AVSD                          | 353                    | 14                              | 397                           | 4.55 | 2.73-7.61 | 154                    | 6                               | 390                           | 4.55 | 2.08-9.96 | 1.00       | 0.38-2.66 | 0.99    |
| APVR                          | 51                     | <5                              | 196                           | 2.16 | 0.31-15.0 | 29                     | 0                               | 0                             |      |         |             |        |         |
| LVOTO                         | 598                    | 14                              | 234                           | 2.84 | 1.69-4.76 | 811                    | 21                              | 259                           | 3.15 | 2.07-4.81 | 0.90       | 0.45-1.78 | 0.75    |
| RVOTO                         | 305                    | 9                               | 295                           | 3.47 | 1.82-6.60 | 173                    | 8                               | 462                           | 5.50 | 2.80-10.8 | 0.62       | 0.23-1.64 | 0.33    |
| Septal defects§               | 3,286                  | 63                              | 192                           | 3.60 | 2.82-4.69 | 2,073                  | 29                              | 140                           | 2.65 | 1.85-3.81 | 1.37       | 0.88-2.13 | 0.17    |
| ASD                           | 1,550                  | 33                              | 213                           | 2.75 | 1.95-3.89 | 1,078                  | 23                              | 213                           | 2.76 | 1.83-4.18 | 1.00       | 1.71-0.99 | 0.99    |
| VSD                           | 1,516                  | 29                              | 191                           | 2.87 | 1.99-4.15 | 904                    | 13                              | 144                           | 2.18 | 1.26-3.77 | 1.32       | 0.68-2.55 | 0.41    |
| Complex                       | <5                     | 0                               | 0                             |      |         | <5                     | <5                              | 2,500                         | 27.2 | 5.0-148   |             |        |         |
| Valve defects                 | 628                    | 30                              | 478                           | 5.53 | 3.90-7.84 | 635                    | 17                              | 268                           | 3.11 | 1.95-4.98 | 1.82       | 0.99-3.34 | 0.05    |
| Other specified               | 514                    | 25                              | 486                           | 5.60 | 3.82-8.21 | 320                    | 5                               | 156                           | 1.79 | 0.75-4.26 | 3.34       | 1.26-8.88 | 0.02    |
| Unspecified                   | 1,131                  | 49                              | 433                           | 5.61 | 4.26-7.37 | 652                    | 9                               | 138                           | 1.78 | 0.92-3.38 | 3.31       | 1.61-6.79 | 0.001   |
| PDA||                          | 481                    | 13                              | 270                           | 3.30 | 1.93-5.65 | 125                    | 0                               | 0                             |      |         |             |        |         |

Abbreviations: Atrioventricular septum defect (AVSD); anomalous pulmonary venous return (APVR); left ventricular outflow tract obstruction (LVOTO); right ventricular outflow tract obstruction (RVOTO); atrial septum defect (ASD); ventricular septum defect (VSD); persistent ductus arteriosus (PDA). *Twins and chromosomal defects excluded. Among 2,341,061 births, there were altogether 20,868 births with any congenital heart defect (birth prevalence 89 per 10,000). †Risk ratio (RR) with 95% confidence interval (CI) adjusted for year of birth (5 years intervals) and heart defects in other family members (first-, second-, and third-degree relatives) in log-linear binomial regression analyses. ‡Approximated RR estimated as OR in logistic regression analyses adjusted for year of birth (5-year intervals). §Including ASD+VSD, unspecified septal defects. ¶Persistent ductus arteriosus (PDA)
Supplemental Figure I
**Supplemental Figure I.** Forest plot with the ratio between the female birth rate ratio and the male birth rate ratio among individuals with congenital heart defects relative to the general population*.

*The ratio compared birth rate ratios for women affected with congenital heart defects (relative to the female birth rate in the population) and birth rate ratios for men affected with congenital heart defects (relative to the male birth rate in the population) among 1,635,823 women and 1,692,165 men born in Denmark, 1970 onwards.

Atrioventricular septum defect (AVSD); anomalous pulmonary venous return (APVR), left ventricular outflow tract obstruction (LVOTO); right ventricular outflow tract obstruction (RVOTO); atrial septum defect (ASD); ventricular septum defect (VSD); persistent ductus arteriosus (PDA).
Figure Legend

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