# CONGENITAL ANOMALIES: STATE OF THE ART AND THE NEW PARADIGMS FOR A PRECISION PUBLIC HEALTH APPROACH

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# CONGENITAL ANOMALIES: STATE OF THE ART AND THE NEW PARADIGMS FOR A PRECISION PUBLIC HEALTH APPROACH

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# Editorial: Congenital anomalies: State of the art and the new paradigms for a precision public health approach

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**congenital anomalies (CAs), epidemiology, precision medicine, public health, outcomes**

Editorial on the Research Topic
Congenital anomalies: State of the art and the new paradigms for a precision public health approach

Congenital anomalies (CA) can be broadly defined as structural, anomalies, chromosomal anomalies, and syndromes occurring in the early stages of embryonic development that may be diagnosed prenatally or postnatally. They are a major cause of perinatal mortality, childhood morbidity and childhood disability. The scholarly activities concerned with CA comprise a complex set of scientific questions and public health challenges. A rich mix of disciplines as diverse as genetics, embryology, pathology, physiology, public health, epidemiology, biostatistics but also social sciences including sociology, psychology, medical ethics and anthropology can and do address important questions concerning fetuses and newborns with CA.

Our topic includes 16 manuscripts that address a limited but coherent set of topics as may be appropriate for a Research Topic on CA coordinated by our colleagues at Frontiers in Pediatrics. Our focus was, broadly speaking, public health issues with important contributions from population-based epidemiological studies, systematic reviews and meta-analyses. We also included interesting clinical case studies and crucial considerations on data quality in studies of CA. We were also fortunate to include a qualitative study on the important topic of parents' perspectives on newborns with CA. Finally, we included articles that included questions related to CA but were not exclusive to these anomalies. Even if they went beyond the field of CA, they also shared important overlapping features with the literature on CA.

Precision public health is a relatively recently used term with, forgive the pun, at times imprecise definitions and connotations. Our goal here was to be precise about the research questions tackled and the methods required to address them and their limitations. In general, as proposed by Khoury et al. precision public health refers to a population approach (i.e., the population is the unit of intervention rather than the individual) for providing the right "treatment" at the right time to the right people.

Of course, from a population perspective, for conditions such as CA, "treatment" must be expanded to include the triple focus on causes (e.g., prevention), occurrence, and outcomes (including the evaluation of care and the societal impact on families and health care services).

An important and often quite difficult issue to address in studies of CA is the inherent, multi-dimensional heterogeneity of these anomalies. Indeed, CA represent a diverse set of pathologies in terms of their empirical attributes and epidemiology—wide variations in their total and live birth prevalence, known environmental risk factors and teratogenic effects of medications, surveillance methods, coding schemes, anatomy, pathophysiology, fetal vs. newborn physiology (notably in the case of congenital heart defects), embryology and organogenesis. There are multiple and myriad combinations of the modalities for medical and surgical management of the anomalies, substantial differences in the short- and longer-term health care and educational requirements of the different anomalies and varying degrees of progress in the prenatal screening, pre and post-natal management and the attendant trends in the short and long-term health and developmental outcomes of CA.

Even within groups of CA, perhaps most notably, for congenital heart defects, there are great heterogeneities with respect to the aspects noted above for CA in general. The article concerned with the trends in the prognosis of hypoplastic left heart syndrome (Best et al.) represents one "extreme" case of CHD as compared to the fortunately far more frequent minor ventricular septal defects that almost always resolve spontaneously with no health repercussions for the newborns with this particular type of CHD. There are also "intermediate" but certainly major subtypes of CHD, most notably Tetralogy of Fallot, Transposition of Great Arteries and Coarctation of Aorta. For the CHD in general and the subtypes mentioned above, substantial progress has been made for their prenatal diagnosis, postnatal screening (by pulse-oximetry in particular) and in their medical and surgical management. As a result, temporal trends clearly show improved outcomes for these anomalies even if long-term neuro-developmental and other health and education outcomes remain important questions that are being actively studied as part of long-term, prospective, population-based cohort studies.

With the exponentially increasing rate of accumulation of literature in general, and specifically in the field of CA, systematic reviews and meta-analyses, when appropriate, can make important contributions to the literature as represented by the systematic review of studies on the relation between CA and growth restriction at birth (Ghanchi et al.) in this Research Topic. Such reviews also have the added advantage of pointing out limitations in the currently available literature and can suggest fruitful venues for future research.

An important area of research reflects sociodemographic differences in relation to access to health care services such as prenatal screening (Santoro et al.). Other important areas of public health and epidemiological research relate to "Prevention" of CAs such as introducing folic acid fortification in Europe. The effect of not introducing mandatory fortification on the prevalence of neural tube defects is highlighted in the European study by Morris et al.

Ebert et al. demonstrated the value of health care data to determine the prevalence of rare diseases such as bladder exstrophy and epispadias. However, for research related to congenital heart defects, population-based registry data were obtained to assess the risk of growth restriction at birth (Ghanchi et al.) and to demonstrate the improved prognosis in children with hypoplastic left heart (Best et al.).

In conclusion, overall, CA represent relatively frequent events (3% of all births) with important, broadly speaking public health issues. As evident in this sample of studies, there is great heterogeneity within CA and most CA are rare diseases however (prevalence <1 per 2,500 births) and hence their study represents important conceptual and empirical challenges. In this context, it is particularly important for studies to be as precise as possible about the scientific question(s) at hand, appropriate study design and analysis and the limits of each study. It is also essential that we understand whether and to what extent results a given study on a specific type or subgroup of CA may or may not be applicable to other CA. Notwithstanding these caveats, the literature on CA continues to grow and diversify in ways that move the field forward in both its scientific and public health endeavors.

## Author contributions

A first draft of the Editorial was prepared by BK and LB. All authors contributed to revision of the text and have read and approved the final version of the Editorial.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

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# Maternal and Neonatal Complications After Natural vs. Hormone Replacement Therapy Cycle Regimen for Frozen Single Blastocyst Transfer

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**Objective:** To evaluate the maternal and neonatal complications after frozen-thawed blastocyst transfer cycles utilizing different endometrial preparation regimens.

**Design:** This is a retrospective cohort study and a secondary analysis of a multicenter, randomized, controlled trial comparing live birth rate after fresh vs. frozen single blastocyst transfer (Frefro-blastocyst).

**Setting:** Reproductive medicine centers.

**Patient(s):** A total of 800 women with regular menstrual cycles undergoing their first cycle of *in-vitro* fertilization after frozen-thawed single blastocyst transfer.

**Intervention(s):** Endometrium preparation was performed with a natural cycle regimen or hormone replacement therapy (HRT) cycle regimen, at the discretion of local investigators. All pregnancies were followed up until delivery.

**Main Outcome Measure(s):** Maternal and neonatal complications.

**Result(s):** 513 infertile patients who underwent natural cycles regimen and 287 who underwent HRT cycles regimen were analyzed. The incidences of maternal and neonatal complications were comparable between the natural cycle and HRT cycle regimen. Regarding the risk of gestational diabetes, gestational hypertension, pre-eclampsia, preterm delivery, small for gestational age and large for gestational age, the HRT cycle was still not a significant risk factor after adjusting for potential confounders. The natural cycle regimen yielded an insignificant higher total live birth rate [59.45 vs. 50.17%, $P = 0.001$, adjusted odds ratio (AOR) 1.366, 95% confidence interval (CI) 0.975–1.913], clinical pregnancy rate (68.23 vs. 58.89%, $P = 0.008$, AOR 1.406, 95% CI 0.992–1.991)

and ongoing pregnancy rate (62.18 vs. 52.61%, $P = 0.008$, AOR 1.387, 95% CI 0.988–1.948) than did the HRT cycle regimen. However, compared to natural cycles, HRT cycles were associated with a significantly higher risk of biochemical miscarriage (6.86 vs. 18.18%, $P < 0.001$, AOR 0.328, 95% CI, 0.176–0.611).

**Conclusion(s):** The incidence of maternal and neonatal complications in natural cycle and HRT cycle regimens after frozen single blastocyst transfer were comparable. Frozen-thawed single blastocyst transfer in a natural cycle was associated with lower biomedical miscarriage than the use of the HRT cycle.

**Clinical Trial Registration Number:** Frefro-blastocyst was registered at Chinese Clinical Trial Registry, ChiCTR-IOR-14005405.

**Keywords: biomedical miscarriage, frozen embryo transfer, natural cycle, hormone replacement therapy cycle, single embryo transfer**

## INTRODUCTION

The goal of assisted reproductive technology (ART) is to achieve a live, healthy, full-term singleton baby. Single embryo transfer (SET) is the most efficient approach to reduce the risk of multiple pregnancies (1). With the development in cryopreservation technology, especially the introduction of vitrification, the application of frozen embryo transfer (FET) has become increasingly popular (2). Studies have shown that frozen embryo transfer by avoiding a supra-physiological environment for embryo implantation after ovarian stimulation increased pregnancy rate (3, 4). By prolonging the *in vitro* culture, only embryos of excellent quality with the highest potential for implantation will reach blastocyst stage selection. Blastocyst transfer has resulted in significantly higher rates of pregnancy and delivery than cleavage-stage embryo transfer (5).

Our previous trial suggested that frozen single blastocyst transfer was better for achieving singleton live birth than fresh single blastocyst transfer in women with good prognosis (6). However, frozen single blastocyst transfer was associated with a higher risk of pre-eclampsia (6). Further, higher rates of hypertensive disorders and placenta accreta in pregnancy were noted after frozen embryo transfer (7). FET singletons may be at an increased risk of being born large for gestation age (LGA) (7, 8). Whether these differences are due to the protocol used in frozen cycles remains unknown.

A crucial aspect of FET cycles is the preparation of the endometrium to receive the transferred embryo. The most commonly used endometrial preparation methods for FETs include hormone replacement therapy (HRT) cycle and natural cycle (NC). Many retrospective analyses were performed of frozen blastocyst transfers, and different conclusions were drawn about the implantation, pregnancy, and live birth rate (9–12). The results of meta-analysis and systematic review show that no sufficient evidence has been found to support the superiority of one method over the other (2, 13). While few studies have investigated the neonatal and maternal outcomes comparing these strategies, compared to natural cycle protocols, higher rates of hypertensive disorders in pregnancy (14, 15), postpartum hemorrhage (14, 16), post term birth (14), macrosomia (14), and cesarean section (15) were detected in the HRT cycle. It seemed that the HRT cycles of FET have a negative effect on obstetric outcome.

In this study, a secondary analysis was performed to see whether the method of endometrial preparation for transfer of vitrified blastocysts was associated with obstetric complications outcomes.

## MATERIALS AND METHODS

### Study Population

The Frefro-blastocyst study was conducted during August 1, 2016, to June 3, 2017 in 21 academic fertility centers in China. The original study was approved by the ethics committees of all study sites and was registered at Chinese Clinical Trial Registry (number ChiCTR-IOR-14005405) (6). The design and main outcomes of this trial have been previously reported in detail (17). Briefly, 1,650 women with regular menstrual cycles undergoing their first cycle of *in vitro* fertilization were enrolled, and eligible women were randomly assigned to either fresh or frozen single blastocyst transfer. For those assigned to frozen blastocyst transfer, all blastocysts were cryopreserved, and a delayed frozen-thawed single blastocyst transfer was done. Considering the condition of switched groups in the randomized clinical trial (RCT), 724 patients adhered to frozen protocol and 87 women assigned to the fresh embryo transfer group who actually had single frozen blastocyst transfer were included. However, only natural cycle and HRT cycle regimens for endometrium preparation of FET were analyzed.

### Study Procedure

After ovarian stimulation with a gonadotropin-releasing hormone antagonist protocol, women who obtain four or more than four embryos on day three of the embryo culture were randomized into two groups: fresh single blastocyst transfer group and frozen single blastocyst transfer group. The selection of the single blastocyst gave priority to the score of the inner cell mass, and the score of trophectoderm was also considered. The rank of blastocyst grade from top to good was AA, AB,

BA, BB, AC, and BC. If two or more blastocysts were of equal grade, their early scores at cleavage stage were referred for the selection of the single blastocyst. Supernumerary embryos were frozen on day 5 or 6 according to embryo development. On day 5 or 6 of embryo culture, women who were assigned to the frozen blastocyst transfer group had their blastocysts vitrified and a deferred frozen blastocyst transfer. The selection of the frozen blastocyst for thawing was based on the blastocyst grade before freezing.

## Endometrial Preparation Protocols

At least 4 weeks after blastocysts vitrified, endometrium preparation was performed with a natural cycle regimen or HRT cycle regimen. At the discretion of local investigators, this assignment was not randomized. For the natural cycle regimen, when it was detected that the dominant follicle and the endometrial thickness reached 7 mm or more, local investigators decided whether to use human chorionic gonadotropin (hCG) for ovulation triggering according to their clinical routine. Ovulation was determined by ultrasound monitoring and a single frozen-thawed blastocyst, either day 5 or day 6, was transferred on the 5th day after ovulation. Details of embryo transfer procedure were shown in a previous study (6). Luteal phase support was started after the ovulation day with oral dydrogesterone 10 mg three times daily. If the patient was pregnant, luteal phase support was continued until 10 weeks' gestation. For the HRT cycle regimen, the endometrium prepared with oral estradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 4–8 mg daily was started on days 1–3 of the menstrual cycle. Vaginal progesterone gel (Crinone, Merck Serono) 90 mg/day and oral dydrogesterone 10 mg twice daily were added when the endometrial thickness reached 7 mm or more. A single frozen-thawed blastocyst was transferred on the 5th day after progesterone initiation. Estradiol valerate at the dose for endometrium preparation was continued until the day of the serum hCG test, 2 weeks after embryo transfer. If pregnancy was achieved, estradiol valerate stopped gradually at 8–9 weeks of gestation; vaginal progesterone gel and oral dydrogesterone was continued until 10 weeks of gestation.

## Outcome Measures

The study outcomes included clinical pregnancy, ongoing pregnancy, pregnancy loss, live birth, ectopic pregnancy, perinatal complications and neonatal complication gestational, e.g., gestational diabetes mellitus (GDM), pregnancy-induced hypertension (PIH), pre-eclampsia, gestational age at birth, preterm birth, small for gestational age (SGA), large for gestational age (LGA), and neonatal hospitalization for more than 3 days. Preterm birth was defined as delivery before 37 complete weeks of gestation. The outcomes SGA and LGA were, respectively, defined according to birth weight for the 10th and 90th percentile of gender-specific birth weight reference for Chinese (18).

## Statistical Analysis

Continuous data were expressed as mean (SD) and compared by the Student's *t*-test. Categorical data were represented as frequency and percentage; differences in these variables between the treatment groups were assessed by $\chi^2$ analysis, with Fisher's exact test for expected frequencies less than five. A $P < 0.05$ was considered statistically significant. Crude odds ratios (OR) with 95% confidence intervals (CI) for each primary outcome were calculated. Logistic regression analysis was used to compare adjusted odds ratios (AOR) and 95% CI for the effect of natural cycle vs. HRT cycle regimen on obstetric and perinatal complications. Goodness of fit for logistic regression models were calculated by the Hosmer-Lemeshow (HL) test, if the $P > 0.05$, the model passed the test. All analyses were performed with the use of SPSS software 21.0.

# RESULTS

Seven hundred and twenty-four women who were assigned to the frozen embryo transfer group actually adhered to the protocol, while 87 women assigned to the fresh embryo transfer group actually had a frozen embryo transfer. Of these, 11 women were excluded due to a stimulation cycle regimen for endometrial preparation of FET. 513 infertile patients who underwent natural cycles regimen and 287 who underwent HRT cycles regimen were analyzed (**Figure 1**).

Baseline characteristics are listed in **Table 1**. Maternal age, body mass index, duration of infertility, proportion of previous conception, the proportion of IVF indications, antral follicle count (AFC), baseline follicle stimulating hormone (FSH), baseline luteinizing hormone (LH), oestradiol ($E_2$), days of ovarian stimulation, endometrial thickness on day of trigger, number of oocytes, fertilization method and incidence of ovarian hyper-stimulation syndrome (OHSS) were comparable between the natural and HRT cycles. However, in the natural cycles compared to the HRT cycles, total testosterone level, total gonadotropin dose, $E_2$ and progesterone level on the day of trigger were significantly lower ($P < 0.001$, $P = 0.02$, $P = 0.006$, respectively), the number of 2PN embryos, cleavage embryos and day-3 suitable for transferred embryos were less ($P = 0.015$, 0.032, 0.015, respectively), and the endometrial thickness was thinner ($P < 0.001$).

The pregnancy outcomes after FET are shown in **Table 2**. The natural cycle regimen yielded a higher total live birth rate including singleton and twin than the HRT cycle regimen (59.45 vs. 50.17%, $P = 0.011$). Singleton live birth per woman was also higher in natural cycles (57.50 vs. 48.78%, $P = 0.017$), whereas twin live birth per woman was similar between the two protocols ($P = 0.565$). Clinical pregnancy (68.23 vs. 58.89%, $P = 0.008$) and ongoing pregnancy (62.18 vs. 52.61%, $P = 0.008$) were significantly higher in the natural cycles. Furthermore, the rate of biochemical miscarriage was significantly lower in the natural cycles (6.86 vs. 18.18%, $P < 0.001$). No significant differences were observed in terms of birth weight, gestational weeks, biochemical pregnancy and clinical pregnancy loss rate.

Maternal and neonatal complications stratified by FET method are shown in **Table 3**. There were no significant differences in the incidence of ectopic pregnancy, GDM, PIH, pre-eclampsia, preterm rupture of membrane,

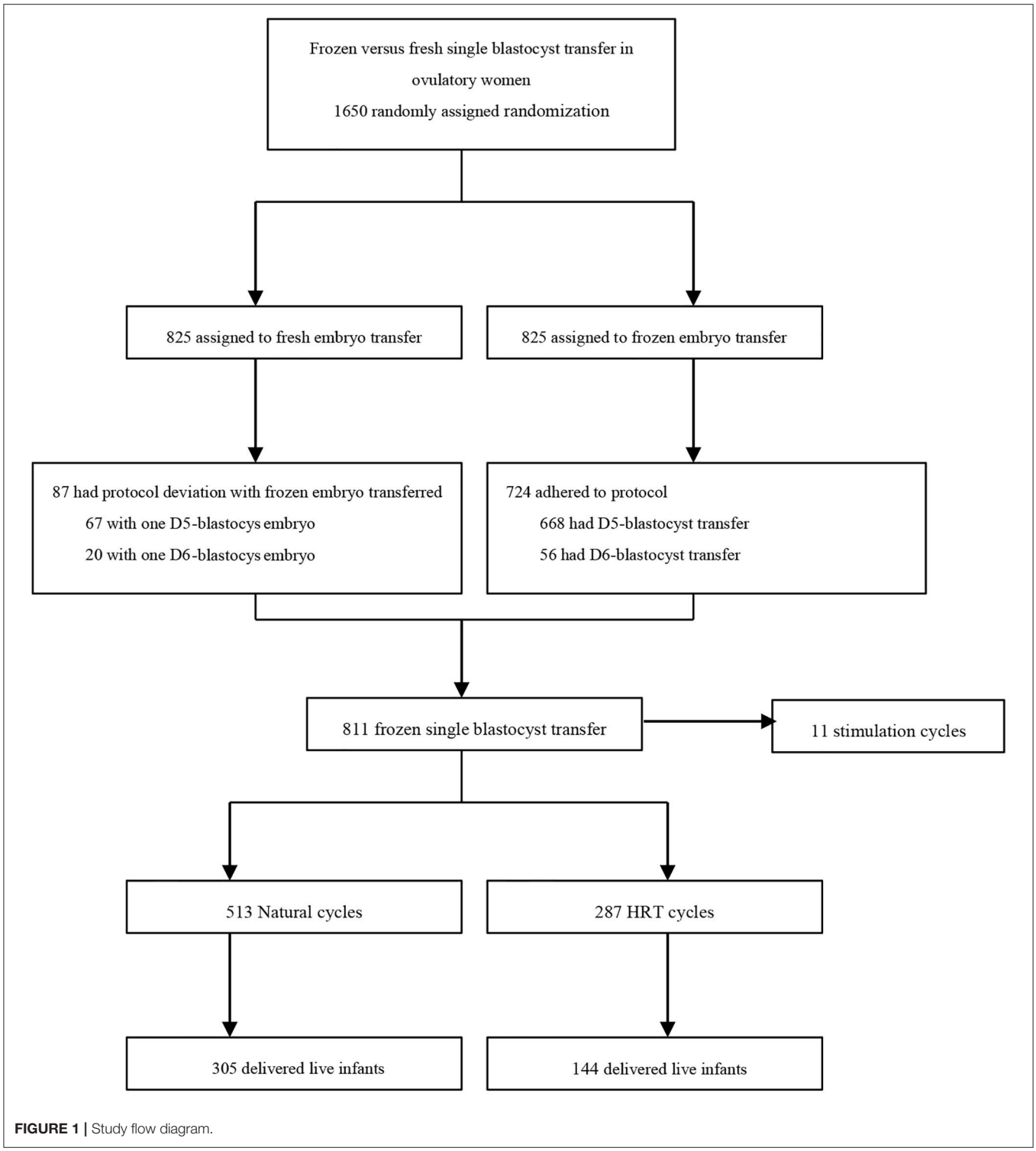


**FIGURE 1 |** Study flow diagram.

preterm delivery and post-partum hemorrhage between the two groups. In addition, no significant between-group difference was found in the risks of small for SGA, LGA, neonatal hospitalization for more than 3 days, neonatal infection among live newborn, nor birth defect.

Regarding the risk of gestational diabetes, gestational hypertension, pre-eclampsia, preterm delivery, SGA and LGA, the HRT cycle was still not a significant risk factor after adjusting the results for potential confounders. A multiple logistic regression analysis adjusted for potential confounders showed that natural cycle FET (vs. HRT cycle-FET) was a

**TABLE 1 |** Baseline characteristics.

| Characteristics | Natural cycles ($n$ = 513) | HRT cycles ($n$ = 287) | $P$-value |
|---|---|---|---|
| Age (years) | 28.74 ± 2.89 | 29.08 ± 3.01 | 0.117 |
| Body-mass index (kg/m) | 22.32 ± 3.07 | 22.52 ± 3.30 | 0.379 |
| Duration of infertility (years) | 3.26 ± 2.11 | 3.29 ± 2.35 | 0.846 |
| Previous conception *n* (%) | 219/513 (42.69) | 133/287 (46.34) | 0.318 |
| Indications for IVF | | | 0.364 |
| Tubal factor | 276/513 (53.80) | 156/287 (54.36) | |
| Male factor | 113/513 (22.03) | 64/287 (22.30) | |
| Unexplained infertility | 20/513 (3.90) | 18/287 (6.27) | |
| Combined factors | 104/513 (20.27) | 49/287 (17.07) | |
| Antral follicle count in both ovaries | 16.46 ± 5.46 | 16.87 ± 5.99 | 0.339 |
| Baseline sex hormone | | | |
| FSH (IU/L) | 6.34 ± 1.50 | 6.32 ± 1.46 | 0.812 |
| LH (IU/L) | 4.78 ± 1.93 | 4.83 ± 2.21 | 0.717 |
| Oestradiol (pg/mL) | 37.07 ± 14.60 | 37.94 ± 15.64 | 0.430 |
| Total testosterone (ng/dL) | 28.46 ± 14.03 | 35.96 ± 17.82 | <0.001 |
| Fresh cycle variables | | | |
| Days of ovarian stimulation | 9.28 ± 1.40 | 9.49 ± 1.68 | 0.056 |
| Total gonadotropin dose (IU) | 1559.32 ± 479.52 | 1634.78 ± 500.26 | 0.036 |
| Oestradial level on HCG trigger day (pmol/L) | 3431.55 ± 1810.08 | 3758.37 ± 1801.59 | 0.020 |
| Progesterone level on HCG trigger day (nmol/L) | 1.14 ± 0.52 | 1.26 ± 0.58 | 0.006 |
| Endometrial thickness on HCG trigger day (cm) | 1.07 ± 0.20 | 1.06 ± 0.20 | 0.512 |
| No. of oocytes retrieved | 14.29 ± 5.74 | 14.69 ± 5.68 | 0.339 |
| No. of 2PN embryos | 9.43 ± 3.97 | 10.17 ± 4.53 | 0.015 |
| No. of cleavage embryos | 9.29 ± 3.96 | 9.94 ± 4.36 | 0.032 |
| Fertilization method | | | 0.333 |
| IVF *n* (%) | 359/513 (69.98) | 210/287 (73.17) | |
| ICSI *n* (%) | 127/513 (24.76) | 68/287 (23.69) | |
| IVF +ICSI *n* (%) | 27/513 (5.26) | 9/287 (3.14) | |
| No. of day-3 embryos suitable for transfer | 6.96 ± 2.99 | 7.59 ± 3.69 | 0.015 |
| OHSS *n* (%) | 3/513 (0.58) | 4/287 (1.39) | 0.258 |
| FET cycle variables | | | |
| Endometrial thickness before transfer (cm) | 1.00 ± 0.17 | 0.94 ± 0.16 | <0.001 |
| Timing of embryo transfer—no./total no. (%) | | | 0.853 |
| Day 5 | 465/513 (90.64) | 259/287 (90.24) | |
| Day 6 | 48/513 (9.36) | 28/287 (9.76) | |
| No. of remaining frozen blastocysts | 4.19 ± 2.90 | 4.27 ± 3.00 | 0.720 |

**TABLE 2 |** Livebirth, birthweight, pregnancy, and pregnancy loss.

| | Natural cycles ($n$ = 513) | HRT cycles ($n$ = 287) | $P$-value |
|---|---|---|---|
| Total live birth per woman | 305/513 (59.45) | 144/287 (50.17) | 0.011 |
| Singleton live birth per woman | 295/513 (57.50) | 140/287 (48.78) | 0.017 |
| Twin live birth per woman | 10/513 (1.95) | 4/287 (1.39) | 0.565 |
| Birth weight | | | |
| Singleton (g) | 3395 ± 476.14 | 3417.39 ± 480.02 | 0.654 |
| Monozygotic Twin (g) | 2424.50 ± 501.58 | 2365.00 ± 525.03 | 0.782 |
| Gestational weeks (week) | 38.9 ± 1.66 | 39.04 ± 1.74 | 0.464 |
| Pregnancy | | | |
| Biochemical pregnancy—no. (%) | 379/513 (73.88) | 209/287 (72.82) | 0.745 |
| Clinical pregnancy—no. (%) | 350/513 (68.23) | 169/287 (58.89) | 0.008 |
| Ongoing pregnancy—no. (%) | 319/513 (62.18) | 151/287 (52.61) | 0.008 |
| Pregnancy loss-no./total no. (%) | | | |
| Total pregnancy loss among biochemical pregnancies | 67/379 (17.68) | 62/209 (29.67) | 0.001 |
| Biochemical miscarriage | 26/379 (6.86) | 38/209 (18.18) | <0.001 |
| Clinical pregnancy loss | 41/350 (11.71) | 24/169 (14.20) | 0.422 |
| First trimester pregnancy loss | 31/350 (8.86) | 18/169 (10.65) | 0.513 |
| Second trimester pregnancy loss | 10/350 (2.86) | 6/169 (3.55) | 0.669 |

higher risk factor for live birth rate (AOR 1.411; 95% CI 1.011–1.970), clinical pregnancy rate (AOR 1.444, 95% CI 1.022–2.041), ongoing pregnancy rate (AOR 1.443, 95% CI 1.031–2.020) and a significant lower risk factor for biochemical miscarriage (AOR 0.323; 95% CI 0.174–0.599) in Model 1, which did not pass the HL test (**Table 4**). When adding age and FSH to adjust in Model 2, only the rate of biochemical miscarriage (AOR 0.328; 95% CI 0.176–0.611) was still significantly lower in natural cycles than in HRT cycles (**Table 4**).

## DISCUSSION

In ovulatory women with a good prognosis, we found that the incidence of maternal and neonatal complications was similar in natural cycle and HRT cycle regimens after frozen single blastocyst transfer. HRT FET was associated with increased risks of biochemical miscarriage in comparison to the natural FET. However, the natural cycle regimen did not result in a higher rate of live birth, clinical pregnancy or ongoing pregnancy than in the HRT cycle regimen after adjusting for potential confounders.

HRT cycle is also called hormone replacement cycle (HRC), programmed cycle (PC) or artificial cycle (AC) in different literatures (10, 19–21). For anovulatory women, exogenous hormone preparation is often preferred. In ovulatory women, artificial endometrial preparation may benefit from minimal monitoring and ease of scheduling transfers. However, the universal application of HRT cycles may have potential disadvantages including an increased cost, inconvenience and the potential adverse events (e.g., increased thrombotic risk) associated with estrogen supplementation (22). Natural cycle of endometrium preparation can only be offered to patients

**TABLE 3 |** Maternal and neonatal complications.

| | Natural cycles ($n = 513$) | HRT cycles ($n = 287$) | *P*-value |
|---|---|---|---|
| Maternal complications | | | |
| Ectopic pregnancy | 3/350 (0.79) | 2/169 (0.96) | 0.663 |
| Gestational diabetes | 40/350 (11.43) | 19/169 (11.24) | 0.950 |
| Gestational hypertension | 8/350 (2.29) | 5/169 (2.96) | 0.765 |
| Pre-eclampsia | 9/350 (2.57) | 6/169 (3.55) | 0.580 |
| Placenta previa | 6/350 (1.71) | 0 | 0.184 |
| Preterm rupture of membrane | 36/350 (10.29) | 18/169 (10.65) | 0.898 |
| Preterm delivery | 22/350 (6.29) | 10/169 (5.92) | 0.870 |
| Post-partum hemorrhage | 3/308 (0.97) | 5/144 (3.47) | 0.117 |
| Neonatal complications | | | |
| Small for gestational age | 16/310 (5.16) | 10/144 (6.94) | 0.447 |
| Large for gestational age | 58/310 (18.71) | 27/144 (18.75) | 0.992 |
| Neonatal hospitalization >3 days | 39/303 (12.87) | 13/142 (9.15) | 0.255 |
| Neonatal jaundice among live newborns | 55/303 (18.15) | 26/142 (18.31) | 0.968 |
| Neonatal infection among live newborns | 11/303 (3.63) | 4/142 (2.82) | 0.783 |
| Birth defect | 10/318 (3.14) | 5/148 (3.38) | 1.000 |

*Values are number (percentage).*

with an ovulatory cycle. Developing follicle and urine or serum luteinizing hormone levels are needed to be monitored. Detection of an LH surge, thawing and transfer can be planned accordingly. While ovulation is triggered by hCG administration, the dominate follicular formation could reduce cancellation rates, shortening the duration of monitoring and improving corpus luteum function. No statistically significant difference for live birth rate was noted between spontaneous and induced ovulation (13, 23). Groenewoud et al. summarizes the differences between the two regimens, no optimal minimal monitoring regimen in NC–FET has been determined, routine use of luteal phase support in NC–FET has not been shown to be advantageous but to increase treatment burden. Furthermore, the costs of both protocols were comparable (2).

In this multicenter clinical trial, local investigators decide endometrium preparation protocols and whether to use hCG for ovulation triggering according to their clinical routine. The baseline characteristics of total gonadotropin dose, $E_2$ and progesterone level on day of trigger, the number of 2PN embryos, cleavage embryos and day-3 suitable in the natural cycles were different from the HRT cycles. Thus, it can be hypothesized that more follicular developed during IVF procedure in the HRT cycle regimen. Although hyperandrogenism have a higher risk of maternal and neonatal pregnancy complications (24, 25), total testosterone in both regimens was within the normal range, regardless of its effect on obstetric outcomes. In multivariate analysis, the effects of these differences were adjusted. We observed that endometrial thickness before transfer was significantly thicker in natural cycles compared with HRT cycles, and it is consistent with previous reports (9, 26). Natural cycles yielding an optimal endometrial thickness may compromise the window of implantation (WOI) (9).

Thus, optimal endometrial preparation and identification of the receptive window are important factors in the process of embryo implantation (27). Recent data from both simple histologic endometrial dating and transcriptomic microarray had shown that the window of implantation in hormonally prepared cycles for FET be delayed in about 25% women (28). Cluster analysis demonstrated that natural cycles were associated with a better endometrial receptivity transcriptome than HRT cycles (29).

As only a few high quality RCTs on the optimal preparation for FET are available (22), no substantial difference in live birth was obtained from a recent meta-analysis (30). Many studies did not conduct subgroup analysis of cleavage stage embryo and blastocyst transfer; no statistically significant difference for both clinical pregnancy and live birth were noted between natural cycles and HRT cycles (15, 19). In the patients with frozen cleavage embryo transfer, natural cycle and HRT cycle protocols yielded a comparable clinical pregnancy rate and live birth rate (10), while in frozen blastocyst transfer HRT cycles were associated with higher live birth rate (10, 31). Nonetheless, our study suggests that the live birth rate of frozen blastocyst transfer was better with the natural cycle preparation protocol before adjustment, supporting previous findings (9, 12). The increase of live birth rate in natural cycles may potentially be due to the lower risk of biochemical miscarriage. A prospective and observational cohort study by Cerrillo et al. (20) was performed and a higher miscarriage rate was observed in the hormone replacement cycles when compared to the natural cycles.

Many factors influence the live birth and clinical pregnancy rate following FET: female age and basal FSH level, the number of top-quality embryos and maximal endometrial thickness were the significant factors (9, 32). Therefore, when model 1 did not pass the Hosmer-Lemeshow test after adjusting the potential confounding factors, we added age and FSH to adjust. In NC-FET, the serum level of progesterone elevation present for 2 or more days before the LH surge (33), HCG trigger or not, and luteal support type may affect live birth rate (34). In HRT-FET, duration of both estrogen and progesterone supplementation and various routes of estrogen and progesterone administration are necessary to be considered (2). In our study, the disproportionate number of NC and HRT cycle was due to the discretion of local investigators, and this can lead to an inherent bias.

Numerous studies have tried to identify the optimal regimen of FET to obtain better pregnancy outcomes and to avoid or reduce adverse obstetric and neonatal outcomes. A large-scale registry-based study (7) on maternal and neonatal outcome of pregnancy after single embryo transfer showed that FET was associated with improved outcomes of preterm birth (PTB), low birth weight (LBW), and SGA compared with fresh transfer. Moreover, FET was associated with a statistically significant higher rate of placenta accreta and PIH. Compared to natural frozen cycles, higher rates of hypertensive disorders and preeclampsia in pregnancy were detected in HRT cycles (14,

**TABLE 4 |** Logistic regression for the effect of natural cycle vs. HRT cycle regimen on obstetric complication.

| | | | Model 1 | | Model 2 | |
|---|---|---|---|---|---|---|
| | **Unadjusted OR (95% CI)** | ***P*-value** | **Adjusted OR (95% CI)** | ***P* value** | **Adjusted OR (95% CI)** | ***P*-value** |
| Total live birth | 1.456 (1.089–1.948) | 0.011 | 1.411 (1.011–1.970) | 0.043 | 1.366 (0.975–1.913) | 0.070 |
| Clinical pregnancy | 1.499 (1.111–2.023) | 0.008 | 1.444 (1.022–2.041) | 0.037* | 1.406 (0.992–1.991) | 0.055 |
| Ongoing pregnancy | 1.481 (1.105–1.984) | 0.008 | 1.443 (1.031–2.020) | 0.033 | 1.387 (0.988–1.948) | 0.059 |
| Biochemical miscarriage | 0.331 (0.195–0.564) | <0.001 | 0.323 (0.174–0.599) | <0.001* | 0.328 (0.176–0.611) | <0.001 |
| Gestational diabetes | 1.019 (0.570–1.819) | 0.950 | 0.850 (0.428–1.688) | 0.642 | 0.886 (0.446–1.762) | 0.730 |
| Gestational hypertension | 0.767 (0.247–2.382) | 0.765 | 0.652 (0.186–2.289) | 0.505 | 0.662 (0.187–2.348) | 0.523 |
| Pre-eclampsia | 0.717 (0.251–2.048) | 0.580 | 0.732 (0.238–2.250) | 0.586 | 0.749 (0.244–2.300) | 0.613 |
| Preterm delivery | 1.066 (0.493–2.306) | 0.870 | 1.273 (0.518–3.130) | 0.599 | 1.274 (0.520–3.124) | 0.596 |
| Small for gestational age | 0.729 (0.322–1.649) | 0.447 | 0.712 (0.293–1.728) | 0.452 | 0.700 (0.285–1.715) | 0.435 |
| Large for gestational age | 0.997 (0.601–1.655) | 0.992 | 1.064 (0.602–1.881) | 0.832 | 1.084 (0.613–1.916) | 0.782 |

*Modal 1 is adjusted for total testosterone, total gonadotropin dose (IU), oestradial level on HCG trigger day (pmol/L), progesterone level on HCG trigger day (nmol/L), No. of 2PN embryos, No. of cleavage embryos, No. of day-3 embryos suitable for transfer and endometrial thickness before transfer (cm). Modal 2 is adjusted for age, FSH, total testosterone, total gonadotropin dose (IU), oestradial level on HCG trigger day (pmol/L), progesterone level on HCG trigger day (nmol/L), No. of 2PN embryos, No. of cleavage embryos, No. of day-3 embryos suitable for transfer and endometrial thickness before transfer (cm). HRT cycle regimen was set to be the referent group.*
**Value of Hosmer-Lemeshow Test goodness-of-fit <0.05.*

15, 21). Recent studies focused on the number of corpus luteum (CL) (14, 35), which can impact obstetric outcomes. Programmed frozen embryo transfer without ovulation is absent of CL, and natural cycle FET due to spontaneous ovulation has a CL. There is an increased rate of preeclampsia in programmed FET cycles where no CL is present. The study showed highly increased rates of preeclampsia in HRT FET cycles compared to other FET protocols (14). Vascular health in early pregnancy was altered in women with aberrant numbers of CL (0 or >3) and might represent insufficient cardiovascular adaptation contributing to an increased risk of preeclampsia (35).

The average birth weight from HRT was significantly greater compared with NC (36). A higher risk of post-term delivery and Cesarean section were noted in patients who conceived singletons after HRT cycle compared with those who conceived after NC-FET (21, 36). FET singletons had an increased risk of being born LGA (8), but the frequencies of macrosomia were comparable between patients after NC-FET and HRT-FET (21). Artificial hormone circumstances in frozen embryo transfer during the HRT cycle may change the placental basal plate and be causatively associated with the amount of bleeding in deliveries (16). The research indicated the association of the thinned decidual layer with pregnancies after the frozen-thawed embryo transfer (16).

Although our study had a relatively large sample, it was neither designed nor powered to show differences in obstetric and neonatal complications. The strengths of this study included the multicenter source of the data, which enhanced the generalizability of our results. In addition, data collection on maternal and neonatal outcomes was obtained in a consistent way and adjustment was made for several confounders. There are also limitations to this study. First, though it was a relatively large-sample study from a randomized clinical trial, the selection of endometrial preparation regimen for frozen single blastocyst is not random. Our results need to be confirmed by future randomized controlled trials. Second, there were differences in baseline characteristics between the two protocols, which reduced comparability. Although multivariate analysis was performed, the interaction between the confounders was not clear. Additionally, only young women with a good prognosis were included, thus we should also be cautious to extend the results to patients with older age, poor ovarian response, or repeatedly previous failed IVF cycles whose risks of obstetric complications may be higher than good prognosis patients. Finally, elements of the pragmatic design such as whether to use HCG trigger in natural cycles, duration of both estrogen and progesterone supplementation, or choice of luteal support could have affected results. Thus, whether endometrium preparation with hormone replacement acts as an unfavorable factor for the occurrence of biomedical miscarriage warrants further study. If so, what the mechanism is also needs to be investigated.

## CONCLUSION

In summary, in women with ovulation there were no significant differences of maternal and neonatal complications in single frozen blastocyst transfer after natural cycle or HRT cycle regimens. Natural cycle was associated with a lower biomedical miscarriage rate.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review

Board of Center for Reproductive Medicine of Shandong University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

YS was in charge of the trial conduct. JL, JZ, GH, JT, and YS designed the study. YS, JZ, GH, JT, ZW, and NX acquired the data. ZW, YP, and QJ performed the statistical analyses. YP, ZW, and QJ interpreted the data. JL wrote the first draft of the report with inputs from JZ, GH, and JT. YS provided comments, participated in additional discussions, and revised the paper. All authors approved the final version.

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# SMART Work Design: Accelerating the Diagnosis of Rare Diseases in the Western Australian Undiagnosed Diseases Program

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The accurate and efficient diagnosis of rare diseases, many of which include congenital anomalies, depends largely on the specialists who diagnose them – including their ability to work alongside specialists from other fields and to take full advantage of cutting-edge precision medicine technologies and precision public health approaches. However, highly specialized clinicians operating within a historically-siloed healthcare system is antithetical to the multi-disciplinary, collaborative, and creative approach that facilitates the diagnosis of rare diseases. The Western Australian Undiagnosed Diseases Program (UDP-WA) successfully re-designed the work of the involved clinicians to facilitate teamworking across silos. To understand the effectiveness of the Western Australian program, we draw on a SMART work design perspective (i.e., work that involves Stimulation, Mastery, Agency, Relations, and Tolerable demands). We propose that the redesign was successful in part because it improved crucial psychosocial work characteristics that are less prevalent in the broader work system, as identified in the SMART model. Based on the effectiveness of UDP-WA and its SMART design, we provide a framework that clinicians, healthcare managers, and policymakers can consider when they re-design work so that they can create SMART jobs within healthcare.

**Keywords: rare disease (RD), diagnos*, work redesign, teamwork, congenital abnomarlities, genomics**

Individually, rare diseases are uncommon, each affecting fewer than 200,000 people in the U.S. (Orphan Drug Act of 1983). However, collectively, the ∼10,000 different rare diseases will affect 1 in 12 people in their lifetime (1, 2)—which is an estimated 25–30 million Americans (3). Advances in genomic sequencing technology have facilitated a rapidly accumulating body of scientific knowledge on rare diseases, with new rare diseases appearing in the medical literature each week (4). Yet, despite these technological and scientific advancements, diagnosing which of the 10,000 rare diseases a given patient might have is an incredibly challenging task.

In addition to the vast amount of scientific literature on rare diseases, many rare diseases affect multiple physiological systems, thus requiring expertise from across multiple clinical specialties; this includes those with knowledge of congenital anomalies, as rare developmental defects during embryogenesis are the largest class of rare diseases (5). However, the structure of clinical knowledge within the health system is notoriously and increasingly siloed (6). Further, since ∼70% of rare diseases have a genetic basis (7), clinical genetic expertise and expertise about advancements in (gen)omic testing technology is often required in the diagnosis of rare disease patients. In fact, genomic testing has recently been shown to be significantly more cost effective than traditional diagnostic approaches (8, 9)—yet, many clinicians lack this (gen)omic literacy, or the ability to access this expertise for their patients.

Consequently, and characteristically, patients with rare diseases embark on what is known as a "diagnostic odyssey," whereby no single practitioner is looking at them "as a whole" (10). In Australia, ∼30% of patients wait more than 5 years to reach a diagnosis (and 30% wait between 5 to upwards of 20 years), 30% of patients see more than six specialists, and 50% of patients receive at least one incorrect diagnosis (10). This "chaos that coexists with being undiagnosed" (11) often causes suffering, frustration, and uncertainty for patients and their families. It is also expected to be financially costly for the health system; just a *subset* of the patients with rare diseases (in the total Western Australian population of 2.5 million people) incur an estimated annual cost of AUD395 million to the Western Australian Health System in hospital admissions alone. In Western Australia, this subset represented 2% of the total population, yet accounted for 10.5% of hospital *inpatient* expenditure (12). While it is challenging to quantify exactly, it is likely that the resource-intensive nature of diagnosis bears a similarly disproportionate cost. It certainly bears a professional and emotional cost to clinicians, who must struggle against the siloed health system, an overload of clinical and scientific information, and a limited amount of time and resources to find answers for their patients—often for years, and often unsuccessfully.

## THE UNDIAGNOSED DISEASES PROGRAM

In 2008, the United States' Undiagnosed Diseases Program (UDP-US) was conceived to address the unmet needs of individuals living with undiagnosed rare diseases. In the UDP, selected patients undergo a series of diagnostic tests and expert consultations, following which an interdisciplinary team of clinical and research experts examine the clinical and laboratory results for diagnostic clues. The cornerstone of the UDP concept is the use of *expert panel meetings*, in which the team of experts pool their expertise and discuss the patient's case in real time, generating creative ideas through a process of collaborative information processing and brainstorming. Thus, the UDP concept integrates previously siloed interdisciplinary expertise, and (gen)omic and other emerging precision medicine technologies, offering a disease-agnostic approach to diagnosis that capitalizes on the multi-disciplinary collaboration that is often necessary in the context of rare diseases. Starting as a single site at the National Institutes of Health (NIH), the NIH Common Fund initiative has since supported an expanded version of the program—the Undiagnosed Diseases Network—which now has 12 sites across the country.

Following the success of the UDP-US, and the identification of substantial patient need in Australia, the Western Australian UDP (UDP-WA) was implemented in March 2016 by a team of clinicians (from Genetic Services WA) and policymakers (from the Office of Population Health Genomics). While the UDP-WA functions with broadly the same structure as the UDP-US, it was implemented as a clinical service program within the WA public health system (13) to supplement existing genomic diagnostic work flows and multidisciplinary clinics (14)—whereas the UDP-US is supported by NIH research funding. After 1 year of operating, the percentage of families receiving a definitive diagnosis had nearly doubled, from 30% prior to the UDP-WA to 55% in the following year (14). The UDP-WA focuses on pediatric and pediatric-to-adult transition age groups; the majority of patients served by the UDP-WA have at least one congenital anomaly. For further detail on the UDP-WA please see Baynam et al. (13, 14).

Why is this approach so successful? We argue that a crucial part of the UDP's success is **good "work design**," which fosters the psychological processes—social, motivational, and cognitive—for those engaging in the diagnostic work (i.e., the clinicians) that are necessary for diagnosing rare and complex diseases. For the past 3 years, we have been conducting research to *empirically* substantiate this claim [e.g., (15, 16)]. Here, we aim to introduce the theoretical background and conceptual basis of our new perspective on this program, and the interdisciplinary collaboration that occurs therein, which is rooted in organizational psychology. By drawing on the broader psychology literature, as well as our published research on the UDP (and quotes from this research), we aim to convince readers that the UDP is one example of how such a work design-focused perspective can elicit a deeper understanding of how to solve complex challenges in genetics and broader healthcare work. As research on work design spans a complex history of literature in the field of organizational psychology [e.g., see (17)], we rely on the SMART work design framework (18) to synthesize and convey our perspective, proposing that: *SMART work design could help to facilitate effective multi-disciplinary collaboration in healthcare.*

## INTRODUCING: WORK DESIGN

*Work design* refers to "the content and organization of one's work tasks, activities, relationships, and responsibilities" (19). Issues in healthcare relating to team work, multiskilling, and the use of protocols in delivering patient care are all about work design because they are concerned with who does what, when, and how, in the work system. For example, poorly structured work in intensive care units was shown to predict high levels of workload which, in turn, was associated with lower perceived safety of patient care (20). While much of the technology and

**TABLE 1 |** The SMART work design framework (18).

| Dimensions | Sub-dimensions |
|---|---|
| Stimulating | • Adequate skill variety |
| | • Sufficient task variety |
| | • Problem solving demands |
| Mastery | • Role clarity |
| | • Feedback from others |
| | • Task identity |
| Agency | • Control over scheduling |
| | • Control over work methods |
| | • Decision making control |
| Relational | • Support from supervisor and peers |
| | • Sense of task significance |
| | • Perceived social worth |
| Tolerable demands | • Moderate time pressure and workload |
| | • Manageable emotional demands |
| | • Low role conflict |

scientific knowledge that supports the delivery of patient care is rapidly advancing; historically, much less deliberate, evidence-based effort has been devoted to advancing the design of the work systems and structures that support healthcare workers in using these technologies [cf. (21)].

A great deal of theory and evidence in the organizational psychology literature has identified several key psychosocial "job characteristics" that define well-designed work, and that have been shown to lead to superior outcomes for both employees and organizations (17). The "SMART" model of good work design (18) synthesizes this literature into a simple framework. In what follows, we outline the core components of the SMART work design model—Stimulation, Mastery, Agency, Relations and Tolerable demands (see **Table 1**)—and use the UDP-WA as an example to illustrate the importance of well-designed work in interdisciplinary healthcare settings. All quotes are from Hay et al. (15).

## SMART WORK DESIGN IN THE UDP-WA

The first important concept in the SMART model is that work should be "Stimulating"; jobs should involve a high degree of mental complexity and variety, including the opportunity to use a variety of skills (*skill variety*), engage in a wide variety of tasks (*task variety*), and to "think outside of the box" (*problem solving demands*). The UDP-WA cultivates a high level of stimulation, offering clinicians the opportunity to collaborate on cases that often fall outside their own specialty, thus also giving them the opportunity to develop new knowledge and skills related to other specialty areas (including genetics): *"… you just learn just by being there… I think it's such a good learning experience and so interesting."(Pediatrician).* Further, finding diagnoses for chronically-undiagnosed patients with a complex constellation of symptoms within the expert panel meetings requires engaging in a process of intensive problem solving, which many clinicians have described as highly stimulating: *"…like the art of medicine… I think everyone finds it really refreshing to actually do that, to have a real puzzle and to have to use all the medical knowledge that you've got to kind of piece it together." (Pediatrician).*

"Mastery" refers to the degree to which a job provides individuals with a sense of *role clarity* (e.g., about tasks and expectations), performance *feedback*, and *task identity* (i.e., completing a piece of work from beginning to end); and is an area in which the UDP-WA is particularly well-designed. Traditionally, clinicians who work with rare disease patients may receive very limited feedback; in some cases, they never receive updates about the outcomes of their clinical contribution, which generates a sense of chronic unease and uncertainty for the clinicians: "*Feedback is critical. Otherwise, it's like any human endeavor; if you're banging your head against the wall, it's always nice to know that there was some reason for it." (Clinical Biochemist).* Through the UDP-WA, clinicians receive frequent feedback about the outcomes of the diagnostic tests that were suggested by the team, and the impact of this on the progression toward a diagnosis for the patient.

Relatedly, members of the expert panel are able to follow the diagnostic process from beginning to end (i.e., they have high *task identity*). Prior to the implementation of the UDP-WA, this diagnostic process (and the ongoing patient care and management process more broadly) was marred by the tendency for rare disease patients to be treated as individual body systems: *"They'll see an endocrinologist for that problem, and they'll see a neurologist for that problem – but these two doctors don't talk to each other, so they don't get holistic treatment, because they're not seen as a whole person, they're seen as their individual body parts" (Policy officer).* This is not only inefficient for the patient; it is also de-motivating and frustrating for the clinicians. Through the introduction of interdisciplinary panel meetings in the UDP-WA, clinicians are presented with a holistic picture of the patient, and the opportunity to see, and contribute to, the whole diagnostic process. This gives the clinicians a sense of task identity. Finally, in our continuous shadowing of the meetings over a 2 year period, we have observed that that the Program Director repeatedly reinforces psychological safety and reminds participants of all types and career stages to voice their ideas (even perceived "silly" ones) as they could potentially lead to a diagnosis. At the same time, clinicians are recruited into the expert panels based on their existing specialty role (which enhances their role clarity in this regard).

The third theme in the SMART model is "Agency," which refers to the extent to which work provides employees with autonomy and decision-making opportunities —including, for example, deciding on one's work schedule (*work scheduling autonomy*) and which work methods to use (*work methods autonomy*). The UDP-WA offers the participating clinicians a substantial degree of autonomy with regards to their choice to attend the meeting (in person or via telehealth), as well as through offering them a variety of means through which they can choose to engage with the program (e.g., patient summaries, contributions via the digital health platform Patient Archive, e-mail comments). However, and perhaps more importantly, for clinicians who have an undiagnosed patient, the UDP-WA empowers them with the resources needed to diagnose

their patient—thus increasing their work methods autonomy. Typically, the structure of the broader system (i.e., the typical structure of clinical work whereby clinicians have limited time with their patients, and typically little access to multidisciplinary diagnostic teams) inhibits clinicians' ability to get their patients the in-depth attention that they require; the availability of the UDP-WA empowers clinicians to give their patient the care they need, outside of the traditional confines of the health system (which, in the case of rare diseases, impedes the diagnostic process). Thus, while clinical work typically involves a high degree of autonomy to begin with, the empowering nature of the UDP-WA serves to increase this further by creating more options for clinicians.

The fourth concept that is crucial in the design of the UDP-WA is the opportunity to form relationships. Such "Relational" work is defined as the extent to which individuals experience *social support* (from supervisors and colleagues), *task significance* (i.e., a sense of purpose in relation to the lives of others and society more broadly), and *social worth* (i.e., the sense that their work is appreciated) in their role. Prior to the implementation of the UDP-WA, the opportunity to build relations between clinicians from different specialty areas was challenged by increasing siloing and fragmentation: "*a lot of the human interactions and the efficiencies of the stuff you used to do in the corridor when you walked past someone, didn't happen anymore*" (*Program Director*). The UDP-WA fosters positive team experiences, allows for opportunities to network with professionals outside of one's discipline, and provides the opportunity for multidisciplinary collaboration and support. While the work of clinicians is arguably already quite high on task significance, the way in which the UDP-WA streamlines and increases the efficiency of the diagnostic process serves to further amplify clinicians' perceptions that they are having a positive impact through their work: "*this collective real time thinking and investigating is the thing that gives it life" (Clinician).*

Finally, "Tolerable demands" refers to the extent to which a job involves manageable levels of work demands, such as *time pressure and workload*, *emotional demands*, and *role conflict* (i.e., job feedback, instructions, and demands are inconsistent). The demands of clinical work are high—particularly in the context of ongoing and increasing institutional pressures to cut costs and "do more with less" [e.g., (22)]. However, the demands of finding diagnoses for patients with rare diseases are extraordinarily high; the sheer quantity of potential diagnoses, and the ever-increasing volume of scientific literature on such diseases, leads to incredible demands being placed on clinicians—who were previously facing such demands in relative isolation. The UDP-WA distributes the intellectual challenges and complexities of the diagnosis of rare diseases across the expert panel team, thus reducing the workload and emotional demands experienced by any one clinician; one clinician, in referring to their newly-diagnosed patient, said "that case has been weighing on my mind for *years*." The UDP-WA also supports the team with the technical infrastructure and Nurse Coordinator support to alleviate the time burden associated with consolidating extensive clinical information. The role of the Nurse Coordinator in enabling teams to support rare disease patients is crucial: a recent study employing the Delphi method found a Nurse Coordinator to be the single most important factor in enabling a functional multidisciplinary team (23).

## MOVING FORWARD

We argue that technological and scientific innovation—alone—are insufficient to address the challenges posed by rare disease diagnosis. Instead, further attention should be paid to the way in which the relevant tasks, activities, and work relationships are structured (i.e., *work design*) and the way in which this facilitates the collaborative, stimulating, and supportive environment necessary for clinicians to successfully diagnose rare diseases. This includes further documentation and investigation of the effects of SMART work design on key outcomes in healthcare. However, evidence thus far suggests that transformative work design is a critical element of sustainably addressing unmet needs and implementing precision medicine approaches for precision public health. Further, improving work structures is a critical element of sustainably implementing new (precision medicine) technologies, including through an equitable lens for those that need them the most, and for the most (i.e., precision public health). Thus, we encourage clinicians, managers, and policymakers who are working in the context of undiagnosed diseases—and other complex healthcare challenges that require interdisciplinary approaches—to proactively consider the role of SMART work in their organization. To this end, we also propose further widespread collaboration between people working in the healthcare sector and organizational psychologists, to ensure the design of a better system of healthcare work that is more effective, more engaging, and more equitable; for patients, employees, organizations, and economies.

## WEB RESOURCES

For more information about SMART work design, please visit: https://www.smartworkdesign.com.au/.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

SP developed the SMART work design model (see transformativeworkdesign.com). GH and FK developed the conceptual material relating to SMART work design, with support and additional ideas from CT and SP. AB and GB contributed perspectives on healthcare policy and rare diseases, informing the application of the SMART work design concept to the specific context of rare disease diagnosis. CT wrote the first draft of the paper, with guidance from GH and FK. GH significantly refined the manuscript (through multiple iterations), with input from all other authors. All authors contributed to the conceptual basis of this paper.

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# Why Does Child Mortality Decrease With Age? Modeling the Age-Associated Decrease in Mortality Rate Using WHO Metadata From 14 European Countries

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**Background:** Mortality rate rapidly decreases with age after birth, and, simultaneously, the spectrum of death causes show remarkable changes with age. This study analyzed age-associated decreases in mortality rate from diseases of all main chapters of the 10th revision of the International Classification of Diseases.

**Methods:** The number of deaths was extracted from the mortality database of the World Health Organization. As zero cases could be ascertained for a specific age category, the Halley method was used to calculate the mortality rates in all possible calendar years and in all countries combined.

**Results:** All causes mortality from the 1st day of life to the age of 10 years can be represented by an inverse proportion model with a single parameter. High coefficients of determination were observed for total mortality in all populations (arithmetic mean = 0.9942 and standard deviation = 0.0039).

Slower or no mortality decrease with age was detected in the 1st year of life, while the inverse proportion method was valid for the age range [1, 10) years in most of all main chapters with three exceptions.

a) The decrease was faster for the chapter "Certain conditions originating in the perinatal period" (XVI).
b) The inverse proportion was valid already from the 1st day for the chapter "Congenital malformations, deformations and chromosomal abnormalities" (XVII).
c) The shape of the mortality decrease was very different for the chapter "Neoplasms" (II) and the rates of mortality from neoplasms were age-independent in the age range [1, 10) years in all populations.

**Conclusion:** The theory of congenital individual risks of death is presented and can explain the results. If it is valid, latent congenital impairments may be present among all cases of death that are not related to congenital impairments. All results are based on published data, and the data are presented as a supplement.

**Keywords: mortality rate, age, childhood, congenital anomalies, WHO database**

## INTRODUCTION

Mortality rate is one of the most important indicators of population health. It decreases with age after the birth and increases with age in adults. This increase in adults is approximately exponential and is a curious phenomenon observed in several higher organisms, including *Homo sapiens* (1–9). It is usually interpreted as a manifestation of aging and affects all individuals (10–18). From the statistic point of view, age is a deterministic variable in this relationship and coefficients of determination may be higher than 0.99 (4, 18–23).

Faster changes in mortality rates with age are during childhood (5, 7–9). The fast mortality decrease in childhood is accompanied by age-based changes in the spectrum of death causes (24–28). The dominant death causes which reached more than 10% during the 1st weeks of life are insignificant after the age of 5 years. **Figure 1** illustrates the proportions of deaths from diseases categorized in specific chapters of the 10th revision of the International Classification of Diseases (ICD10) (28, 29). The proportions are shown in the age categories during the age interval [0, 10) years and 100% corresponds to all deaths in a specific age range. Chapters whose diseases were responsible for more than 1% of the deaths in at least one age category are shown. The proportions were calculated for all 14 countries together and they were very similar in individual countries.

The aims of the study may be split into two main levels:

a) What type of model could describe the decrease of mortality with age after birth? Is it exponential, power or some other type of mathematical function? The questions lead to the parametric description of age trajectory of mortality (ATM). The historical list of studies on this topic is not big and the models are described more detailed in the **Appendix** (the relationships 17–24). The exponential Gompertz model used by Thiele was the first historical attempt (30). Bourgeois Pichat composed the model describing the decrease of death in the age of $n$ months for the age interval 28–365 days (31–34). A model of ATM for all age categories was designed later by Heligman and Pollard (7). The simple linear model with two parameters in the log-log scale could be also used and it corresponds to the Weibull distribution [the relationships (23) and (24) in the **Appendix**]. For example, Bebbington et al. used the reduced additive and flexible Weibull distributions with the separation of mortality to exogenous or endogenous causes over the entire life span (8, 9). It has also been shown that the decrease may be represented by an inverse proportion model with a single parameter, and the model may be valid for higher ages (it has been assessed for congenital anomalies of the central nervous system within the age range [0, 90) years) (28). All-causes ATM and ATM for the main chapters of ICD10 were constructed here and convenient parametric models were found.

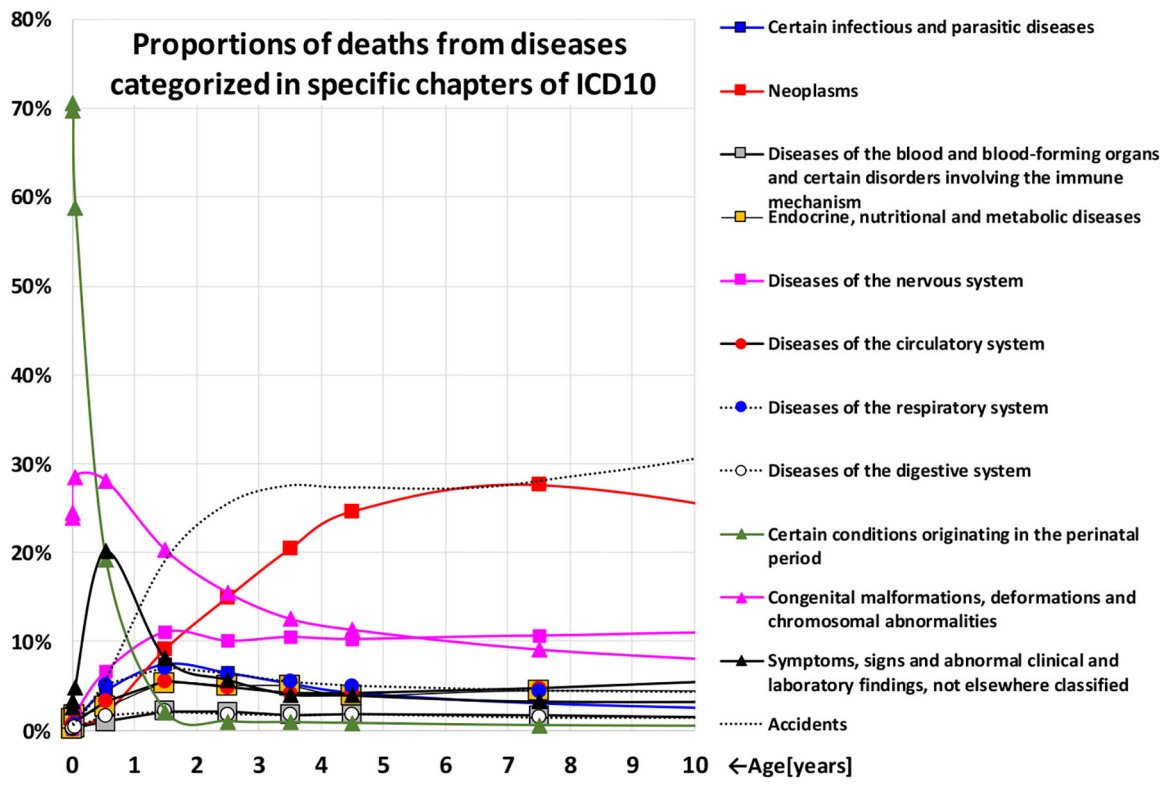


**FIGURE 1 |** Proportions of deaths from diseases categorized in specific chapters of ICD10.

b) The attempt to explain the steep decline in mortality rate with age after birth represented the second level of the study. The first view could lead to the assumption, that population is homogenous and the development of every individual caused the steep decrease of mortality with age. The alternative assumption that population is very heterogeneous was used here. Heterogeneity from the point of view of ATM was used also in the "frailty model of mortality" in some demographic studies (35, 36). The more theoretical discussion is done for example in Vaupel et al. (36). Vaupel et al. proposed a model of heterogeneity in individual frailty, estimating the gamma distribution of frailty in a population at birth (35). An individual with a frailty of 1 represents a "standard" individual, while an individual with a frailty of 2 is twice as likely to die at any particular age as the standard individual (an individual with a frailty of 0.5, on the other hand, is only one-half as likely to die). It has to be stressed that the theoretical shape of ATM was common to all subpopulations in the theory. Heterogeneity consisted in differences in one of the two parameters of the exponential increase with age and the parameter corresponded frailty. The hypothesis was created to explain the exponential increase of ATM in adults. It was not related to the decrease of mortality with age after birth and the extinction of subpopulations with age was not considered. On the other hand, we have tried to explain the decrease of child mortality with age. We hypothesized that the mortality decrease with age was the result of the extinction of more sever patients. Individuals with more severe congenital impairment typically died during the 1st hours or days of life, and the majority of these severe cases were classified as congenital anomalies or other impairments originating in the perinatal period (28). The proportion of severe congenital anomalies and impairments originating in the perinatal period was very small after the 1st year and decreases to zero as age increases (26). The coincidence between All-causes ATM and ATM constructed for congenital anomalies was the other empirical finding which was assumed here (28). It means that the model of inverse proportion was valid for All-causes ATM, and simultaneously for ATM constructed for congenital anomalies up to 90 years. Furthermore, investigating ATM constructed for other specific group of diseases may help to elucidate how the resulting All-causes ATM was composed. The similarity between ATM constructed for congenital anomalies and other ATM may show that "the sequential exclusion of individuals with more severe impairments was responsible for the steep decrease in total mortality with age."

From methodical point of view, age was the main explanatory variable here. The age at which an individual died was a very reliable information and it could be assumed that the number of deaths is a well-known information in developed countries. On the other hand, the determination of the death cause may be related to some uncertainty.

The present study used data of 14 European countries and the calendar periods in which ICD10 was used. The calendar periods are shown in **Table 1** and the studied population represents ∼570 million living persons in one calendar year (the combined population of all the 14 countries is labeled as P14). The total mortality rate decreased three orders of magnitude with age within the age range of [0, 10) years. For example, the highest mortality rate was 39,543.8 per 100,000 living persons per year in P14, in the age range 0–24 h, and the ATM reached the minimum value of 10.5 per 100,000 living persons per year within the age range of [5, 10) years. Mortality rates increased beyond the minimum value, reaching 19,788.2 deaths per 100,000 living persons per year within the age range [90, 95) years (similar figures were seen in each country and in each calendar year).

P14 represents the biggest aggregate for which ATM were constructed and enables smoother curves. P14 may also eliminate zero cases for some diseases in specific age category. Initially, the decrease in ATM was analyzed in the largest population P14. Total mortality rates and mortality rates from diseases of specific ICD10 chapters were used to construct ATM. ATM were also constructed for each country, three specific country groups, and for ICD10 chapters.

## MATERIALS AND METHODS

The 1-year period is a typical time unit used in epidemiology, biology, and social sciences. Biological events (e.g., death or disease) in different ages may be studied in one specific calendar year across age categories (transversal description). Other possibility is the longitudinal observation of individuals born in the same year (generation study or longitudinal study). If the time unit "1 year" is replaced with the time unit "1 day" (e.g., for some biological, social, or cultural reasons), events observed for 1 year represent the aggregation of the events that occurred in 365 days. Aggregation of more calendar years has a similar meaning. Age was here assumed as the main factor and all other factors were assumed to be less significant. Furthermore, the existence of general mechanisms was assumed, as demonstrated in the ATM after birth. The mechanism could be unknown and was identified as aging in higher age ranges, as mortality rate increased with age (37). For these reasons, ATM were primarily constructed in as large population as possible (P14). Data and results of specific countries and of three country groups were also studied. Including more regions and calendar years within the analysis may eliminate all factors other than age, rendering the impact of age more visible. The following standard definition of the force of mortality at age x was used:

$$\mu(x) = \lim_{h \to 0} \frac{D(x+h)}{L(x)} = -\frac{dS(x)}{dx} \Big/ S(x) \cong \frac{Di}{Li} \cdot \frac{1}{(Bi - Ai)} \quad (1)$$

where $D(x+h)$ is the number of deaths in a small age range, [x, x+h), the infinitesimal increment $h$ is positive. $S(x)$ is the survival function (percentage of living people at age $x$), which is valid in principle: $S(x) = 1\text{-}F(x)$, where $F(x)$ is the cumulative distribution function of the probability of death. The empirical value $Di$ is the number of deaths within an age range [$Ai$, $Bi$), while $Li$ is the size of the population among which the deaths occurred. Empirically, changes in $Li$ within an age interval [$Ai$, $Bi$) were very small, when compared with changes in $Di$, and

**TABLE 1 |** Calendar periods and population sizes.

| Country | France | Germany | Italy | Spain | UK | Czech Republic | Austria |
|---|---|---|---|---|---|---|---|
| Years | 2000–2014 | 1998–2015 | 2003–2014 | 1999–2015 | 2001–2015 | 1994–2015 | 2002–2016 |
| Population | 61,466,098 | 80,892,654 | 59,026,383 | 44,137,863 | 61,569,167 | 10,384,837 | 8,420,447 |
| **Country** | **Hungary** | **Poland** | **Slovakia** | **Sweden** | **Norway** | **Denmark** | **Finland** |
| Years | 1996–2015 | 1999–2015 | 1996–2014 | 1997–2015 | 1996–2015 | 1994–2009 | 1996–2015 |
| Population | 10,055,552 | 38,570,112 | 5,407,663 | 9,215,809 | 4,708,433 | 5,357,073 | 5,265,968 |

*Size of specific population is mean of population sizes in specific calendar years.*

the number of living people *Li* within an age interval [*Ai*, *Bi*) was used instead of the average number of living people in the region and calendar period. Namely, population *Li* goes through the "window" in time or through the age interval [*Ai*, *Bi*) and it is the meaning of the product *Li*•(*Bi*-*Ai*) in Equation (1). The unit corresponding to the mortality rate is "person-years," which means the number of years lived by members of the population between ages *Ai* and *Bi*. Uncertainty or possible demographic error of Li were discussed in the previous studies in detail (20–23) and they were assumed to be negligible with the respect to resulting ATM.

ATM were assumed to be unknown theoretical curves, and were constructed using the right side of the Equation (1). Mortality rates in different age groups describes the groups Li in the same way a decay constant describe the force of a radioactive decay on different radionuclides, while the different radionuclides correspond to the groups Li.

## Population of 14 Countries (P14)

Zero deaths in a given age group may be the major problem for the construction of ATM from a disease or from a set of diseases grouped in some ICD10 chapters. While zero deaths due to some disease may occur within a specific age category in a specific calendar year, at least one death may occur within the same age category in other calendar year. Consequently, the inclusion of additional calendar years may remove this obstacle. The method was first utilized by the well-known astronomer and mathematician Edmond Halley (38–40). In 1693, he elaborated a population life table for the city of Breslau using data based on his analysis of the number of births and deaths recorded in parish registers over several calendar years. This method enables the calculation of the mortality rate within one age category based on the number of deaths and living persons in several years (26, 28, 39, 40). It simply means that, in each age interval, the number of living people and the number of all died in more than one calendar year and more than one region are added together. It is noteworthy that standard epidemiologic and demographic interpretations of the specific value of mortality rate within a specific age category differ from the values calculated for one calendar year. However, the interpretation is the same for all age categories, and the resulting ATM may be interpreted as a hallmark of the population changes with aging. It has also been used in paleodemography, where a population and its corresponding life table were constructed (40). Besides, including more regions and calendar years may eliminate factors other than age.

The WHO mortality database contains the number of deaths within specific age categories in different countries (41). It is crucial that the database uses the following four age categories for the 1st year of life: [0, 24) hours, [1, 7) days, [7, 28) days, and [28, 365) days. Unfortunately, these four categories are not used in all countries and calendar years. The death cause is determined using the specific revision of the International Classification of Diseases in the database. The database contains the 7–10th revisions of ICD and specific revisions are used for different calendar years by different countries. Here, the calendar period used in each country corresponds to the period when the recent 10th revision (ICD10) was applied (29).

The present study used WHO data collected in 14 European countries. Calendar periods and these populations are shown in **Table 1** (42). The population of the 14 European countries combined (P14) represents ∼570 million living persons in one calendar year. Besides being constructed for populations of specific countries and for P14, ATM were also constructed for the following populations: (a) the five big EU countries (France, Germany, Italy, Spain and the U.K.), labeled as "P1," (b) the five Central European countries (Austria, Czech Republic, Hungary, Poland and Slovakia), labeled as "P2," and (c) the four Nordic countries (Denmark, Finland, Norway and Sweden), labeled as "P3." The relevance order was: P14, the three subgroups of countries (P1, P2, and P3), and the 14 countries separately. A total of 18 ATM could be constructed for specific groups of diseases in 18 different populations. If no deaths were registered within a specific age category in any population, ATM for this population was not constructed. For example, no cases in the chapter "Pregnancy, childbirth, and the puerperium" (XV) were found within the age range [0, 10) years in all populations. Thus, this chapter was not relevant to the study.

As the WHO database (41) is not user-friendly, "txt" files were imported into Microsoft (MS) Excel 2016. Moreover, a simple program was developed using Visual Basic for Applications to perform the following manipulations (the calculation of the sum of cases in each chapter and each age category). MS Excel was used at the first level of processing, along with standard packages in R 3.3.2 for Windows. The level of statistical significance was set to 0.05 for all tests. The arithmetic mean of the interval endpoints was used as a representative value for each age category. The time unit "1 year" was used in all age categories and in all calculations.

**TABLE 2 |** Results of total mortality calculated in the log-log scale.

| Population | Test of linearity | γ | Lower CI 95% | Upper CI 95% | $\bar{R}^2$ | $R_b^2$ | $R_b^2$-$\bar{R}^2$ | Test of c/x |
|---|---|---|---|---|---|---|---|---|
| France | 0.31 | −0.984 | −1.034 | 0.934 | 0.9963 | 0.9965 | 0.0002 | 0.47 |
| Germany | 0.70 | −0.987 | −1.036 | 0.938 | 0.9964 | 0.9967 | 0.0003 | 0.54 |
| Italy | 0.82 | −1.007 | −1.072 | 0.942 | 0.9941 | 0.9948 | 0.0007 | 0.81 |
| Spain | 0.36 | −0.960 | −1.022 | 0.898 | 0.9941 | 0.9931 | −0.0010 | 0.17 |
| UK | 0.88 | −1.030 | −1.082 | 0.977 | 0.9963 | 0.9959 | −0.0004 | 0.22 |
| **P1** | 0.72 | −0.997 | −1.046 | 0.948 | 0.9965 | 0.9970 | 0.0004 | 0.87 |
| Czechia | 0.18 | −0.918 | −1.004 | 0.831 | 0.9873 | 0.9810 | −0.0064 | 0.06 |
| Austria | 0.14 | −1.006 | −1.069 | 0.944 | 0.9944 | 0.9951 | 0.0007 | 0.82 |
| Hungary | 0.21 | −1.008 | −1.075 | 0.941 | 0.9937 | 0.9945 | 0.0007 | 0.79 |
| Poland | 0.93 | −1.023 | −1.086 | 0.961 | 0.9946 | 0.9948 | 0.0002 | 0.41 |
| Slovakia | 0.20 | −0.951 | −1.030 | 0.871 | 0.9901 | 0.9887 | −0.0014 | 0.19 |
| **P2** | 0.73 | −1.003 | −1.063 | 0.942 | 0.9948 | 0.9955 | 0.0006 | 0.92 |
| Sweden | 0.99 | −0.968 | −1.023 | 0.912 | 0.9953 | 0.9948 | −0.0005 | 0.22 |
| Norway | 0.78 | −0.966 | −1.012 | 0.920 | 0.9967 | 0.9959 | −0.0008 | 0.13 |
| Denmark | 0.29 | −1.014 | −1.060 | 0.967 | 0.9970 | 0.9972 | 0.0002 | 0.51 |
| Finland | 0.08 | −0.960 | −1.032 | 0.888 | 0.9919 | 0.9913 | −0.0007 | 0.23 |
| **P3** | 0.45 | −0.975 | −1.027 | 0.924 | 0.9960 | 0.9959 | −0.0001 | 0.30 |
| **P14** | 0.78 | −0.996 | −1.048 | 0.945 | 0.9962 | 0.9967 | 0.0005 | 0.88 |

*The column labeled as "Test of linearity" contains p-value of the test of the null hypothesis for the quadratic element ($H_o$: $\delta = 0$) in model (2) (all trajectories were linear in the log-log scale). The column labeled as "γ" contains point estimation of the slope in model (3) and the next two columns contain the limits of 95% confidence intervals of the parameter γ. $\bar{R}^2$ is the adjusted coefficient of determination calculated for two parameters and nine points in the model (3). $R_b^2$ is the coefficient of determination of the inverse proportion (4) in the log-log scale and it was calculated using formula (6). The last column labeled as "Test of c/x" contains p-value of the standard Fisher's test, which determined that the model (3) (with two parameters) does not provide a significantly better fit than the model (4) (with a single parameter). The inverse proportion (4) was a successful model in all cases.*

## Model Assessment

The decrease in the mortality rate with age was visually linear in the log–log scale. At first, the linearity of the trajectory within the age range [0, 10) years was statistically examined by the full quadratic model (2) using the method of least squares (LS):

$$ln\left[\mu(x)\right] = constant + \gamma.\ln(x) + \delta.\ln(x).\ln(x) \quad (2)$$

The null hypothesis for the quadratic element ($H_o$: $\gamma = 0$) was not rejected ($P > 0.05$), while the parameter γ was significant ($P < 0.0001$) in all populations. The F-test, in which the full model (2) does not provide a significantly better fit than the restricted model without the quadratic element, provided the same result. Consequently, it was assumed that the decrease in total mortality with age was linear in the log–log scale. Following this assumption, a linear submodel (3) was developed:

$$ln\left[\mu(x)\right] = constant + \gamma.\ln(x) \quad (3)$$

The two parameters $\ln[\mu_1]$, γ, their standard deviations, and the adjusted coefficients of determination $R^2$ in the linear model (3) were calculated using the LS method for the age interval of [0, 10) years. The hypothesis that the residuals were age-independent was not rejected ($P > 0.05$), and our data further confirmed that the residuals were not *U*-shaped. The results are shown in **Table 2**.

Because the slope γ was close to the value−1, the null hypothesis $H_o$: $\gamma = -1$ was examined using the model (3) and was not rejected ($P > 0.05$). The specific value −1 for the parameter γ corresponds to the inverse proportion between mortality rate and age. If $\gamma = -1$, the following is formally valid:

$$\begin{aligned}\mu(x) &= \frac{\mu_1}{x} \text{ is on the log}-\text{log scale}: \ln[\mu(x)] \\ &= \ln(\mu_1) - \ln(x) \end{aligned} \quad (4)$$

The parameter $\mu_1$ in model (4) can be estimated using the Equation (5) for *n* pairs of values $\ln[\mu(x_i)]$ and $\ln(x_i)$, via the LS method:

$$ln(\mu_1) = {\sum\{ln[\mu(x_i)] + ln(x_i)\}}/{n} \quad (5)$$

Furthermore, the standard coefficient of determination $R_b^2$ in model (4) can be described as follows:

$$\begin{aligned} R_b^2 &= 1 - {SS_{resid}}/{SS_{Total}} = \\ &= 1 - {\sum\{ln[\mu(x_i)] - [ln(\mu_1) - ln(x_i)]\}^2}/{\sum\left\{ln[\mu(x_i)] - {\sum ln[\mu(x_i)]}/{n}\right\}^2} \end{aligned} \quad (6)$$

The inverse proportion (4) is a nested model that includes the two-parameter linear model (3). In the next step, the null hypothesis that model (3) (with two parameters) does not provide a significantly better fit than model (4) (with a single parameter), was tested using a standard Fisher's test. Resulting *p*-values are shown in **Table 2** (the null hypothesis was not rejected in any

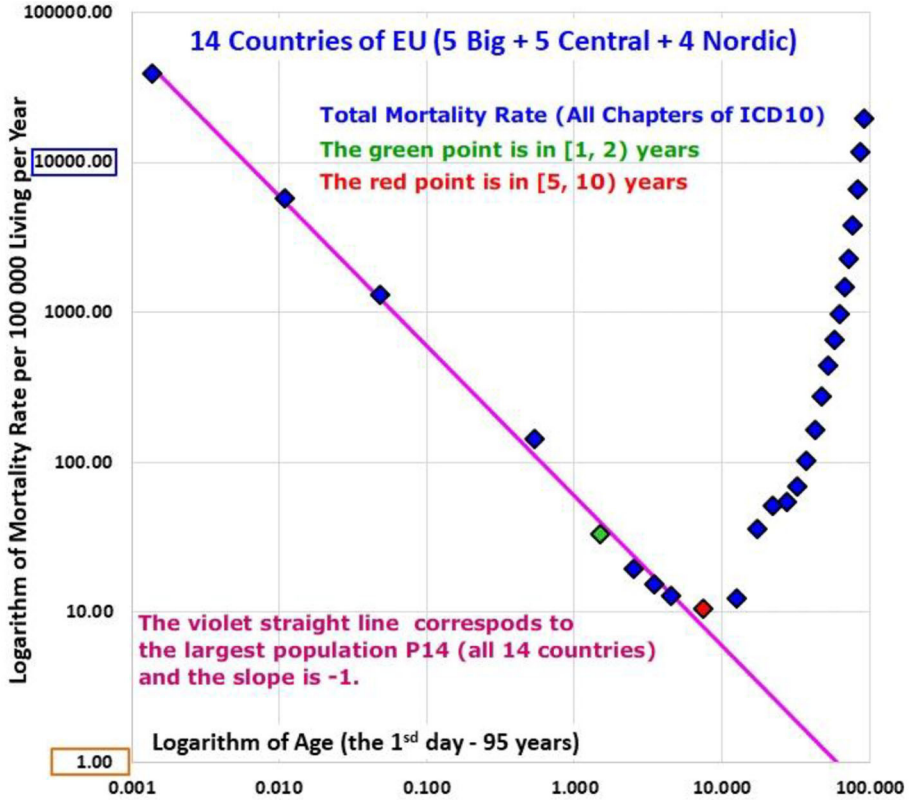


**FIGURE 2 |** (Animation 1) Age trajectory of total mortality.

population). Consequently, the inverse proportion between total mortality and age was the resulting model within the age range of [0, 10) years. Besides, the standard coefficients of determination $R_b^2$ in model (4) were higher than 0.99 in all populations, except for Czech Republic (0.981). Arithmetic mean of coefficients of determination $R_b^2$ in model (4) was 0.9942 and standard deviation was 0.0039. Such values are uncommon and the inverse proportion between total mortality and age may be considered as a deterministic relationship. It implies that, compared to the 1st day of life, mortality rate was 10 times lower after 10 days, 100 times lower after 100 days, and 3,650 times lower after 10 years.

# RESULTS

## Age Trajectory of Total Mortality

Age trajectory of total mortality is more important than ATM due to specific groups of diseases. It is also more reliable because the determination of the cause of death may be burden with some uncertainty (for example, it may be expected for cases registered in the 18th chapter of the ICD10: "Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified"). The age trajectories of total mortality are expected to be smoother and show more significant findings than the investigations of specific diseases.

The age trajectories of total mortality reached the minimal value within the age range [5, 10) years in each country separately, as well as in P1, P2, P3, and P14. Slovakia was an exception, where the minimal value was found within the age range [10, 15) years. Furthermore, the age range [0, 10) years was used to evaluate the total mortality decrease with age. At first, the age trajectories of total mortality were investigated visually. All age trajectories of total mortality are shown in Animation 1 and one age trajectory of total mortality for P14 is shown in **Figure 2** in the log-log scale.

One of *p*-values of the Test of linearity in 14 separate countries was only 0.08 in Finland and one of *p*-values of the Test of c/x was 0.06 in Czechia in **Table 2**. The relatively small values near the significance level 0.05 showed that the null hypothesis of the linearity was more in danger in Finland. The null hypothesis that the model c/x described data was more in danger in Czechia. On the other hand, *p*-values of both tests were very high in aggregated populations P1, P2, P3, and P14 in **Table 2** (it simply corresponded to the law of large numbers and to the fact that populations P1, P2, P3, and P14 were composed from smaller populations). It has to be note that all results and conclusions were limited to the fact that the investigation populations originated from Central, Western and Northern European countries and may not be representative of non-European countries. The partly similar study was done in the six countries of the South America and it had showed that the model of inverse proportion was valid for All causes ATM (specific chapters of ICD10 were not investigated in the study) (23). It indicated that the conclusion of the presented study may be valid

in less developed countries. Naturally, other study has to confirm or reject such findings.

## Chapters of the ICD10

Cause of death is a less reliable information than the determination of age. On the other hand, the inspection of ATM from specific diseases may provide an important viewpoint to the decrease of mortality rate with age. It may show the composition of age trajectory of total mortality. The WHO database (41) uses the ICD10 classification (29), and the four age categories during the 1st year of life were used in some calendar years and regions. If the four age categories are used, the ATM may be constructed (constructing ATM with <4 age ranges for the 1st year of life is also possible; however, essential information about decreased mortality rate after birth is lost in such case).

The main chapters of the ICD10 were used here to study the spectrum of diseases related to the decrease in mortality rate with age after birth. Besides, ATM due to congenital anomalies of the central nervous system because higher coefficient of determination in some previous studies [the category is the subset of the chapter "Congenital malformations, deformations and chromosomal abnormalities" (XVII.)].

**Table 3** contains the number of deaths within the age interval [0, 10) years and the proportions of diseases of main chapters of the ICD10 calculated for the P14. If non-zero cases were present in each age category, ATM could be constructed, and such chapter was labeled as "yes" in the column "ATM." All external causes (accidents) were mentioned in the chapters XIX, XX, and XXI, and were grouped together in a single category labeled as "Accidents." The two dominant chapters "Certain conditions originating in the perinatal period" (XVI) and "Congenital malformations, deformations and chromosomal abnormalities" (XVII) addressed cases of congenital abnormality or any other defect developed during the perinatal period (diseases of the two chapters accounted for 39 and 23% of all deaths in the P14 within the age interval [0, 10) years, respectively). The chapter "Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified" (XVIII) addressed cases with unclear diagnosis and was evaluated separately (it may be assumed that visible congenital abnormalities were not related to death in this chapter).

The inverse proportion model (4) fits ATM of total mortality with very high coefficients of determination. Coefficients of determination lower than 0.99 were found only in Czech Republic and Slovakia (arithmetic mean = 0.9942 and standard deviation = 0.0039 and a value of 0.9967 was reached in P14). Simultaneously, total mortality rates were the sum of deaths registered in all chapters of the ICD10. Thus, the whole spectrum of ATM due to the specific chapters shows the composition of the age trajectory of total mortality. For these reasons, preference was given to the linear relationship in the log-log scale, which was used in the visual inspection. Straight lines with the slopes −1 or 0 were used in the age ranges [0, 365 days) and [1, 10) years, for preliminary assessment.

ATM from diseases of the main ICD10 chapters [i.e., the chapters marked with "yes" for the P14 in the column "ATM" in **Table 3**, were constructed for each population (the first column of **Table 2** shows the list of populations)]. ATM from accidents represents non-biological causes of death and were not investigated (this category comprised about 8% of deaths in P14 in the age range [0, 10) years). Initially, ATM were visually inspected in the log-log scale for all populations and the ATM of P14 are shown in Animation 2. The first ATM of Animation 2 is in **Figure 3** [ATM from "Certain infectious and parasitic diseases" (Chapter I)]. ATM from "Certain infectious and parasitic diseases" (Chapter I) of all populations are shown in Animation 3 and the ATM of France is shown in **Figure 4** (some of these ATM had undefined mortality rates within some age categories, due to the absence of death).

ATM from neoplasms (Chapter II) differed significantly form ATM of other ICD10 chapters. Results of all populations are shown in Animation 4 and ATM of P14 is shown in **Figure 5**. These ATM decreased during the 1st year of life but became age-independent from the 1st year to the 20th year of life. The decreased mortality rate from neoplasms during the 1st year may be described by the inverse proportion model (4) for all populations, except for Spain, U.K., Czech Republic, and Slovakia. The inverse proportion model (4) was not rejected in all other populations within the age interval [0, 365) days. All $p$-values calculated for neoplasms are shown in **Table A1** in the Appendix.

Unlike neoplasms, mortality rates from other conditions had a faster decline after the 1st year of life than during the 1st year (see Animation 2). The mortality decrease was described by the inverse proportion (4) in six chapters within the age interval [1, 10) years in population P14 (the results are in the last column in **Table 3**), while the inverse proportion (4) was rejected only in two chapters of ICD10 in P14. A faster decrease in mortality from "Certain infectious and parasitic diseases" and a slower decrease in mortality from "Diseases of the genitourinary system" within the age range [1, 10) years were observed. More detailed figures are shown in **Table 3**. Age-independence (null hypothesis Ho: slope = 0) and the specific value of slope −1 were tested separately for the age ranges [0, 365) days and [1, 10) years. The bending ATM are also studied and discussed in the section "Theory of Congenital Individual Risks."

ATM from conditions of chapters I, III, IV, VI, IX-XI, and XIV were bending in the log-log scale, and these causes of death are not related to congenital impairment (Animation 2). Diseases of all chapters for which mortality decrease was slower in the 1st year of life than after the 1st year, and were not related to congenital impairment were aggregated in the single group "Other diseases," which comprised conditions of chapters I-XV without the second chapter "Neoplasms." The results of ATM from "Other diseases" of all populations are shown in Animation 5 (ATM of P14 is shown in **Figure 6**).

## Age Trajectory of Mortality From Certain Conditions Originating in the Perinatal Period (Chapter XVI) and From Congenital Anomalies (Chapter XVII)

These two chapters differ from the other ICD10 chapters in a very important aspect: cases in the two chapters are related to

**TABLE 3 |** Results calculated in main chapters of ICD10 during the first 10 years in P14.

| Chapter | ∑Di | P | ATM | the 1st day-365 days | | | 1–10 years | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $\gamma_1$ | $\gamma_1 = 0$ | $\gamma_1 = -1$ | $\gamma_2$ | $\gamma_2 = 0$ | $\gamma_2 = -1$ |
| Certain infectious and parasitic diseases (I.) | 9,224 | 2.49 | Yes | −0.22 | 0.011 | 0.022 | −1.28 | 0.000 | 0.006 |
| Neoplasms (II.) | 20,754 | 5.61 | Yes | −0.65 | 0.026 | Ho | −0.001 | Ho | 0.0002 |
| Diseases of the blood and blood–forming organs and certain disorders involving the immune mechanism (III.) | 2,871 | 0.78 | Yes | −0.50 | 0.011 | 0.011 | −0.88 | 0.003 | 0.295 |
| Endocrine, nutritional and metabolic diseases (IV.) | 8,306 | 2.25 | Yes | −0.45 | Ho | Ho | −0.85 | 0.007 | Ho |
| Mental and behavioral disorders (V.) | 215 | 0.06 | No | x | | x | x | | x |
| Diseases of the nervous system (VI.) | 16,854 | 4.56 | Yes | −0.25 | 0.020 | 0.002 | −0.73 | 0.006 | Ho |
| Diseases of the eye and adnexa (VII.) | 4 | 0.00 | No | x | x | x | x | x | x |
| Diseases of the ear and mastoid process (VIII.) | 158 | 0.04 | No | x | x | x | x | x | x |
| Diseases of the circulatory system (IX.) | 8,374 | 2.26 | Yes | −0.49 | 0.006 | 0.005 | −0.83 | 0.014 | Ho |
| Diseases of the respiratory system (X.) | 10,176 | 2.75 | Yes | −0.22 | Ho | 0.016 | −1.01 | 0.001 | Ho |
| Diseases of the digestive system (XI.) | 3,454 | 0.93 | Yes | −0.43 | 0.012 | 0.007 | −0.92 | 0.002 | Ho |
| Diseases of the skin and subcutaneous tissue (XII.) | 46 | 0.01 | No | x | x | x | x | x | x |
| Diseases of the musculoskeletal system and connective tissue (XIII.) | 331 | 0.09 | No | x | x | x | x | x | x |
| Diseases of the genitourinary system (XIV.) | 758 | 0.20 | Yes | −0.46 | 0.013 | 0.009 | −0.64 | 0.005 | 0.023 |
| Pregnancy, childbirth and the puerperium (XV.) | 0 | 0.00 | No | x | x | x | x | x | x |
| Certain conditions originating in the perinatal period (XVI.) | 144,469 | 39.06 | Yes | −1.16 | 0.005 | Ho | −1.52 | 0.004 | Ho |
| Congenital malformations, deformations and chromosomal abnormalities (XVII.) | 85,252 | 23.05 | Yes | −0.91 | 0.0001 | Ho | −1.22 | 0.001 | Ho |
| Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (XVIII.) | 29,132 | 7.88 | Yes | −0.62 | 0.042 | Ho | −1.29 | 0.003 | Ho |
| Accidents (XIX,.XX.,XXI.) | 29,487 | 7.97 | Yes | x | x | x | x | x | x |
| "Other diseases" | 60,771 | 16.43 | Yes | −0.36 | 0.007 | 0.002 | Ho | 0.003 | Ho |
| CACNS (ICD10 codes: Q00–Q07) | 10,596 | 2.86 | Yes | −0.99 | 0.007 | Ho | Ho | 0.002 | Ho |

*Diseases from chapters for which the decrease in mortality was slower during the 1st year than after the 1st year and were not related to congenital impairment were added to the group labeled as "Other diseases." This group contained the chapters I-XV, without the chapter II (neoplasms). The last three chapters (XIX, XX, and XXI) were aggregated into a category labeled as "Accidents." The column labeled as "∑Di" contains number of deaths within the age range [0, 10) years. The column labeled as "P" contains proportions of diseases of specific chapter or chapters in all deaths within the age interval [0, 10) years. The column labeled as "ATM" indicates if all age categories contained a non-zero number of deaths and if ATM could be constructed for the age range [0, 10) years. If all age categories contained a non-zero number of deaths, the row was marked with "yes" in the column "ATM". If one age category contained zero deaths, the row was marked with "no" in the column "ATM." Slope $\gamma_1$ was calculated in the log-log scale for the first four age categories (during the first year). Slope $\gamma_2$ was calculated in the log-log scale for the age range [1, 10) years. The column labeled as "$\gamma_1 = 0$" contains p-value of the test of the null hypothesis Ho: $\gamma_1 = 0$. The column labeled as "$\gamma_1 = -1$" contains p-value of the test of the null hypothesis Ho: $\gamma_1 = -1$. The column labeled as "$\gamma_2 = 0$" contains p-value of the test of the null hypothesis Ho: $\gamma_2 = 0$. The column labeled as "$\gamma_2 = -1$" contains p-value of the test of the null hypothesis Ho: $\gamma_2 = -1$. If the null hypothesis was not rejected, the symbol "Ho" was used in the last four columns (a value of 0.05 indicated statistical significance for all tests).*

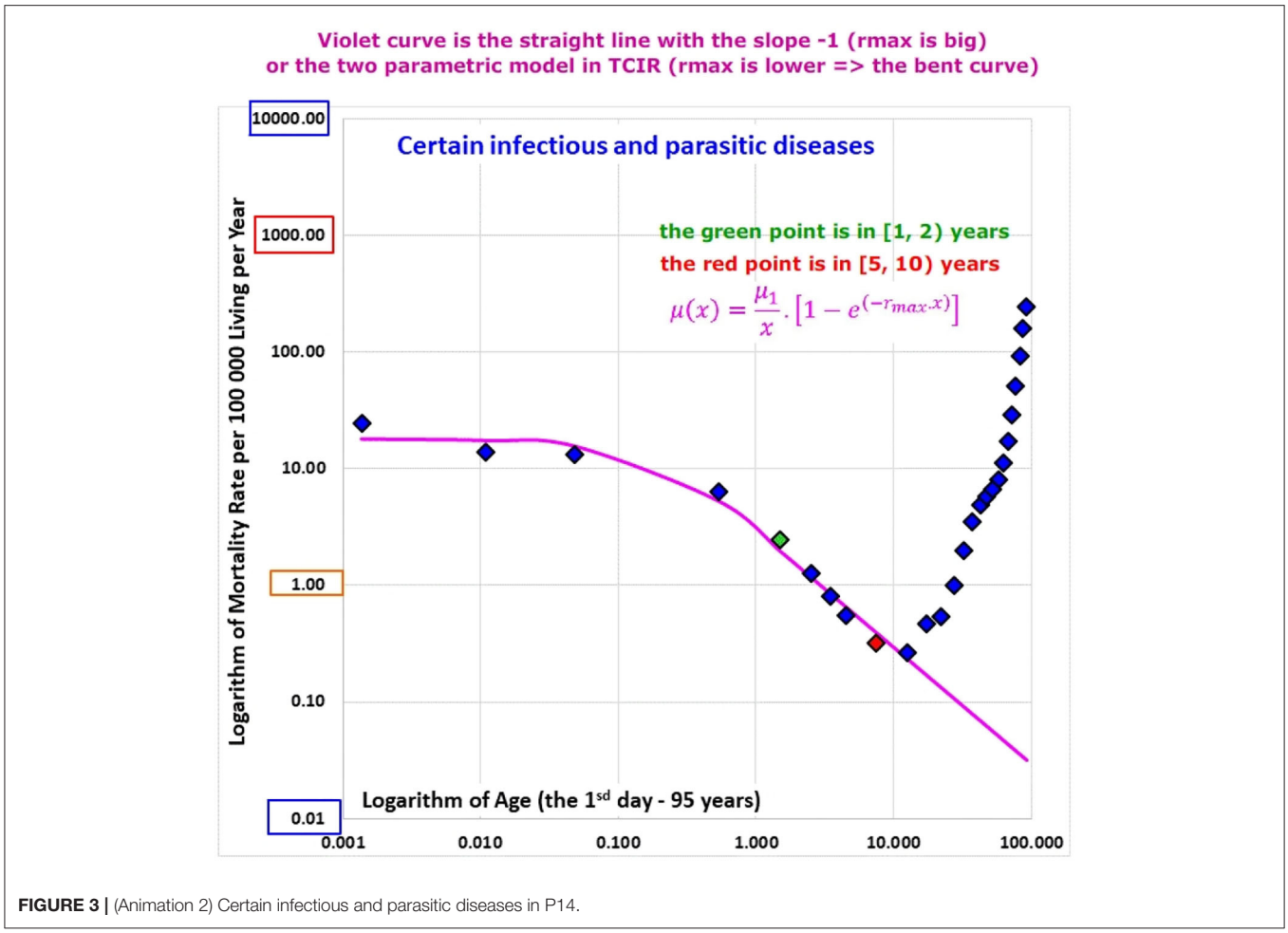


**FIGURE 3 |** (Animation 2) Certain infectious and parasitic diseases in P14.

congenital impairment or any impairment that was developed during the perinatal period. At first, ATM from these conditions were also visually inspected in the log-log scale; results of all populations are shown in Animations 6 and 7, and the ATM of P14 are shown in **Figures 7**, **8**. No case was found in some age categories in some populations in the chapter "Certain conditions originating in the perinatal period" (XVI.). Consequently, 14 results of ATM from conditions of chapter XVI were available, while all 18 results of ATM from conditions of chapter XVII were available.

Bending ATM were not observed for conditions of the two chapters and ATM decreased to higher ages (see Animation 6 and 7). The shortest age range in which mortality decreased with age was [0, 15) years, and the age interval was used to the description of the mortality decrease unlike the inspection in the two age intervals in **Table 3** (ATM decreased in the age range [0, 15) years also in P14).

Visual inspection of Animations 6 and 7 show that the variation in the linear model is larger for conditions of the chapter XVI and smaller for conditions of the chapter XVII. The linearity was rejected only in the U.K. and Poland, while it was not rejected in the rest 12 ATM in the chapter XVI (it means that the null hypothesis Ho: $\delta = 0$ in the model (2) was tested and 14 ATM were obtained in the chapter). Regarding ATM from chapter XVII conditions, linearity was observed in all populations, except for those from Czech Republic, Slovakia, and Denmark.

In the next step, two specific slopes−1 and−2 in the log-log scale were tested within the age range [0, 15) years (only the linear ATM in the log-log scale were tested in the next step). The inverse proportion model (4) was rejected in all populations, regarding ATM from chapter XVI conditions (mortality decrease was steeper in the log-log scale). The specific value of−2 in the model (3) was accepted in three populations (France, Poland, and P2) but rejected in P14 and in the remaining populations. Interpretation and the meaning of the value−2 are discussed in the following text.

Different results were obtained for ATM from chapter XVII conditions (Congenital malformations, deformations and chromosomal abnormalities). The inverse proportion model (4) was not rejected in 12 out of 15 populations (the linearity was rejected in three populations). The inverse proportion model (4) was not rejected in the most important population P14.

In summary, the decrease in mortality from chapter XVI conditions with age was steeper, while the inverse proportion model (4) better fitted ATM from chapter XVII conditions

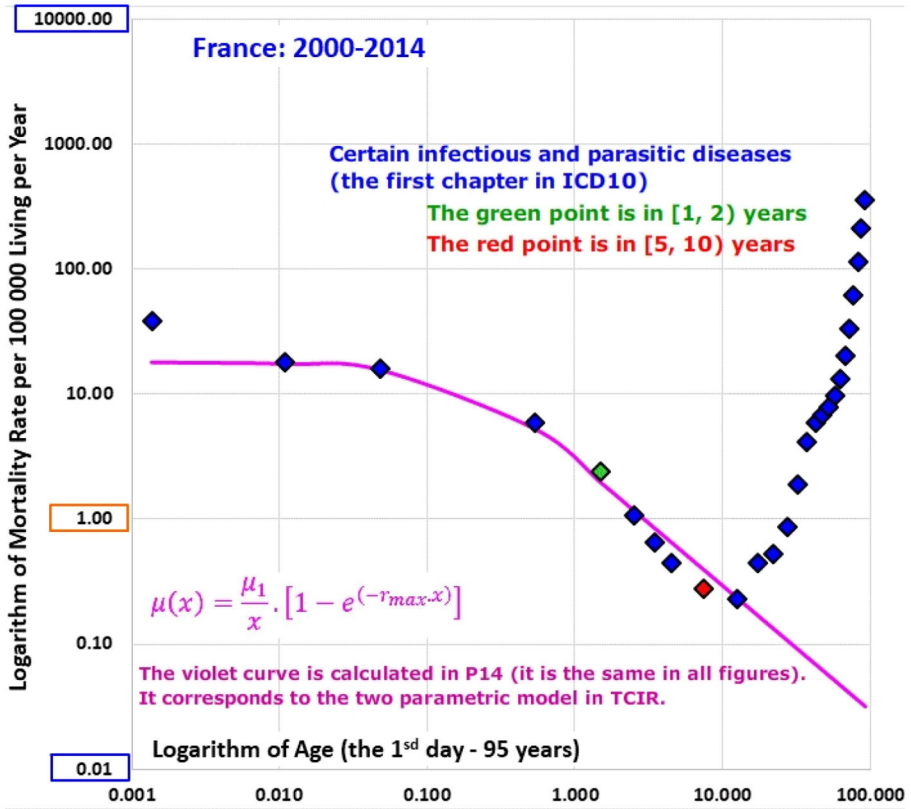


**FIGURE 4 |** (Animation 3) Certain infectious and parasitic diseases in France.

(Animations 6 and 7). All detailed figures are shown in **Tables A2, A3** in the Appendix.

## Age Trajectory of Mortality Due to Congenital Anomalies of the Central Nervous System (CACNS)

It was previously shown that the inverse proportion model was valid up to higher ages for congenital anomalies of the central nervous system (CACNS) with high coefficients of determination in other populations (26, 28). CACNS are designated by the Q00-Q07 codes in the ICD10 and were studied here as an individual disease group. Data on ATM from CACNS of all populations are shown in Animation 8, and the ATM of P14 is shown in **Figure 9**. The age range [0, 15) years was used to compare ATM from CACNS and from conditions of chapters XVI and XVII, as, in all populations studied, this was the lowest age range in which mortality decreased (results of the shorter age range [0, 10) years were very similar). All 18 ATM due to CACNS were available in the age range [0, 15) years.

Linearity was rejected in six countries (Germany, Italy, Austria, Poland, Slovakia and Finland), while it was not rejected in all other countries and in all aggregated populations P1, P2, P3, and P14. Consequently, the inverse proportion model (4) was tested in 12 populations and it was rejected only in Czech Republic and Hungary. The coefficients of determination calculated in the model (4) were very high, in general, reaching the maximum value of 0.9964 in P1, and the value of 0.9958 in P14. All these results are shown in **Table A4** in the Appendix. Arithmetic mean of $R_b^2$ calculated for 9 ATM in the age range [0, 15) years was 0.9868 (standard deviation = 0.0078).

The coefficients of determination $R_b^2$ calculated for ATM from CACNS in the model (4) were almost as high as those calculated for total mortality (**Table 2**). For example, the coefficient of determination was 0.9967, for total mortality within the age range [0, 10) years in P14, while a value of 0.9958 for ATM from CACNS was found in P14 in the same age range. The inverse proportion model (4) was not rejected in 12 populations in the age range [0, 10) years. Arithmetic mean of $R_b^2$ calculated for 12 ATM from CACNS in the age range [0, 10) years was 0.9873 with standard deviation 0.0076.

Overall, the mortality decrease with age was slower within age ranges above 20 years in some populations (see Animation 8).

## Age Trajectory of Mortality Due to Symptoms, Signs, and Abnormal Clinical and Laboratory Findings, Not Elsewhere Classified (Chapter XVIII)

A bending ATM was also detected for conditions of chapter "Symptoms, signs and abnormal clinical and laboratory findings,

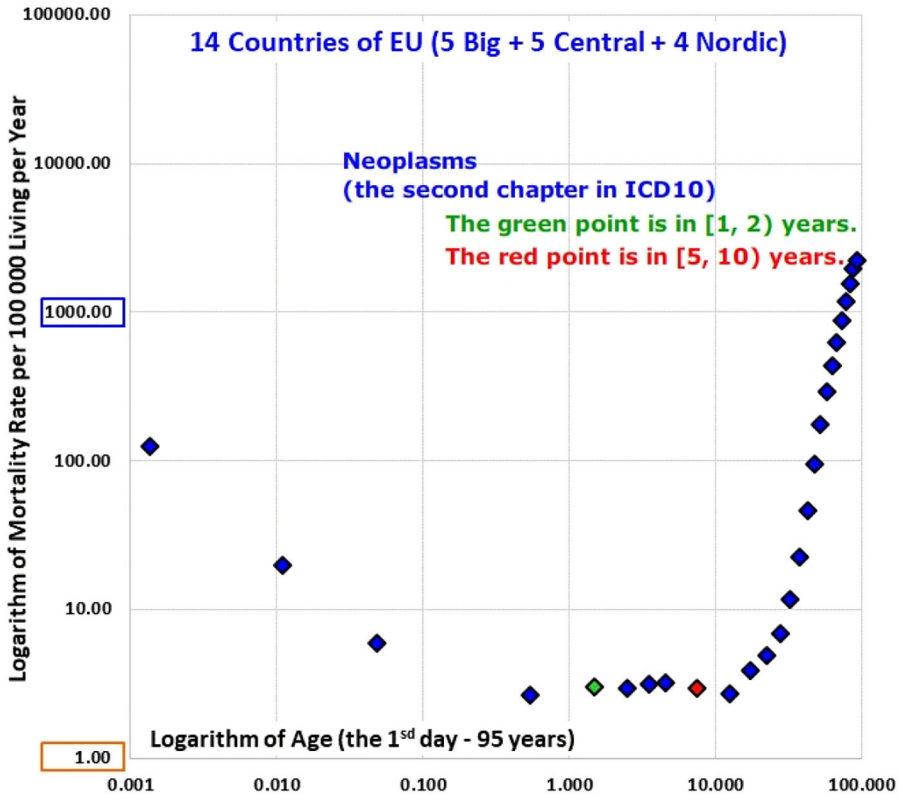


**FIGURE 5 |** (Animation 4) Neoplasms in P14.

not elsewhere classified" (XVIII), which contains cases with problematic diagnosis. ATM in P14 was age-independent in the 1st year of life and decreased within the age range [1, 10), according to the inverse proportion model (4) (**Table 3**). A total of 7.88% of all deaths due to these conditions in P14 occurred within the age range [0, 10) years.

## Theory of Congenital Individual Risks (TCIR)

The previous results may be explained by the theory that mortality decrease with age is caused by the extinction of individuals with severe congenital impairment. This theory is based on the assumption that some children had a congenital impairment, and this was approximately age-independent. The majority of the population had a very mild impairment or none impairment, which was not relevant to the ATM calculation. Age-independence is a rough approximation, and it is used to describe heterogeneity in the whole population. The main reason for the aforementioned assumption is that the decrease in the mortality rate from CACNS with age is possibly caused by the extinction of severe impairments. In addition, the decrease in mortality from CACNS with age was previously indicated by the inverse proportion model (25, 26, 28). Herein, total mortality also decreases with age, according to the same model, with high coefficients of determination (**Table 2**). In fact, a congenital impairments leading to a congenital individual risk of death may change with age. Such changes are not addressed here, considering the whole spectrum of impairments in the born population. Simultaneously, the majority of the population has very small or absent congenital individual risks of death, which render them irrelevant to ATM.

A population is composed of subpopulations characterized by specific impairment levels, and each congenital impairment level is described by a continuous value ($r$), which corresponds to the congenital risk of death or mortality rate per one person-year. It is an empirically unknown value for concrete person and, furthermore, some distribution of r may be assumed in born population. In other words, it is assumed that the shape of ATM is a hallmark of the $r$ value distribution at the moment of birth. Survival function S(r, x) in the subpopulation of individuals with similar impairments and with the same value of r is:

$$S(r, x) = \exp(-r.x) \tag{7}$$

This is probably the simplest survival curve and it is also valid for the proportion of specific radionuclide in time x (if the r value in the formula (7) is replaced by the decay constant). Derivation of relationships between mortality rate and x is presented in the appendix in the first paragraph Equations in TCIR.

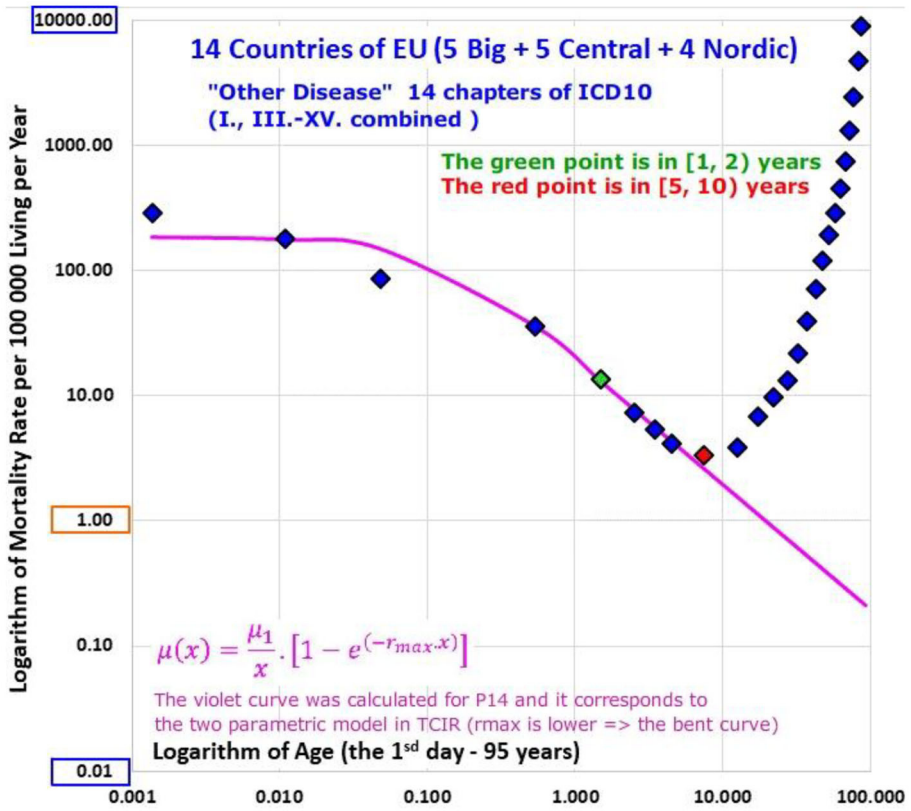


**FIGURE 6 |** (Animation 5) Other diseases in P14.

If it is assumed that "the more severe the impairment, the less frequently it occurs in the born population," mortality rate decreases with age according to the inverse proportion model (4) and the Equation (15) in the appendix is valid. Such distribution of r may be explained by the biological selection of higher values of r, which could proceed during the previous generations or during pregnancy.

The interpretation of the formula (16) in the appendix is: "if the incidence of different *r* values is approximately the same," the slope of mortality decrease with age in the log-log scale is about−2. For example, such situation may happen after a huge catastrophe. In a more general way, it may happen when the selection of serious impairments is not present. This may be approximately valid for impairments developed in the perinatal period, which are grouped within the chapter "Certain conditions originating in the perinatal period (XVI)." This is a possible explanation for the steeper decline in mortality due to chapter XVI conditions with age.

## Bending Age Trajectory of Mortality

Bending ATM represent the next empirical verification of TCIR (see Animations 2, 3, and 5). At first, it is difficult to assume that any hypothetical individual development is more significant after the age of 1 or 2 years (it has to be valid if any homogenous description of population is assumed). Such hypothetical homogenous development has to be significantly slowed down during the 1st year and it is more significant after the 2nd year up to the age of 10 years.

In contrast, the TCIR explains the mortality decrease with age based on the sequential extinction of more severe impairments, and it could also explain the bending ATM. Mathematically, if maximal value $r_{max}$ of congenital individual risks in the born population is not big and infinity is replace by $r_{max}$ in Equation (13) in the appendix, the following approximation is valid (25, 26):

$$\mu(x) = \int_0^{r_{max}} c.e^{(-r.x)}dr = c.\left[\frac{e^{(-r.x)}}{-x}\right]_0^{r_{max}} = c.\frac{e^{(-r_{max}.x)}}{-x} - c.\frac{1}{-x} = \frac{\mu_1}{x}.\left[1 - e^{(-r_{max}.x)}\right] \quad (8)$$

Furthermore, if the product $r_{max}.x$ is smaller than 1 in Equation (8), the theoretical mortality rate is approximately constant. It follows from the approximation (9) (25, 26):

$$\mu(x) = \frac{\mu_1}{x}.\left[1 - e^{(-r_{max}.x)}\right] \cong \frac{\mu_1}{x}\left[1 - (1 - r_{max}.x)\right] = \mu_1.r_{max} \quad (9)$$

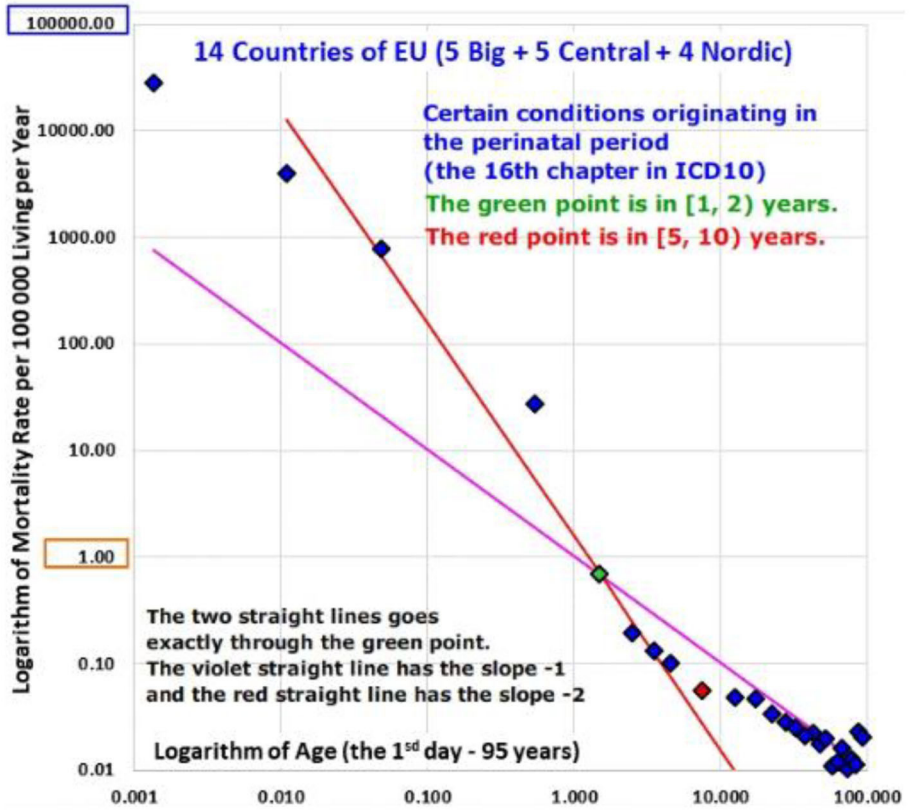


**FIGURE 7 |** (Animation 6) Certain conditions originating in the perinatal period in P14.

If a time unit of 1 year is used, and if r is per year, then the product $r_{max}.x$ is unit less. The approximation (9) is valid if the product $r_{max}.x$ is smaller than 1. On the other hand, if age x is higher ($x > 2$), the exponential term in the squared brackets can be neglected in Equation (8), and the inverse proportion (15) in the appendix is valid. Consequently, the theoretical relationship (8) may explain the bending ATM, and it was used to fit bending ATM.

Why may congenital risks be limited in some categories of diseases and why may the limit be higher for congenital anomalies? For example, if an individual case of death is related to infectious disease and no visible congenital impairment is determined, a hypothetical congenital impairment is latent. Some ICD10 chapters do not automatically contain cases with severe and visible congenital impairments (unless such impairments exist). If TCIR is valid for diseases that are not related to congenital anomalies, the spectrum of r should be limited by relatively small value of $r_{max}$ for the diseases. This explanation may account for the empirical ATM in Animations 2, 3, and 5, or in **Figures 3**, **4**, **6**.

For these reasons, the model (8) was used here and the two parameters $r_{max}$ and $\mu_1$ were estimated. The two values were numerically calculated by minimizing the residual sum of squares with the coefficients of determination. The values for P14 are shown in the two last columns in **Table A5** in the Appendix.

On the other hand, no such small limit of r may exist in the all-cause category, and this is the explanation for why ATM were not bending and the model (4) fits them in the whole age interval [0, 10) years (**Table 2**). The limit $r_{max}$ may correspond to a medical definition of a specific ICD10 chapter and may also differ according to the country. The relationship (8) could explain the decrease in the total mortality rate with age, according to an inverse proportion following the 1st day of life, which may be age-independent during the 1st year of life.

As higher the $r_{max}$ the straighter the ATM is in formula (8) in the log-log scale (empirically, the highest value of $r_{max}$ was in the chapter XVIII. in **Table A1**). It should be emphasized that the inverse proportion (4) was valid for total mortality from the 1st day of life and the coefficients of determination in **Table 2** were very high.

As the bending ATM may be explained by TCIR for conditions of all chapters with the bending ATM were grouped in one category of diseases and the category is labeled as "Other diseases." The category "Other diseases" does not contain Neoplasms (II) and the two chapters XVI. and XVII. related to some impairment present after the perinatal period (this set of chapters contained the chapters I and III–XV). The results calculated for the category "Other diseases" are shown in the last row of **Table A5** in the Appendix.

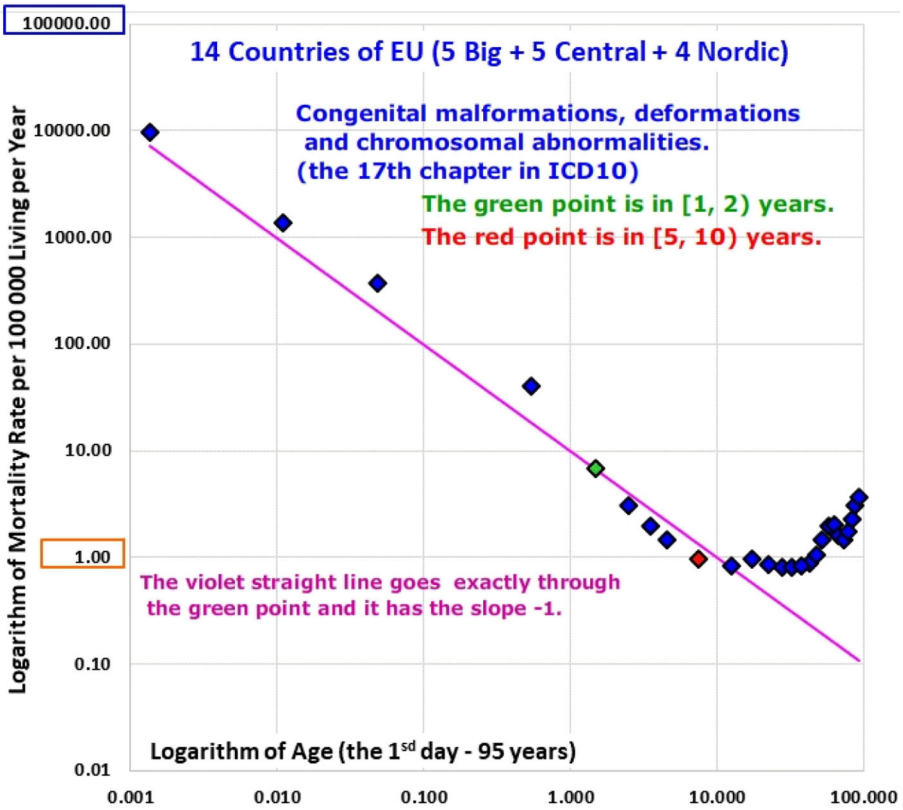


**FIGURE 8 |** (Animation 7) Congenital malformations, deformations and chromosomal abnormalities in P14.

The most important coefficients of determination $R_b^2$ calculated for P14 with in the age interval [0, 10) years in the model (4) are: 0.9967 (total mortality), 0.9958 (CACNS), 0.9815 (the chapter XVII), and adjusted coefficients of determination calculated by the model (15) for the bending ATM are shown in the last column of **Table A5** in the Appendix. The highest values of the bending model (15) were 0.977 for conditions of the first ICD10 chapter and 0.968 for conditions of the "Other diseases" category. The results are also shown visually in Animations 1, 5, and 8.

All the results show that the possible mechanisms responsible for the model of the inverse proportion (4) may be general biological process. The values of $R_b^2$ were very high for CACNS, where the mechanisms were well-visible and where the extinction of more severe impairments is obvious.

## Other Historical Models of Age Trajectories of Total Mortality After Birth

The list of all parametrical attempts to describe age trajectories of human mortality after birth is not long. Four models, which are described in the appendix, were published in the scientific literature. Their ability to fit nine age categories in the age interval [0, 10) years, which were used in the WHO database, is not high. Age trajectory of total mortality in P14 was fitted in the present study using these models and the LS method. The resulting curves are shown in **Figure 10** and the inverse proportion model (4) fits data preferably. The adjusted coefficient of determination was 0.9967 in the inverse proportion model (4), 0.9396 in the HP model, 0.4241 in the Exp model, and was negative in the BP model (the sum of squares was bigger than in the trivial model without parameters).

Consequently, the ability of models Exp and BP to explain the data is very weak (model BP is even increasing in lower ages). Model HP showed a greater ability, but the coefficient of determination is much lower (0.9396), when compared with the inverse proportion method (0.9967). Besides, model HP is concave in the log-log scale and linearity was not rejected in all populations, regarding total mortality. The parameter $\delta$ in the model (2) may closely estimate the ATM curvature in the log-log scale. Its estimation was positive in seven countries, and negative in other populations, including P1, P2, P3, and P14 (statistically, the null hypothesis Ho. $\delta = 0$ was not rejected in all populations). The results show that the models Exp, BP, and HP were not suitable to describe age trajectories of total mortality after birth.

The Weibull model (WM) with two parameters is linear in the log-log scale and may be identified with the model (3). It may fit linear data in the log-log scale but absolute value of the slope of mortality decrease in the log-log scale should be $<1$ (the slope has to be $> -1$). Consequently, WM may not fit steeper decrease with slope equals to $-1$ or $< -1$. WM is identical with

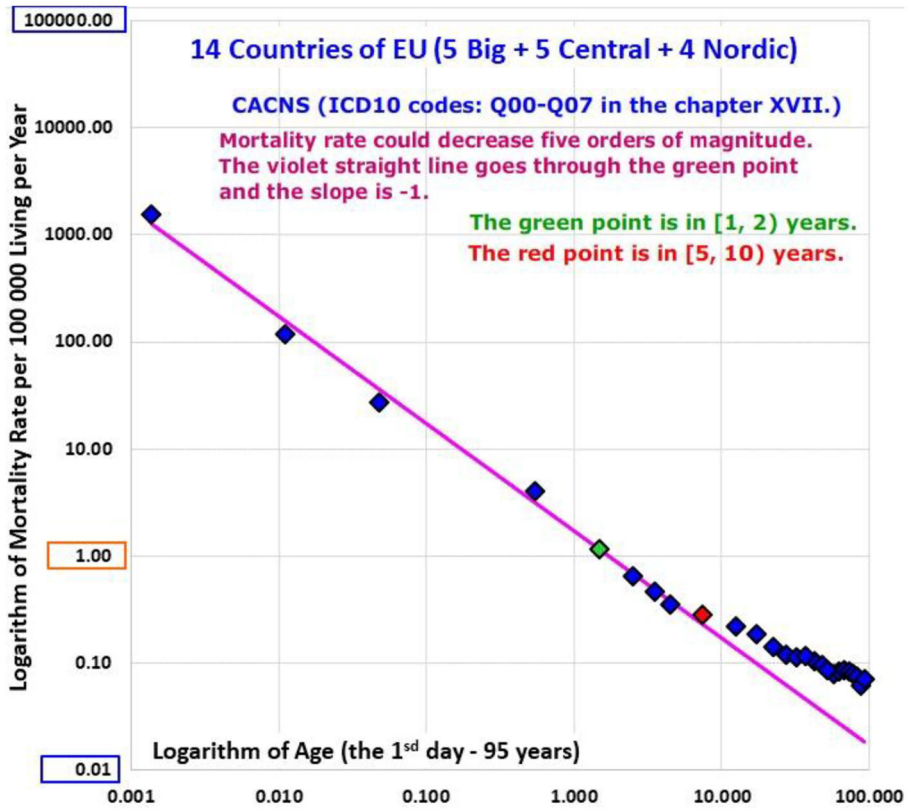


**FIGURE 9 |** (Animation 8) Congenital anomalies of the central nervous system.

the linear model (3) if slope is $> -1$. Because the slope calculated in WM was very close to-1 (the estimation calculated in WM was −0,9964) the straight line calculated in the model (3) and the straight line calculated in the model (4) are graphically identical and both models are represented by one line in **Figure 10**.

## DISCUSSION

The relationship between mortality rate and age was the main finding in the present study and shapes of ATM were used for these evaluations. Generally, shapes come from the world of platonic ideas according to the Greek "theory of forms," and for example, no physical object has, at first, the shape of a perfect circle. Statistically, proofing a linear relationship is not possible, but it may be rejected. Likewise, it is not possible to distinguish the two values of slope−1 and −1.000001 in the linear model. The value−1 is simply assumed because it may be interpreted easily. On the other hand, if the percentage of an explained variation is bigger than 0.99 or even bigger than 0.998 then such model is very successful. It was observed here and the empirical relationships were very deterministic. Such values of coefficients of determination were observed for total mortality and CACNS mortality.

The log-log scale was used for two reasons. At first, the shape was more visible in this scale and, secondly, residuals were not dependent on the explanatory variable, which was the logarithm of age (the basic assumptions of regression model was not satisfied in the standard scale). The inverse proportion model (4) has one parameter and the estimation of the parameter has the same degrees of freedom (n−1) as the standard estimation of the arithmetic mean, and the only parameter may be estimated using the formula (5).

### General Look at Change Over a Period of Time

There are two different ways to the description of change in time. The first possibility is to express the change regardless of the original value of an observed parameter. The second possibility is to relate the change to the original value. The two ways represent the background of the difference between intensive and extensive quantities. For example, if the weight of an individual is increased by 5 kg, the value of 5 kg represents the first possibility. If the baseline individual's weight was 50 kg, for instance, the second possibility is to state that the weight had a 10% increase. If the baseline weight was 100 kg, the increase was 5%. The first way was primarily chosen in mathematics in the definition of derivation (value of derivation does not depend on the original value of the function but only on the function's "shape" or "behavior"). The value of the derivation of the function divided by the original value of the function represents the second way, and it is one

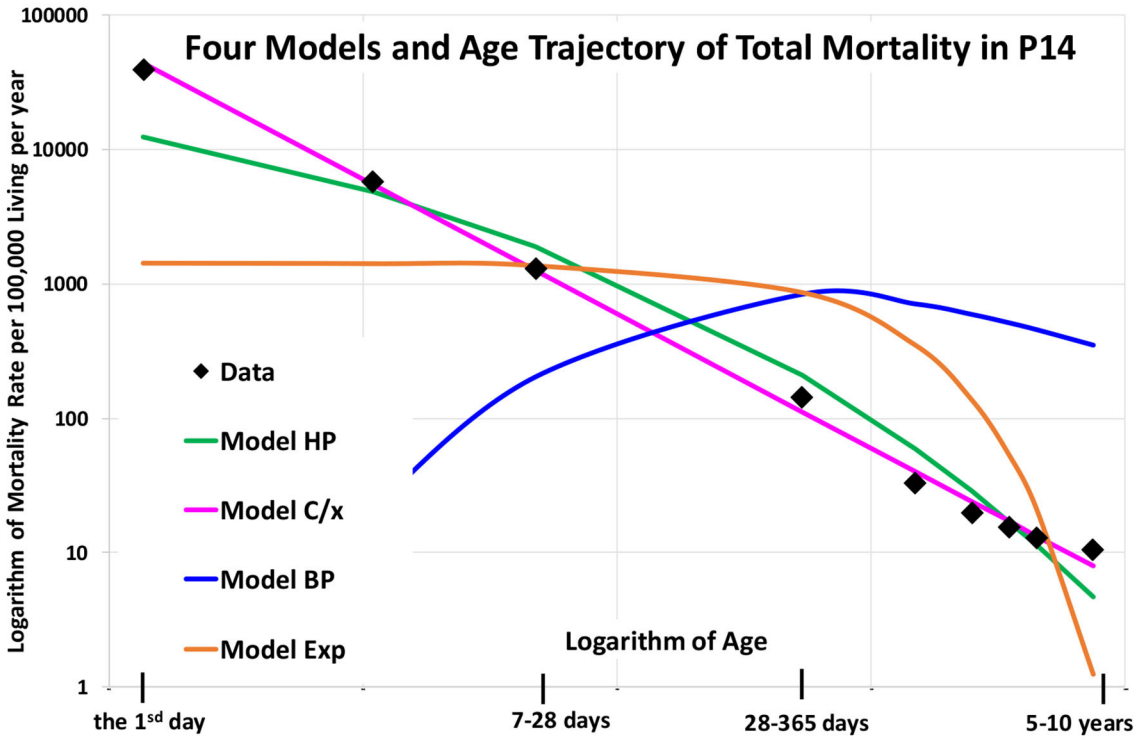


**FIGURE 10 |** (Animation 9) Four historical models and age trajectory of total mortality in P14.

interpretation of the definition (1). Regardless of the use of S(x) or L(x) in the equation (1), the changes are expressed in relation to the original values. If the formula (1) (the mathematical operator) is applied on ATM once more, interesting results are obtained. If it is applied on the standard Gompertz formula, a simple constant is obtained. The well-known exponential increase of mortality with age is valid for adults (4, 18, 19, 21, 22) The resulting constant is the slope of the linear mortality increase in the semi-logarithmic scale (one of the two Gompertz parameters). It is probably the more important parameter describing the shape of ATM (the dynamics of the increase in mortality of adults with age). If the formula (1) is applied to the inverse proportion model (4), a simple inverse proportion "1/x" without any parameter is obtained. The two curiosities may have been important to the interpretation of ATM in all ages and to general mechanisms responsible for ATM constructed for all ages.

## Two Kinds of Time

If the change in time is related to living individuals, two kinds of time are usually used. They may be labeled as "inner time" and "outer time." Standard Lexis diagram applied to demography is one example of the use of the two kinds of time. Calendar year is considered outer time in Lexis diagram, while age is considered inner time. Usually, the two kinds of time are not distinguished in science. If the outer time is used, the onset of time axis usually has a different meaning, if compared to the use of the inner time. Zero has different meaning in different calendar systems, and, usually, the onset is not important. It may be situated to the first Greek Olympic games or to any other time point. If ATM are studied, the birth or the onset of time axis has a more important role, and the onset should not be arbitrarily defined. For example, based on these considerations, age-independence of ATM during the 1st year and within the age range [1, 20) years have different interpretations. If the onset of time axis has a crucial meaning (e.g., the birth), the "inner time" is used and change in time means that the change proceeds inside the system. For example, ATM of a given population corresponds to "inner time" and correspond to the change proceeds inside the system. On the other hand, physical trajectories of the same individuals correspond to "outer time" (trajectories describing the movement of studied people during their life) and correspond to the change proceeds outside the system. Such considerations may explain the use of the logarithmic scale for describing ATM because the changes corresponds to inner time.

## Halley Method

The relationship between mortality and age had a crucial meaning here and it was the first reason to construct ATM using the Halley method in P14 (impact of age may be more visible in shapes of the ATM) (38–40). The second reason to use the method was the fact that no deaths from some diseases were detected in some calendar years.

The assumption that some general mechanisms exist, which are responsible for the mortality decrease with age after birth, was strongly confirmed (see Animations 1–8). Regardless of TCIR, inverse proportion model (4) is dominant through the

whole spectrum of diseases and it should be a hallmark of some biological mechanisms. Observations also supported the statement that ATM constructed for the large populations P1, P2, P3, and P14 were smoother, and the coefficients of determination were higher in these populations, for total mortality and for mortality from CACNS (**Table 2**, **Table A4**).

Generally, the determination of cause of death may differ within 14 studied countries and it was not clear if the same case was put in the same subcategory of ICD10. It was assumed here that such uncertainty was less probable for the main chapters of ICD10 because they were relatively extensive. Besides, it was empirically confirmed here that the shapes of ATM were almost identical in studied 14 European countries.

## Extinction of Individuals With More Severe Impairments

The explanation of the steep decrease of mortality with age represented the second viewpoint had mentioned in the introduction. The assumption of TCIR that the decrease in mortality with age was caused by the extinction of individuals with more severe impairments may be indirectly supported by the two following results:

1) Total mortality rates decrease according to the inverse proportion model (4) and, simultaneously, the significance of the two chapters "Certain conditions originating in the perinatal period" (XVI.) and "Congenital malformations, deformations and chromosomal abnormalities" (XVII.) decrease dramatically with age in **Figure 1**.
2) The CACNS category contains the following eight subcategories: Anencephaly and similar malformations (Q00); Encephalocele (Q01); Microcephaly (Q02); Congenital hydrocephalus (Q03); Other congenital malformations of the brain (Q04); Spina bifida (Q05); Other congenital malformations of spinal cord (Q06); Other congenital malformations of nervous system (Q07). ATM from CACNS showed higher coefficients of determination and the ATM decreases up higher ages. The proportions of specific subcategories of CACNS are shown in **Table A6** in the **Appendix**, and the proportions of Q00, which includes more severe congenital anomalies, strongly decrease with age.

Extinction of individuals with more severe impairments is acceptable during the 1st year of life and it is not self-evident in higher ages. Unfortunately, the incidence of congenital anomalies among living people were usually registered only at the moment of birth (27, 43). The incidence of congenital anomalies after birth is difficult to find in scientific literature, and only the proportions of congenital anomalies among individuals who died is available. The assumption that mortality decrease with age is caused by the extinction of individuals with more severe impairments is supported by the results found for ATM from congenital anomalies.

The main problem of explaining the ATM shapes using any homogenous process, which is assumed to be valid for each person, is acceptance of such explanation for CACNS and other congenital anomalies. The shapes of ATM due to CACNS and congenital anomalies were the same in the 1st years and after the 1st year of life. Besides, ATM due to CACNS was described by the inverse proportion model with a single parameter from birth up higher ages. In addition, such hypothetical mechanisms should be suppressed during the 1st year and it should cause faster mortality decrease in the age interval [1, 10) years for diseases grouped in the category "Other diseases." Simultaneously, the mechanisms is in operation in the two chapters "Certain conditions originating in the perinatal period" (XVI) and "Congenital malformations, deformations, and chromosomal abnormalities" (XVII) form birth up to higher ages. If homogenous process is assumed then the fact that the inverse proportion is valid for the group "Other diseases" within the age range [1, 10) years and for congenital anomalies within the age range [0, 10) years is a random coincidence (see Animations 5, 7, and 8). Consequently, hypothesis that the shapes of ATM were due to any development of each individual is difficult to accept. An alternative explanation of the observed results is that the shapes of ATM were caused by the extinction of individuals with more severe impairments.

## Latent Congenital Risks of Death

If TCIR is correct, significant part of the deaths within the age interval [0, 10) years was, in fact, caused by congenital abnormalities (congenital risk of death), even in the cases registered as "Other diseases." For example, if such individuals were affected by an infectious disease and died, the deaths may have been caused by congenital latent impairment. Most deaths until age 10 years may have been caused by these latent impairments, and the figures in **Table 3** provide simple estimations about the significance of the latent congenital impairments. For example, 16.4% of the deaths until the age of 10 years were due to conditions grouped in the category "Other diseases," and, simultaneously, ATM in this category was bent. According to TCIR, most cases may be caused by latent congenital impairment. If TCIR is correct, the shapes of ATM and the extinction of more sever congenital impairments may be consequence of the interaction between born population and current living conditions.

## Neoplasms

Other results were observed for conditions of the chapter "Neoplasms" (II). Mortality decrease with age occurred in the 1st year of life and was age-independent within the age range [1, 20) years. It may be important for the verification of any hypothesis that a specific type of neoplasm is only significant to a subpopulation with congenital predisposition (e.g., a genetically susceptible subpopulation). According to TCIR, such subpopulation should be larger than the sum of all deaths in the age interval [1, 20) years, since no extinction was observed. The sum of all deaths within the age range [1, 20) years represents the lower limit of the subpopulation assumed to be susceptible to the disease. This suggested limit is based on all ATM results and is very easy to be calculated. On the other hand, such number is only the lower limit of the hypothetical subpopulation. This means that if any predisposition exists then considered disease has to relate subpopulation large than the sum of all death in the whole specific population. For example, the proportion of

all deaths due to conditions of the chapter "Neoplasms" within all living people in P14 was about 0.00003, which means that the incidence of these conditions in a hypothetical subpopulation should be higher than 3 per 100,000 living people. The result is not very significant, as it concerns the whole chapter "Neoplasms" and the proportion is relatively small. If very specific diseases of the chapter "Neoplasms" are analyzed, the age-independence may be empirically confirmed in the first step. It is not necessary to construct ATM for the age range [1, 20) years (no deaths may occur within a specific age range, even in P14). The age range [1, 20) years may be divided into two intervals only, and approximately age-independence may be tested in the two values. The number of all deaths represents thereafter the lower limit of the hypothetical subpopulation.

## CONCLUSIONS

The coincidence between All-causes ATM and ATM constructed for congenital anomalies (or ATM constructed for CACNS) was confirmed in all populations. The model of inverse proportion was found as a general rule. The bending ATM was also described by the inverse proportion behind the age of 1 year (it was age independent or decreased slowly during the 1st year of life). Consequently, child mortality decrease with age may be the result of the sequential exclusion of individuals with more severe impairments. The opposite explanation that homogenous development of every individual was responsible to the decrease was suppressed here.

All results are based on the WHO mortality database (41) and the analysis covers 369,865 deaths at the age range [0, 10) years in P14. The division of the 1st year of life into four age categories is crucial for these observations, and all results are based on published data (39). The sums of death numbers from diseases of all analyzed chapters, within all populations and within all age categories are presented here as a supplement. Sums of living individuals within specific age categories and within specific populations are also shown in a supplement. The sums may be recalculated using the WHO mortality database and all ATM may be verified in the second step (41). The Halley method enables the expansion of the data used in the present study to more countries in the future (38–40). Verification of TCIR for congenital anomalies may be performed by using any register containing incidence of congenital anomalies within more age categories. It may confirm or reject the assumption that the extinction of more severe cases is significant. If TCIR is valid, latent congenital impairments should be present among the deaths from conditions registered in the group "Other diseases" and/or from specific diseases of the group. The latent impairments may be detected among dead from some disease and simultaneously, are not among surviving the same diseases. Consequently, if the detection is successful, the knowledge about latent impairments may help to lower mortality from these diseases.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.who.int/healthinfo/statistics/mortality_rawdata/en/.

## AUTHOR CONTRIBUTIONS

JD realized statistical computations and composed the main text. HH construct and write all parts related to pediatrics and clinical interpretations. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.527811/full#supplementary-material

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# Data Quality Assessment on Congenital Anomalies in Ontario, Canada

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**Background:** Congenital anomalies (CAs) are a major cause of infant morbidity and mortality in Canada. Reliably identifying CAs is essential for CA surveillance and research. The main objective of this study was to assess the agreement of eight sentinel anomalies including: neural tube defects (NTD), orofacial clefts, limb deficiency defects (LDD), Down syndrome (DS), tetralogy of Fallot (TOF), gastroschisis (GS), hypoplastic left heart syndrome (HLHS) and transposition of great vessels (TGA) captured in the BORN Information System (BIS) database and the Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD).

**Methods:** Live birth and stillbirth records between the BIS and CIHI-DAD in the fiscal years of 2012–2013 to 2015–2016 were linked using 10 digit infant Ontario Health Insurance Plan (OHIP) numbers. Percent agreement and Kappa statistics were performed to assess the reliability (agreement) of CAs identified in the linked BIS and CIHI-DAD birth records. Then, further investigations were conducted on those CA cases identified in the CIHI-DAD only.

**Results:** Kappa coefficients of the eight selected CAs between BIS ("Confirmed" or "Suspected" cases) and CIHI-DAD were 0.96 (95% CI: 0.93–0.98) for GS; 0.81 (95% CI: 0.78–0.83) for Orofacial clefts; 0.75 (95% CI: 0.72–0.77) for DS; 0.71 (95% CI: 0.65–0.77) for TOF; 0.62 (95% CI: 0.55–0.68) for TGA; 0.59 (95% CI: 0.49–0.68) for HLHS, 0.53 (95% CI: 0.46–0.60) for NTD-all; and 0.30 (95% CI: 0.23–0.37) for LDD.

**Conclusions:** The degree of agreement varied among sentinel CAs identified between the BIS and CIHI. The potential reasons for discrepancies include incompleteness of capturing CAs using existing picklist values, especially for certain sub-types, incomplete neonatal special care data in the BIS, and differences between clinical diagnosis in the BIS and ICD-10-CA classification in the DAD. A future data abstraction study will be conducted to investigate the potential reasons for discrepancies of CA capture between two databases. This project helps quantify the quality of CA data collection in the BIS, enhances understanding of CA prevalence in Ontario and provides direction for future data quality improvement activities.

**Keywords: the BORN information system (BIS), congenital anomalies, the Canadian institute for health information discharge abstract database (CIHI-DAD), data quality, agreement, kappa test**

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## BACKGROUND

Congenital anomalies (CAs) are a major cause of infant morbidity and mortality in Canada. The overall prevalence rate of anomalies has been estimated as 3.9–4.5% among all births between 1998 and 2009 in Canada (1, 2). CAs have a significant impact on the affected children, their families, and the health care system in Canada (1).

In 1966, the Public Health Agency of Canada (PHAC) established the Canadian Congenital Anomalies Surveillance System (CCASS) to allow passive surveillance of anomaly cases and facilitate analysis and interpretation of Canadian birth population data (1, 3). A primary source of data for congenital anomalies surveillance in Canada is hospital administrative data from the Canadian Institutes for Health Information (CIHI) (4, 5), specifically the Discharge Abstract Database (DAD) (5).

The DAD collects information on obstetrical deliveries, newborns and stillbirths from all acute inpatient hospitals in Canada, except Quebec (6). Newborn and childhood anomalies diagnoses in acute hospitals are recorded and classified according to the 10th International Statistical Classification of Diseases and Related Health Problems (ICD-10), Canadian Adaptation (CA) (6, 7). However, CIHI data has several limitations, e.g., records capturing termination of pregnancy are extremely limited, as are records outlining fetal anomalies identified in the prenatal period. Further, CIHI records do not include environmental exposures data to examine the risk factors for anomalies (4). In an effort to overcome the limitations of CIHI data and to enhance the existing surveillance system, more recently, the PHAC has worked in collaboration with the provinces and territories (PTs) to collect CA surveillance data on a PT level (4).

One example of a provincial contributor to the CCASS is the Better Outcomes Registry & Network (BORN) Ontario. BORN Ontario is a prescribed registry that collects data on every pregnancy and birth in the province (8). As the largest province in Canada (9), Ontario has close to 40% of all births nationally (∼140,000 per year), of which 2.6% are home births (personal communications). All births in Ontario are captured in the BORN registry (8). Hospitals, midwifery practice groups, screening programs, laboratories and clinics across the province contribute data to the registry via the BORN Information System (BIS), which integrates data collected at the point of care across the continuum into a single maternal-child registry (8). BORN has developed rigorous methods for CA capture before birth, at the time of birth and postpartum [birth and neonatal intensive care unit (NICU)/special care nursery (SCN) encounters] (10).

Compared to the DAD, the BORN registry contains more information on maternal obstetrical history, environmental and behavior exposures and characteristics, allowing improved monitoring of anomalies and other health-related outcomes, as well as the ability to explore risk factors for congenital anomalies and evaluate the determinates of prenatal and neonatal health. In addition, fetal anomalies and prenatal screening data are collected in the BIS, which allows monitoring and examination of the status of early termination or pregnancy loss associated with congenital anomalies.

Data quality is essential for CA surveillance and research. The aim of this study is to ensure reliable data and monitor data quality continuously in the BIS (11). Since both the BIS and DAD collect similar newborn anomalies diagnosis information and birth data from acute hospitals in Ontario, we are able to compare the prevalence of anomalies detected in the BIS and DAD, as well as link the BIS and DAD data to compare the reliability of CA diagnoses in both databases (8). The objectives of this study were to: (1) describe the prevalence rates of eight sentinel anomalies including: neural tube defects (NTD), orofacial clefts, limb deficiency defects (LDD), Down syndrome (DS), tetralogy of Fallot (TOF), gastroschisis (GS), hypoplastic left heart syndrome (HLHS) and transposition of great vessels (TGA) in Ontario based on the BIS data and the CIHI-DAD separately; and (2) assess the agreement of these sentinel anomalies identified between the BIS and the CIHI-DAD.

## METHODS

### Study Design and Setting of the Study

For the first objective, all hospital stillbirth (including termination at the infant/fetus's gestational age (GA) at birth ≥20 weeks or birth weight at birth ≥500 g) or live birth records of Ontario residents in the fiscal years of April 1 2012 – March 31 2013 to April 1 2015 - March 31 2016 were captured in the BIS and the CIHI-DAD separately. In the BIS, a data linkage was conducted to identify the eight selected CAs either from a prenatal stage for fetal anomalies or a postnatal stage for newborn anomalies. This internal linkage was performed to link individual records across different encounter databases where CAs might be reported (e.g., prenatal screening, antenatal specialty, birth, or NICU/SCN records). Due to data limitation, we were only able to link singletons with anomalies identified during the prenatal stage but both singletons and high order births at birth and postnatal stages. Anomalies collected in the BIS can be flagged as "Suspected" or "Confirmed" cases. The determination of "Suspected" or "Confirmed" cases depends on the type of CA and is based on clinical assessment performed at each clinic or hospital site. In general, if a CA has not been confirmed yet, the CA is entered as "suspected" in the BIS. Suspected anomalies are identified by indirect means (e.g., prenatal ultrasound). Confirmed anomalies are to be recorded through direct means (e.g., amniocentesis or direct clinical evaluation) (personal communications with clinical experts). At many sites, nurses, genetic counselors or clinic clerks are responsible for data entry by selecting values from a multi-option anomaly picklist; in other centers, birth anomalies are identified through direct upload from the hospital electronic health record. BORN registry adopts a passive case ascertainment method to collect anomaly data in BIS. We calculated both an overall rate which combined "Suspected" and "Confirmed" cases; as well as a "Confirmed" only prevalence rate in the BIS. Among the

**Abbreviations:** BIS, The BORN Information System (BIS); CAs, congenital anomalies; CIHI, the Canadian Institute for Health Information; DAD, Discharge Abstract Database; DS, Down syndrome; HLHS, hypoplastic left heart syndrome; GS, gastroschisis; LDD, limb deficiency defects; NTD, neural tube defects; TGA, transposition of great vessels; TOF, tetralogy of Fallot.

**TABLE 1 |** Picklist values in BIS and ICD-10-CA code in CIHI-DAD for Selected Congenital Anomalies.

| Congenital anomalies | Picklist values in BIS | ICD-10-CA Q code in CIHI-DAD* |
|---|---|---|
| Neural tube defects (NTD)-all | Acrania; exencephaly; Exencephaly; Craniorachischisis; Iniencephaly; Encephalocele; NTD (neural tube defect) with hydrocephalus; NTD (neural tube defect) without hydrocephaluswithout hydrocephalus | Anencephaly and similar anomalies (Q00); Spina bifida without anencephaly (Q05 if not Q00.0); Encephalocele (Q01) |
| Orofacial clefts | MOUTH-Cleft palate; MOUTH-Cleft lip; MOUTH-Cleft lip & palate | Cleft palate only (Q35 excluding Q35.7); Cleft lip only (Q36); Cleft lip with or without cleft palate (Q36, Q37) |
| Limb deficiency defects (LDD) | Generalized/other-Limb reduction defect(s) (LRD) - upper limb; Hands/feet-Clenched hands (persistently); Hands/feet-Radial ray anomaly (absent thumb); Hands/feet-Adactyly (absent fingers/ toes); Hands/feet-Ectrodactyly (lobster-claw / cleft hand); Generalized/other-Limb reduction defect(s) (LRD) - lower limb; Generalized/other-Phocomelia | Q71: Reduction defects of upper limb<br>Q72: Reduction defects of lower limb<br>Q73: Reduction defects of unspecified limb |
| Gastroschisis (GS) | Gastroschisis | Gastroschisis (Q79.3) |
| Down syndrome (DS) | Trisomy 21 (Down syndrome); Trisomy 21 (Down syndrome) - mosaic; Trisomy 21 (Down syndrome) - translocation | Down syndrome (Q90) |
| Tetralogy of Fallot (TOF) | Tetralogy of Fallot | Tetralogy of Fallot (Q21.3) |
| Hypoplastic left heart syndrome (HLHS) | HLHS (hypoplastic left heart syndrome) | Hypoplastic left heart syndrome (Q23.4) |
| Transposition of great vessels (TGA) | Double outlet ventricle (DOV); Transposition of great vessels (TGA); Transposition of great arteries - congenitally corrected (CCTGA) | Transposition of great vessels (Q20.1, Q20.3, Q20.5) |

*BIS, BORN Information System; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database. *The definition and grouping of congenital anomalies was based on the report list provided by the PHAC.*

BIS, 11.3% of these eight anomaly cases were stillbirths and 8.8% of them were terminated. In the CIHI-DAD, we identified cases of the eight CAs among stillbirth and live birth records using ICD-10-CA codes starting with letter "Q" in the report list provided by the PHAC (2). We found 7.9% of these CAs cases were stillbirths in the DAD.

The provincial health card number (Ontario Health Insurance Plan [OHIP]) is a unique identifier, and has been assigned to almost all newborns (8). Therefore, for the second objective, we were able to use the 10 digit infant OHIP number to deterministically link birth records between the BIS and CIHI-DAD in the the 4-year period. Most stillbirths and a small number of livebirths records in both databases do not have an OHIP number. Therefore, we were not able to perform linkage using an OHIP number. Among the unlinked cases, the infants have one or more diagnoses of these eight anomalies. In this linked cohort, we further obtained records with anomaly diagnoses either through the BIS or the DAD. The picklist values for the 8 anomalies in the BIS and corresponding diagnosis ICD-10-CA Q codes used for identifying those eight anomalies in the DAD are listed in **Table 1**.

## Analysis

The overall BIS prevalence rate of one specified CA is defined as the number of live births or stillbirths having a "confirmed" or "suspected" specified CA that was identified from the BIS, expressed as a proportion of the total number of live births and stillbirths delivered in an Ontario hospital with infant residence in Ontario. The BIS "confirmed" prevalence rate of one CA was defined as the number of live births or stillbirths identified having a "confirmed" specified CA that was identified from the BIS, expressed as a proportion of the total number of live births and stillbirths delivered in hospitals with infant residence in Ontario. The DAD prevalence rate of one CA is defined as the number of live births or stillbirths having a specified CA that was identified from the DAD, expressed as a proportion of the total number of live births and stillbirths delivered in an Ontario hospital with infant residence in Ontario in the DAD.

Percent agreement and Kappa statistics were performed to assess the reliability (agreement) of CAs identified in the linked BIS and CIHI-DAD birth records. We applied the following conventional criteria to judge the strength of the agreement: Kappa coefficient $< 0$: less than chance agreement; 0.01–0.20: slight agreement; 0.21–0.40: fair agreement; 0.41–0.60: moderate agreement; 0.61–0.80: substantial agreement; 0.81–0.99: almost perfect agreement (12, 13). For those anomalies with Kappa coefficients $<0.9$, we examined potential reasons for these discrepancies. All data linkages and analysis were performed using SAS 9.4.

## Ethics Consideration

As a quality assurance project, this data quality assessment was exempt from Research Ethics Board review under article 2.5 of the TCPS2 (the overarching ethical framework for research involving human participants in Canada) (14).

# RESULTS

**Figure 1** shows eight CA prevalence rates captured by the BIS overall ("Confirmed" or "Suspected"), BIS "Confirmed" only,

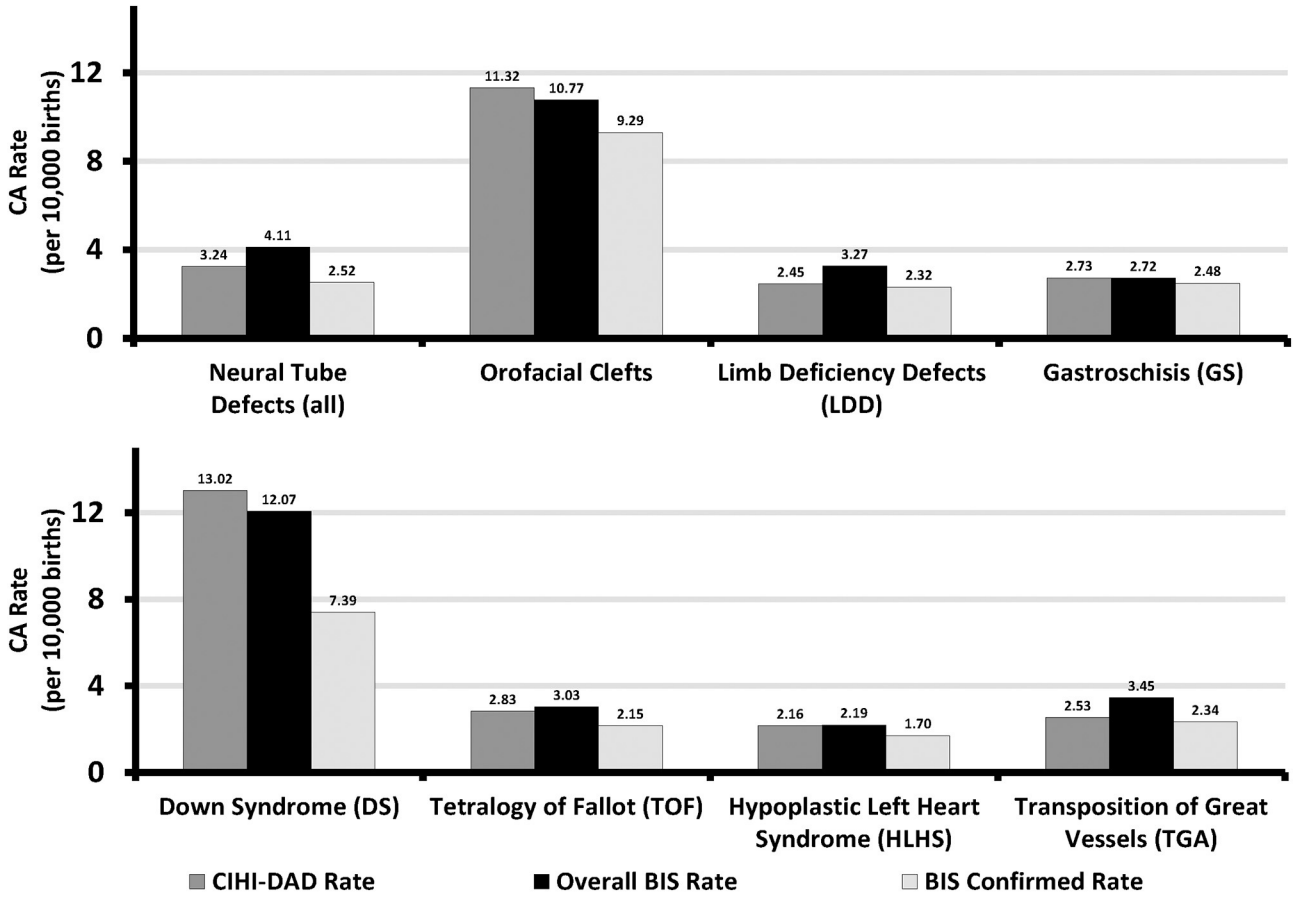


**FIGURE 1 |** Sentinel Anomalies Prevalence Rates in Ontario, Canada (March 31 2012 - April 1 2013 to March 31 2015 - April 1 2016). BIS, BORN Information System; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database.

and CIHI-DAD from April 1 2012 to March 31 2016 including 4.11, 2.52, and 3.24 per 10,000 births for NTD, 10.77, 9.29, 11.32 per 10,000 births for orofacial clefts, 3.27, 2.32, 2.45 per 10,000 births for LDD, 2.72, 2.48, and 2.73 per 10,000 births for GS, 12.07, 7.39, 13.02 per 10,000 births for DS, 3.03, 2.15, 2.83 per 10,000 births for TOF, 2.19, 1.70, and 2.16 per 10,000 births for HLHS, and 3.45, 2.34, 2.53 per 10,000 births for TGA.

Over the study period, a total of 539,272 Ontario hospital birth records from the BIS and CIHI-DAD were linked using the infant's OHIP number. The linkage rates between the two databases were 96.8% (539,272 out of 557,033) in the BIS and 99.3% (539,272 out of 543,001) in the DAD (**Figure 2**). The total number of births from the BIS is higher than those from the DAD because the BIS also includes records of infants whose mothers delivery in Ontario but live outside of Ontario and records of infants are delivered outside of hospitals such as homes and birth centers. The DAD that we receive for analysis only includes records of infants whose mothers delivery babies in Ontario and live in Ontario, and the records of infants who are delivered in hospitals. **Table 2** shows the number of CAs identified from the BIS and the DAD. Although percent agreements of all eight anomalies were over 99.9%, Kappa tests indicate that the degrees of agreement on diagnosis between BIS ("Confirmed" or "Suspected" cases) and CIHI-DAD were varied. The Kappa coefficients (κ) for GS, Orofacial clefts, DS, TOF, TGA, HLHS, NTD-all, and LDD were 0.96, 0.81, 0.75, 0.71, 0.62, 0.59, 0.53, and 0.30, respectively (**Table 2**). Except for the Kappa coefficient on GS ($K = 0.96$), the other seven anomalies had varied degrees of discrepancies. We thus further investigated the CA cases in the linked cohort that were captured in the DAD only (**Table 3**). All births were recorded as live births in both the BIS and DAD and there were only a small number of infants/fetuses whose GA at birth were <30 weeks (<5%). The neonatal transfer rate ranged from 28.36 to 76.03% among the records where CAs were identified in DAD birth records only. Regarding the most frequent and second most frequent ICD-10-CA codes of these seven anomalies identified in the DAD but not the BIS, we found Q05.9 and Q05.8 for NTD; Q35.9 and Q36 for orofacial clefts; Q71.3 and Q72.8 for LDD; Q90.9 and the number of other ICD-10-CA code < 6 for DS; Q20.1 and Q20.38 for TGA. There were no subtypes of ICD-10-CA for TOF and HLHS.

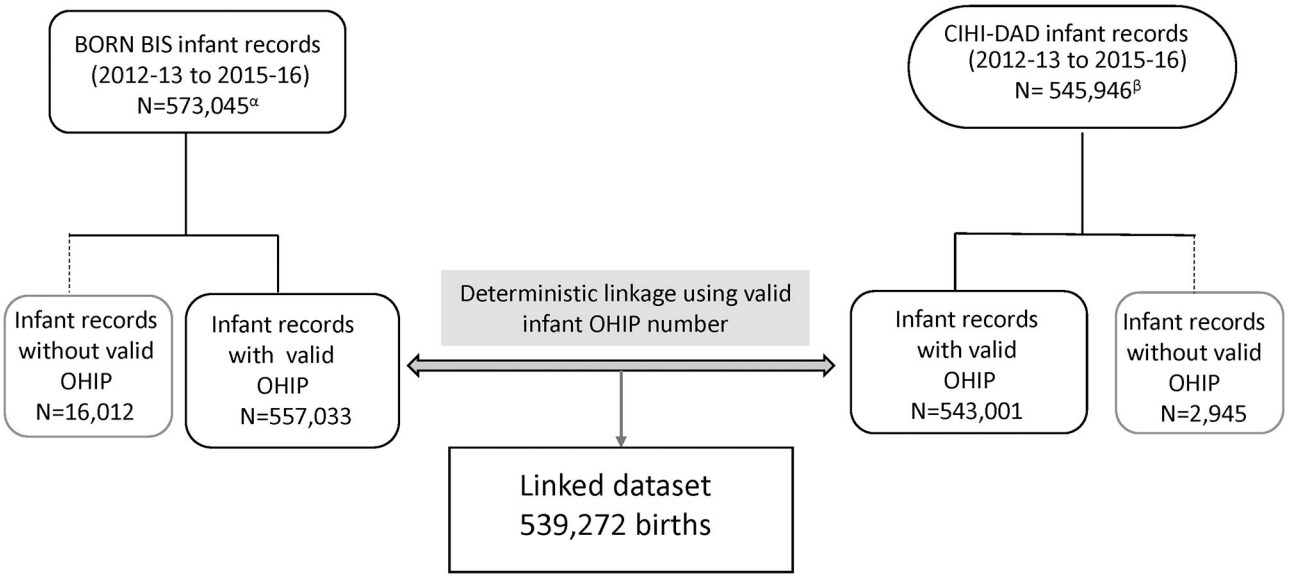


**FIGURE 2 |** Flowchart of linkage for agreement evaluation in Ontario, Canada (March 31 2012 - April 1 2013 to March 31 2015 - April 1 2016). BIS, BORN Information System; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database; OHIP, Ontario Health Insurance plan. $^{\alpha}$BORN BIS infant records include all birth records in Ontario. $^{\beta}$CIHI-DAD infant records include hospital births and infant mother's residence in Ontario only.

**TABLE 2 |** Reliability Assessment on Selected Congenital Anomalies between CIHI-DAD and BIS Birth Records (Ontario, 2012-2013 to 2015-2016)*.

| Selected sentinel congenital anomalies | BIS (N)* | DAD (N)* | Percent agreement | Kappa coefficient | Kappa 95% CI | | Kappa *p*-value |
|---|---|---|---|---|---|---|---|
| | | | | | Lower limit | Upper limit | |
| Neural tube defects (NTD) | 158 | 129 | 99.97 | 0.53 | 0.46 | 0.60 | < 0.0001 |
| Orofacial clefts | 557 | 600 | 99.96 | 0.81 | 0.78 | 0.83 | < 0.0001 |
| Limb deficiency defects (LDD) | 152 | 124 | 99.96 | 0.30 | 0.23 | 0.37 | < 0.0001 |
| Gastroschisis (GS) | 140 | 134 | 100.00 | 0.96 | 0.93 | 0.98 | < 0.0001 |
| Down syndrome (DS) | 591 | 637 | 99.94 | 0.75 | 0.72 | 0.77 | < 0.0001 |
| Tetralogy of Fallot (TOF) | 151 | 136 | 99.98 | 0.71 | 0.65 | 0.77 | < 0.0001 |
| Hypoplastic left heart syndrome (HLHS) | 85 | 72 | 99.99 | 0.59 | 0.49 | 0.68 | < 0.0001 |
| Transposition of great vessels (TGA) | 166 | 117 | 99.98 | 0.62 | 0.55 | 0.68 | < 0.0001 |

**Data was linked between BIS and CIHI-DAD using the baby's valid OHIP number. Most stillbirths were excluded due to lack of the valid OHIP number. In BIS, both "Confirmed" and "Suspected" cases were included. In addition, CA cases were identified from newborn anomalies for both singletons and high-order multiple births and fetal anomalies for singletons only. BIS, BORN Information System; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database.*

## DISCUSSION

In this study we calculated three types of prevalence rates for eight selected anomalies: overall prevalence (suspected + confirmed) in the BIS, confirmed prevalence in the BIS, and prevalence in the DAD. The prevalence rates of these anomalies have varied in the international literature. Certain CA rates identified in the BIS and CIHI-DAD over the study period are comparable with the reported CA rates in other countires (2). The average prevalence of GS and TOF were estimated as 2.75 per 10,000 births in the years of 2014-2015 and 2.82 per 10,000 births in the years of 2003-2012, respectively, in the European Union countries; the overall BIS and CIHI-DAD rates were almost the same (15, 16). Furthermore, the prevalence rates of DS, HLHS, TGA and orofacial clefts were in the range of that reported in the province of Alberta Canada, certain states of the USA and European countries (15–18). The lowest rates of NTD (6.3 per 10,000 births) and LDD (3.3 per 10,000 births) in the literature were still higher than those reported in Ontario, which suggests that both the BIS and DAD may not capture all cases (15, 16, 18).

The prevalence rates for anomalies identified from the BIS (overall or confirmed) and DAD in the 4-year period varied by type of anomaly. These three types of prevalence rates were almost identical for GS. The prevalence rate for orofacial clefts and DS were higher in the DAD than the overall and confirmed prevalence rates in the BIS. Furthermore, for LDD and TGA the

**TABLE 3 |** Distribution of Selected Congenital Anomalies Identified in CIHI-DAD only (Ontario, 2012-2013 to 2015-2016)[¥].

| Selected sentinel congenital anomalies | Cases captured in DAD only, N | Gestational age at birth < 30 weeks, N (%)* | Neonatal transfer, N (%)* | Most frequent ICD-10-CA Q code in DAD, N (%) | Second most frequent ICD-10-CA Code in DAD, N (%) | List of all other ICD-10-CA code in DAD |
|---|---|---|---|---|---|---|
| Neural tube defects (NTD) | 53 | <6 | 16 (30.19%) | Q05.9 (NTD unspecified), *N* = 28 (58.23%) | Q05.8 (Sacral spina bifida without hydrocephalus), *N* = 7 (13.21%) | Q00.0, Q01.1, Q01.2, Q01.8, Q01.9, Q04.8, Q05.4, Q05.6, Q05.7 |
| Orofacial clefts | 134 | 6 (4.48%) | 38 (28.36%) | Q35.9 (Cleft palate, unspecified), *N* = 49 (36.57%) | Q36 (Cleft lip), *N* = 32 (23.88%) | Q35.1, Q35.3, Q35.5, Q37 |
| Limb deficiency defects (LDD) | 83 | <6 | 26 (31.33%) | Q71.3 [Congenital absence of hand and finger(s)], *N* = 21 (25.30%) | Q72.8 [Other reduction defects of lower limb(s)], *N* = 20 (24.10%) | Q71.2, Q71.4, Q71.6, Q71.8, Q71.9, Q72.3, Q72.4, Q72.9, Q73.0, Q73.8 |
| Down syndrome (DS) | 179 | 7 (3.91) | 63 (35.20%) | Q90.9 (Down syndrome, unspecified), *N* = 173 (96.65%) | NA | Q90.1, Q90.2 |
| Tetralogy of Fallot (TOF) | 34 | <6 | 21 (61.76%) | No sub-group Q code | NA | NA |
| Hypoplastic left heart syndrome (HLHS) | 26 | <6 | 19 (76.03%) | No sub-group Q code | NA | NA |
| Transposition of great vessels (TGA) | 30 | <6 | 13 | Q20.1 (Double outlet right ventricle), *N* = 12 (40.00%) | Q20.38 (Other transposition of great vessels NEC), *N* = 8 (26.67%) | Q20.30, Q20.31, Q20.58 |

[¥] *The selected anomalies identified in CIHI-DAD only were all live births. The number of gastroschisis cases identified only in DAD birth records is <6, so gastroschisis cannot be shown in the above table in order to conform with privacy restrictions. Similarly, all numbers < 6 in each cell are not shown. All % represents the percent of all cases identified in each anomaly. The most and second most frequent ICD-10-CA codes are not mutually exclusive. Due to privacy restrictions, only ICD-10-CA codes are listed in the last column. Coding manual can be accessed through the following link: https://secure.cihi.ca/free_products/CodingStandards_v2018_EN.pdf.*

**Based on information from the BIS. Missing values were excluded to calculate rate (%). % of neonatal transfers was not reported for TGA due to missing values >30%.*

*BIS, BORN Information System; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database; NA, not applicable; GA, gestational age.*

prevalence rate in the DAD was lower than the overall BIS rate but close to the confirmed BIS rate. Lastly, for NTD and HLHS the prevalence rate in the DAD was lower than the overall BIS rate but higher than the confirmed BIS rate.

Similarly, the degree of agreement varied on different CAs captured in the BIS and CIHI-DAD. The highest degree of agreement was gastroschisis ($\kappa = 0.96$, close to one), suggesting almost perfect agreement on GS between the two databases. The next highest Kappa coefficient was oral-facial clefts ($\kappa = 0.81$) falling into the almost perfect category as well. DS ($\kappa = 0.75$), TOF ($\kappa = 0.71$), and TGA ($\kappa = 0.62$) fell into the substantial agreement category. HLHs ($\kappa = 0.59$) and NTD ($\kappa = 0.53$) were in the moderate agreement category. The lowest agreement was LDD ($\kappa = 0.30$) which indicated slight agreement.

Both crude comparisons of prevalence rates and Kappa test show that there is a high concordance on GS case ascertainments in the BIS and CIHI-DAD. GS is a birth defect occurring in the early stage of fetal development and is commonly diagnosed at 18–20 weeks of pregnancy by an ultrasound test. The diagnosis is usually easily confirmed after birth when the infant's intestines or other organs are seen outside of the infant's body (19). No additional tests are necessary for diagnosis, it is only very rarely confused with a ruptured omphalocele (Q79.2) and again the diagnosis is made on clinical examination. Thus, compared to most other anomalies, GS is easier to achieve a higher agreement on case ascertainment between clinical workers who enter the clinical information in the BIS and CIHI-DAD.

For the remaining seven anomalies, several factors may explain the discrepancies of anomaly capture between the two databases. First, compared to healthy newborns, infants with anomalies were likely transferred from a labor and birth department to a NICU or SCN for specialized care. Currently, the BIS only captures records in four of eight Ontario level III NICU sites. Therefore, the BIS may not be capturing the anomalies if the diagnosis was completed at a SCN or NICU. Our investigation results in **Table 3** support this hypothesis. The overall neonatal transfer rate was around 10% in this linked cohort; however, a higher neonatal transfer rate to a SCN or NICU rate (28.36–76.03%) was found among the records where CAs were identified only in DAD birth records (**Table 3**).

Second, BORN's CA picklist values, representing specific anomalies, were developed and enhanced by clinical experts. There were around six hundred picklist values (diagnoses) in the BIS. However, the BORN picklist values may not cover

all anomalies such as minor and rare cases. Conversely, CIHI-DAD uses the ICD-10-CA classification to classify anomalies. Anomalies are coded in the categories of Q00-Q99. Each anomaly or anomaly sub-category code starts with a letter "Q," followed by two or three digit numbers. The letter "Q" represents "congenital anomalies" and the next two or three digits represent specific anomalies or a group of anomalies. Since BORN and ICD-10-CA are two different coding systems, there is a discrepancy between clinical diagnoses and the ICD-10-CA classification for certain sub-types of CA, posing a challenge for matching. In this linked cohort, the lowest Kappa value ($k = 0.30$) of LDD suggests most cases captured in the BIS and DAD are different infant records (55% of 152 LDD in BIS and 67% of 124 LDD in DAD). We further compared the BIS picklist values and ICD-10-CA categories for LDD and found there are some discrepancies for diagnoses or classification. In the DAD, the ICD-10-CA classifies LDD into three broader categories. They are Q71 for "Reduction defects of upper limb"; Q72 for "Reduction defects of lower limb"; and Q73 for "Reduction defects of unspecified limb." On the other hand, in the BIS picklist, two general picklist values of "Generalized/other-Limb reduction defect(s) (LRD) - upper limb" and "Generalized/other-Limb reduction defect(s) (LRD) - lower limb" are able to match all subcategories under Q71 and Q72, respectively, in the ICD-10-CA. Specific LDD including "Clenched hands (persistently)" and "Radial ray anomaly (absent thumb)" can be grouped into the ICD-10-CA code Q71.3. The BIS picklist values of "Ectrodactyly (lobster-claw/cleft hand)" and "Generalized/other-Phocomelia" are matched with Q71.6 and Q73.1, respectively. However, the single picklist value of "Adactyly (absent fingers/ toes)" cannot be matched with neither Q71 nor Q72. In addition, there is one specific picklist value of "Generalized/other-Phocomelia" in the BIS to match a specific Q73.1. In addition, the BIS only contains one pick list value that aligns with sub-category Q73.1 but does not contain any pick list values that align with sub-categories Q73.0, Q73.8, or the overarching category Q73. Furthermore, we also found the most frequent ICD-CA Q for LDD is Q71.3 [Congenital absence of hand and finger(s), $N = 21$ (25.30% of 83)] and the second most frequent ICD-10-CA Code in DAD is Q72.8 [Other reduction defects of lower limb(s), $N = 20$ (24.10% of 83)]. Generally speaking, compared with other more severe anomalies, absence of one finger, one toe or not otherwise specified (NOS) reduction defects of lower limb(s) is much milder and less noticeable. Therefore, it is highly unlikely to be captured in both the BIS and DAD (personal communications with clinical experts).

Finally, CA data entry only captures a "snapshot" of CA diagnosis in both the BIS and DAD (personal communication with clinical experts in Ontario). Thus, there is a potential that anomaly diagnosis was corrected after further clinical investigation. However, we lack "follow-up" data to correct the diagnosis. It is possible that there is a misclassification of CA diagnosis in both databases including DS, NTD, TOF, HLHS and TGA, which require additional investigations including genetic testing, neuroimaging or echocardiography, for accurate diagnosis and anomaly classification. **Table 3** shows Q05.9, Q35.9 and Q90.9 are the most frequent ICD-10-CA captured in the DAD but not in the BIS. These codes are all in the "unspecified" categories implying that more specified CA diagnosis may be needed after receiving test results (personal communications with clinical experts during the PHAC annual meeting).

This study has several limitations. First, we assessed the agreement of selected anomalies between two databases and discussed that the way this information is captured in the BIS may contribute to discrepancies. Alternatively, anomaly case ascertainment discrepancies could also be due to how the DAD data are collected, which we are unable to evaluate. In the current study, we were not able to ensure which diagnosis represents the truth; in future work we plan to conduct a chart review to verify the discrepancies in diagnoses between two databases. A re-abstraction study may also be needed to evaluate the validity of anomaly data entry in the BIS. Second, in cases of multiple gestation, there is a challenge with the reliable identification of fetus A/B/C etc. throughout the pregnancy (across scans and across antenatal specialty/prenatal screening follow-up encounters), and subsequent challenges with accurately linking each fetus to the respective infant in the BIS infant datasets. In order to minimize misclassification bias, we only linked fetal anomalies in singletons and all newborn anomalies in the BIS databases. This limitation led to fewer fetal anomalies identified in the BIS. However, the proportion of fetal anomalies (<5%) in multiple gestation pregnancies is small in the BIS prenatal databases. Third, the discrepancies between the two databases on the selected CA diagnoses could be due to a potential linkage error, which may occur when the data entry personnel manually enter infants' OHIP numbers (20). However, in our study we expect the impact of this type of linkage error is minor because both BORN Ontario and CIHI apply strict rules and algorithms to identify correct infant OHIP numbers (5, 21). Finally, unlike the active surveillance system in the province of Alberta, Canada (22), a passive case ascertainment method is being adopted for the Ontario congenital anomaly surveillance. Cases are identified from different data sources. This method is the most feasible and cost efficient method for current surveillance in Ontario. Case reports and data entries are voluntary, which might contribute to issues such as incomplete data entry and inaccurate case ascertainment (23, 24). BORN has strived to develop strategies to improve case ascertainment and enhance the CA surveillance data quality.

## CONCLUSIONS

In summary, we found a high percent agreement between the BIS and CIHI for all anomalies in this study, although the degree of agreement varied among sentinel CAs identified. The potential reasons for discrepancies include incompleteness of capturing CAs using existing picklists, especially for certain sub-types, incomplete NICU data in the BIS, and differences between clinical diagnosis in the BIS and ICD-10-CA coding in the CIHI-DAD. In order to increase the accuracy of anomalies ascertainment and enhance the capture of anomaly data, BORN has developed strategies to find more data sources including recruiting more NICU/SCNsites to

contribute newborn anomaly data, developing a new database to improve the completeness of fetal anomaly data entry, and linking cytogenetics data for verification of chromosomal anomalies ascertainment. BORN is collaborating with additional data partners that can contribute to greater ascertainment of CAs.

The next step will be a data abstraction study to explore and investigate the potential reasons for discrepancies based on hospital chart review. This project helps quantify the quality of CA data collection in the BIS, enhances understanding of CA prevalence in Ontario and provides direction for future data quality improvement activities.

## DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study is held securely at the prescribed registry BORN Ontario. Data sharing regulations prevent this data from being made available publicly due to the personal health information in the datasets. Enquiries regarding BORN data must be directed to BORN Ontario (Science@BORNOntario.ca).

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not provided by the participants' legal guardians/next of kin because as a quality assurance project, this data quality assessment was exempt from Research Ethics Board review in Canada (14).

## AUTHOR CONTRIBUTIONS

QM: project development, data management and analysis, manuscript writing, and editing. SD and AM: project development, manuscript writing, and editing. All authors have read and approved the manuscript.

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# Barriers and Considerations for Diagnosing Rare Diseases in Indigenous Populations

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Advances in omics and specifically genomic technologies are increasingly transforming rare disease diagnosis. However, the benefits of these advances are disproportionately experienced within and between populations, with Indigenous populations frequently experiencing diagnostic and therapeutic inequities. The International Rare Disease Research Consortium (IRDiRC) multi-stakeholder partnership has been advancing toward the vision of all people living with a rare disease receiving an accurate diagnosis, care, and available therapy within 1 year of coming to medical attention. In order to further progress toward this vision, IRDiRC has created a taskforce to explore the access barriers to diagnosis of rare genetic diseases faced by Indigenous peoples, with a view of developing recommendations to overcome them. Herein, we provide an overview of the state of play of current barriers and considerations identified by the taskforce, to further stimulate awareness of these issues and the passage toward solutions. We focus on analyzing barriers to accessing genetic services, participating in genomic research, and other aspects such as concerns about data sharing, the handling of biospecimens, and the importance of capacity building.

**Keywords: Indigenous populations, genomics, diagnosis, rare diseases, equity**

## INTRODUCTION

The number of people worldwide living with a rare disease is estimated between 263 and 446 million (1), and there are an estimated 370–500 million Indigenous peoples in the world, spread across 90 countries and speaking the major share of the world's almost 7,000 languages (2). People with rare diseases frequently suffer from inequitable access to diagnosis and treatment, and the majority of the world's population is currently outside the perimeter of readily accessible diagnostic applications.

Indigenous peoples represent a rich diversity of cultures, religions, traditions, languages, and histories. Globally, the health status of Indigenous peoples frequently varies significantly from that of non-Indigenous people population. While making up <5% of the world's population, Indigenous people account for 15% of the poorest (3). Indigenous people life expectancy is up to 20 years lower than that of the non-Indigenous population (3). Children born into Indigenous families often live in remote areas where there may be relative underinvestment in, or access to, basic social and medical services. Indigenous families, be they metropolitan or more remote, may have limited or no access to culturally appropriate care, due to linguistic, geographic, financial, and other factors.

It takes an average of 5 years to diagnose a child with a rare disease (4). Given known inequities in Indigenous health care, and clinical experience of rare disease diagnosis in Indigenous families (5), the diagnostic odyssey is even more challenging for Indigenous persons living with rare diseases, and there is a need for research assessing the impact of this on the Indigenous communities. These delays often severely limit the benefits of an accurate diagnosis for Indigenous peoples, including psychological relief, reduced isolation, reduction of unnecessary investigations, access to improved or best practice medical care, genetic counseling, clarification of recurrence risk, provision of additional reproductive options, and in some cases access to social and educational services (6).

Over the last 20 years, Indigenous people's rights have been increasingly recognized through the adoption of international instruments and mechanisms, such as the United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP) in 2007 (7), the American Declaration on the Rights of Indigenous Peoples in 2016, 23 ratifications of the Indigenous and Tribal Peoples Convention from 1991, the establishment of the United Nations Permanent Forum on Indigenous Issues (UNPFII), the Expert Mechanism on the Rights of Indigenous Peoples (EMRIP), and the UN Special Rapporteur on the Rights of Indigenous Peoples (UNSR) (8). There is also an increasing awareness and formal recognition of the human rights of people living with rare, including undiagnosed, diseases (9). Collectively, these factors provide a convergent tapestry of rights and recognition to support advancing rare diseases diagnosis for Indigenous people.

Significant diagnostic delays and inequitable access to diagnostic services for people living with a rare disease has prompted a response by initiatives at local, national, and international levels. By way of just one example, the US National Institutes of Health (NIH) established in 2008 the Undiagnosed Diseases Program (UDP) (10), and subsequently, the Undiagnosed Disease Network (UDN; https://undiagnosed.hms.harvard.edu), and similar programs were established globally by countries participating in the Undiagnosed Diseases Network International (UDNI; http://www.udninternational.org) (11). Such programs are advancing the integration of genomics and other "omics" technologies into medical practice to reduce diagnostic delays and improving patient and family journeys. Increasing equity, including that for Indigenous peoples, is a key consideration for this and other diagnostic initiatives.

Increased diagnostic certainty may be provided through various means such as clinical expert opinion and clinical consensus including with application of diagnostic criteria where they exist (for both genetic and non-genetic rare diseases); through other phenotypic approaches such as facial analysis and other imaging; and through the building of Indigenous genomic databases and the identification of rare pathogenic variants in Indigenous Peoples with a suspected rare genetic disorder, or other "omics" technologies (e.g., epigenetic signatures). The International Rare Disease Research Consortium (IRDiRC; www.irdirc.org) has set the goal of enabling all people living with a rare disease to receive an accurate diagnosis within 1 year of coming to medical attention (12). However, significant barriers remain regarding access to diagnosis for Indigenous populations, including but not limited to poorer access to (gen)omic technologies and the research that drives them, which prevent Indigenous peoples from receiving appropriate benefits from (gen)omic and other new knowledge. Hence, IRDiRC has formed a taskforce to identify the barriers for the diagnosis of rare genetic diseases in Indigenous peoples, and areas in which there are opportunities for improvement. This is adding to the knowledge base informing future development of recommendations for improving diagnosis of genetic and rare diseases for Indigenous peoples. In this article, we focus our analysis specifically on the limited access to diagnostic services and clinical expertise, underrepresentation in genomic databases and genomics research in general, concerns with the handling of biospecimens, data use and data sharing, and the importance of building capacity within communities. We provide some examples of initiatives addressing these barriers, which are briefly summarized in **Table 1**.

### Access to Clinical Genetic Services

Clinical genetic service providers are at the front line for rare disease diagnostics and frequently act as the portal to and from translational research. Indigenous access challenges to these services, and associated testing, can include lack of referral and/or referral bias, location, cost, availability, and cultural appropriateness. This includes culturally appropriate education materials that resonate with Indigenous narratives and incorporate indigenous language; meeting places and clinical processes that accommodate for familial and community participation; consent processes adaptable to family structures; involvement of culturally appropriate support and liaison persons for addressing racism and discrimination; and cultural

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competency among genetic health-care staff and capacity building in genetic and rare diseases (be they genetic or environmental) in the primary care workforce. Each of these issues affects engagement in, and delivery of, rare disease diagnosis.

The total burden of rare diseases in Indigenous communities is unknown. Estimates of prevalence of rare disease in "mainstream" (i.e., predominantly non-indigenous ethnicity/ancestry) communities from the Orphanet database range from 3.5 to 5.9%, of which 71.9% have a hereditary basis (1). Assuming similar prevalence in Indigenous communities, this equates to 12–20 million indigenous people worldwide affected by at least one rare disease, based on an estimated worldwide population of 476 million indigenous peoples (8). While many Indigenous people live in metropolitan areas, some Indigenous groups are remote or nomadic with limited geographic access to genetic health care services—and therefore to a rare disease diagnosis. As an example, approximately 1 in 5 Indigenous Australians (Aboriginal and Torres Strait Islander peoples) live in remote areas and about 1 in 3 live in major cities (13). In Canada, half of Indigenous peoples (First Nations, Inuit, and Métis) live in a rural environment (14). For less developed populations globally, even though a doubling of urbanization occurred over the past 50 years, ∼1 in 2 people still live in rural areas (15). Telehealth and outreach clinics are undoubtedly an important part of the matrix of approaches to accommodate geographic access issues. Moreover, there are other challenges for Indigenous families, in terms of technology, internet access, bandwidth, reliability, and cultural appropriateness. In the African context, the needs of people living with rare diseases must also be balanced with basic needs, such as nutrition and communicable-disease prevention (16).

While many rare diseases affect Indigenous populations at similar rates to non-indigenous peoples, some rare diseases are geographically and/or culturally concentrated and this has implications for tailoring research and clinical care. An example is that the Machado-Joseph disease (MJD)—also called spinocerebellar ataxia Type 3 (SCA3)—is estimated to be more prevalent among Aboriginal people from small communities in remote northern Australia than anywhere else in the world (17). Geographical isolation and cultural preference for large, closely tied families have resulted in disproportionately high numbers of people living with this condition. Currently, over 650 Aboriginal Australians are thought to be "at-risk" of developing the disease. The Australian MJD Foundation (MJDF; https://mjd.org.au) was established in 2008, as a community driven response to families affected by MJD, and has pioneered an innovative model of disability service delivery to meet the needs of Aboriginal communities in remote Northern Australia (17). Core to its principles is the implementation of a "two-way," bicultural working model, i.e., the services are delivered in community by an Aboriginal community worker and a non-Aboriginal health or community service professional. The approach allows first language support which minimizes the potential for misunderstanding and disengagement for cultural mistakes. Another example from Canada is work with Gitxsan Health Society to identify the cause of sudden death, often at a young age, in this First Nation's community in northern British Columbia (18, 19). This resulted in the discovery of a mutation in the *KCNQ1* gene, which causes the Long QT syndrome and has now been found in ∼1.5% of the community's population. The team worked with the community to ensure that the research was performed in a culturally safe manner. Long QT syndrome is a rare heart rhythm condition which can be lethal if left untreated, but with appropriate treatment and monitoring patients can live a normal life. Today, anyone with a predisposition to Long QT syndrome receives evidence-based care by a team of northern British Columbia health-care workers supported by the British Columbia Inherited Arrhythmia Program. An African example is glutaric aciduria type 1 (GA1), a rare disease that can be detected by newborn screening and is treatable. The Black South African population, including Indigenous (San and KhoeKhoe) and non-Indigenous peoples, has a predicted prevalence of this disorder that is 20 times higher (1:5184) than global average (1:100,000) (20).

Understanding the total burden of rare diseases in Indigenous populations within the country they reside, the identification of the actual conditions themselves and their individual prevalence and community distribution are significant knowledge gaps to be addressed in terms of tailoring access, and provision of, genetic health care. In addition to diagnostic and epidemiological initiatives, rare disease codification with granular and interoperable systems, such as Orphanet coding (21), in health data sets will be critical, as well as documentation therein of Indigenous status.

Financial access issues are also critical; these may be of particular relevance since poverty is more common among Indigenous, compared to non-Indigenous, people. Also, it is important that funding/reimbursement is sufficient to cover all aspects of diagnostic care, such as pretest and posttest culturally appropriate counseling—and not just the cost of a genetic test.

Given that Indigenous people are more likely to live regionally or remotely when compared to non-Indigenous people, methods to improve access to testing, service, and research will require a combination of approaches that embrace a mixture of telehealth, local capacity building, and outreach models. Approaches that map access closer to home and incorporate cultural, geographic, weather, and language factors will also be beneficial (e.g., Mappa; https://mappanews.org.au).

Poor access to clinical expertise at the point of care and lack of referrals (or referral bias) to genetics specialists is another barrier to diagnosis. Given the diversity and rarity of these disorders, diagnosis of rare diseases is often difficult. Rare in presentation, they can sometimes go unrecognized by practitioners. Moreover, expertise and specialist knowledge may be scarce (22). The development of the European Reference Networks (ERNs) by the European Commission in 2017 was a priority action to compensate for the scarcity of knowledge on rare diseases and improve access to clinical expertise across Europe (23). Preliminary data from the Australian Northern Territory Genetic Service suggested that rates of referral for Indigenous patients were less than half of what would be expected based on the population (24). This may be due to general lack of awareness of indicators of rare diseases by referring doctors, attributing

symptoms to other more common disorders, or a belief that services are not accessible or will not be attended.

Recognizing a patient with a rare disease can be particularly challenging in remote areas where expertise in rare diseases does not exist and for ethnic minorities such as the Irish Traveler population, a distinct Irish ethnic minority. The Irish Travelers are an endogamous nomadic ethnic minority group with a very high incidence of rare diseases. As they practice inter-cousin marriage, a greater incidence of autosomal recessive genetic disorders is reported with many rare diseases occurring in this population with a prevalence threshold >5 in 10,000 (25–27). Clinicians who are less familiar with this population had until recently no resources to support more timely diagnosis of the rare disorders in Irish Traveler patients. A catalog of the disease mutations of known genetic disorders found among this ethnic group is now publicly available (26), and a database of genetic disorders for Irish Traveler is under development to facilitate diagnosis at the point of care (27). National and ethnic specific mutation databases that may be used, for example, to facilitate the provision of genetic services, have been developed for a number of populations worldwide. Some examples include the Mediterranean Founder Mutation Database (28), the Omani National Genetic Database (29), the Israeli National Genetic Database (30), the Moroccan Genetic Disorders Database (31), and the Amish, Mennonite, and Hutterite Genetic Disorder Database (32).

A lack of cultural appropriateness may also challenge Indigenous rare disease diagnosis. This may be due to language barriers, location and setup of clinical rooms, a lack of accounting for gender roles and responsibilities, or lack of awareness of other cultural norms and responsibilities. Communication between Indigenous patients and health-care practitioners' professionals continues to be problematic and is arguably the greatest barrier to the delivery of successful health care and translational research for Indigenous peoples (33). Communication barriers include language issues and the use of medical jargon. These undermine constructive practitioner–patient relationships and result in Indigenous patients and families feeling alienated, non-compliant, and disengaged from health care. Indigenous patients and families want to be informed about health and disease, while information is frequently lacking, inadequate, or presented in ways that are incongruent with indigenous peoples' beliefs, narratives, and experiences (34, 35). Communication issues are compounded when families are uninvolved in communication processes (36, 37). Additionally, a history of negative health-care experiences that parallel other negative colonization experiences results in mistrust and suspicion by Indigenous people of health practitioners and patient information provided (36). Building trust through sustained community partnerships and the use of culturally appropriate language and concepts are critical. One initiative to build the lexicon, narratives and trust to address rare diseases communication issues is Lyfe Languages (www.lyfelanguages.com) (38), which involves translating the standardized description of phenotypic abnormality in man (the Human Phenotype Ontology) into Indigenous languages through community and transgenerational partnerships. Launched with a focus on Aboriginal Australian languages, this has now extended to African and Swedish (Sami) languages. Where Indigenous people may be fluent in English, have English as a second, third, or fourth language, or speak a blended mixture of English and Indigenous languages, such as Aboriginal-English for example, outputs of Lyfe Languages have been adapted to these contexts. Recognizing that some Indigenous people may have no or intermittent internet access, Lyfe Languages has commenced the development of resources (e.g., Apps) that are variably web-based or mobile applications and designed to run with periodic syncing to a web platform, while also supporting scalable and bespoke hard copy (paper) resource development.

While there is a relative dearth of initiatives to improve Indigenous access to genetic and genomic care, from a non-disease-specific basis and an Australian perspective, over the last decade, a number of state-based, multi-state, and more recently national approaches in Australia have begun to address inequitable access to genetic diagnostics and genetic health care across the breadth of rare diseases. These both complement and learn from the extensive work of the MJD Foundation in Australia's Northern Territory. They include initiatives from The National Center for Indigenous Genomics (described further below); the work of the Western Australia (WA) Health Department, the Aboriginal Health Council of WA, and WA Aboriginal Communities (39), ACTG—Aboriginal Community-led Translation of Genomics (40); the work of Genetic Health Queensland and Queensland Aboriginal and Torres Strait Islander Controlled Health Services (41); the Better Indigenous Genetics (BIG) Project, a translational research project for improving genetic health care service delivery in Australia (42); and more recently the work of the POCHE Center for Indigenous Health (43). Each has specific foci and overlaps.

## Underrepresentation in Genomic Research and Reference Databases

The emergence of large-scale databases and biobanks, prompted by the proliferation of large-scale population genomics projects worldwide, has paved the way to genomic medicine. Globally, however, Indigenous peoples remain understudied and underrepresented in genome reference databases (44). For example, a recent analysis revealed that as of 2018, only 22% of individuals in genome-wide association studies (GWAS) were of non-European ancestry (45). People of African and Latin American descent and Indigenous people combined represented <4% of participants. Indigenous participants represented 0.02%, which equates to an alarming 250-fold underrepresentation based on the number of Indigenous people. This under-representation of ethnically diverse populations in human genomic studies has important implications for the interpretability of genomic variants and diagnostic assessments (46). As genome sequencing is increasingly used in the diagnosis of rare and undiagnosed conditions (47), reference genomes from more ethnically diverse populations are needed for reliable interpretation of results and ultimately the effective and responsible implementation of precision medicine in minority populations (16, 46).

A number of initiatives are seeking to address the gap in the availability of Indigenous genome reference databases and ensure culturally appropriate access to data by engaging and co-designing with Indigenous communities, ascertaining the data through trusted partnerships and making it available through mechanisms that embrace both FAIR and CARE principles, discussed further in section Cultural Considerations for the Use of Biospecimens and Data Sharing. The National Center for Indigenous Genomics (NCIG, https://ncig.anu.edu.au) is creating Australia's first database of Indigenous genomes for use in research of benefit to Indigenous Australians (48). The resource is being developed from an existing collection of Indigenous biospecimens held by the Australian National University (ANU) since the 1960s and supplemented by the ongoing addition of new material. Another Australian initiative includes the work of the Telethon Kids Institute to deliver the first whole-exome genomic reference data, and related data access processes, initiating with Western Desert People of Western Australia (40). In Canada, the Silent Genomes project launched in 2018 with the goals of reducing health-care disparities and improving diagnostic success for Indigenous children with genetic diseases plans the development of a database of background genetic variations for Indigenous populations from across Canada (49, 50). In Aotearoa/New Zealand, a similar initiative has commenced, with the goal of developing a catalog of genetic variants within Indigenous Māori, to expedite identification of causative variants and disease diagnoses, as well as development of gene-based diagnostics (51). Like the Silent Genomes project, this is co-led by Indigenous researchers and guided by Indigenous ethical frameworks relevant to the study populations (50).

Other efforts to include minority populations in human genetic studies include the Human Heredity and Health in Africa Initiative (H3Africa; https://h3africa.org), jointly funded by the NIH in the US and the Wellcome Trust in the UK to advance African-led genome research (52), the UK MRC-funded International Center for Genomic Medicine in Neuromuscular Diseases (ICGNMD) (53), and the NIH All of Us research program (https://allofus.nih.gov), a precision medicine initiative which plans to collect DNA and health data from one million people including American Indians and Alaska Natives (54). China has also launched the precision medicine initiative (55), supporting the cohort studies covering healthy population and special disease populations in different geographical areas, where the ratio of Han ethnicity and minority peoples varies among each other. One of the cohort studies with the funding from the precision medicine initiative, covering the registry for over 100 rare diseases, has been performed since 2016 and it recruited patients with a breadth of ethnicities in China (56).

However, aiming for a more diverse population representation in genomic research is not without challenges. In the US, for example, while efforts to recruit tribal nations in the All of Us cohort study are ongoing, a recent report from the Tribal Collaboration Working Group has underlined a range of issues that need to be addressed if tribes are to participate, including a need to recognize tribal sovereignty, rights, and interests (57). The report also raised questions about responsible data use, cultural appropriateness, biospecimen storage and access, and protection and benefits. In another study examining Indigenous Canadians' concerns for contributing their data to a genomic database, issues were raised around lack of trust due to historical transgressions, control and ownership of samples, privacy, and fear of discrimination (58).

Whereas experiences with unethical research practices have caused some individuals to mistrust health research and resulted in Indigenous peoples' hesitancy to participate in genomic research (59), guidelines and ethical frameworks for respectfully engaging with Indigenous communities have emerged in recent years to help build trust and repair relationship. Guidelines for genomics research with Indigenous peoples developed by countries such as the US, Canada, New Zealand, and Australia now provide broad protections for research participants, including protection for sample collection, secondary uses, benefits, and the right to withdrawal from research (59). A framework for enhancing ethical genomic research with Indigenous communities provides a set of principles for conducting respectful engagement, such as understanding tribal sovereignty and research regulation, engaging with the community throughout the research process, building cultural competency of researchers, improving transparency of research practices, building tribal capacity in the research process, and disseminating research findings in a community-accessible format (60). Additionally, the development of culturally appropriate guidelines such as *Te Mata Ira: Guidelines for Genomic Research with Maori* in New Zealand (61) provides directions on the cultural concepts, logic, and values that underpin Maori perspectives on genomic research and outlines a framework to engage communities in a culturally acceptable manner. A culturally informed biobank model that draws on Maori-informed practices has also been developed and provides a framework for considering cultural values in relation to Indigenous peoples in other countries, and society in general, to ensure that biobanking policies, procedures, and practices are culturally acceptable (62).

## Cultural Considerations for the Use of Biospecimens and Data Sharing

Indigenous cultural practices add complexity to the ethical issues surrounding genetic and genomic research, at both the individual and collective levels. Challenges to incorporate local cultural practices into research may negatively influence decisions about participation in a research project (63–65). For instance, indigenous perceptions that blood specimens and other tissue samples remain intimately connected to their ancestors and their lands raise ethical concerns related to the collection, storage, and use of biospecimens (63, 65). It also raises questions about collective ownership, tribal belonging, repatriation, and the use of biospecimens beyond national borders (63). In Australia, the NCIG addresses these challenges by including an Indigenous Majority Board on its governance, which is responsible for the custodianship of biological samples and for its management and use (66). Recently, the NCIG started to offer participants and relatives of deceased people whose historical

samples are in its collection the option of having their remains repatriated to their place of origin (67). Research approaches that promote community participation, such as the application of community-based participatory research (CBPR) principles (68), the adoption of ethical research guidelines (61, 62, 69, 70), and the development of culturally informed policies (71, 72) offer ways to ensure that research involving indigenous peoples is premised on respectful relationships.

Indigenous concerns around data use and sharing is another barrier to research participation (73, 74). This is increasingly important as the current move toward broad data sharing and open data impacts the data governance rights of Indigenous nations (75). As a response, stricter mechanisms of control over biological samples and associated data are emerging by Indigenous Peoples' (e.g., tribal review/oversight boards, research codes, policies), and a global call for "Indigenous data sovereignty"—the right of Indigenous peoples and tribes to govern the collection, ownership, and application of their own data—is growing in intensity and scope (75, 76). Yet, at the same time, it was also acknowledged that stricter mechanisms of control to protect Indigenous rights may deepen the "genomic divide" by excluding them from the potential benefits of data sharing (77). This is especially relevant to rare diseases, where knowledge and expertise are limited and patient populations are geographically dispersed (78). Disparate data is a key barrier to detect rare diseases and the sharing of genomic and clinical data across country borders is imperative to help accelerate the time it takes to diagnose a rare disease. A counterpoint is that solutions that accommodate Indigenous concerns over data management and sovereignty and, through that build trust, may ultimately facilitate the scaling of data sharing for Indigenous and non-Indigenous people.

The reclaiming of Indigenous data sovereignty (IDS) entails Indigenous people being in control of their data and comprises the entitlement to Indigenous data governance (75). As the first Indigenous-governed genome facility in the world, the NCIG in Australia gives control of decision-making back to Indigenous people (66). The Canadian's Silent Genomes is also working in concert with Indigenous Canadian organizations and communities to establish processes for Indigenous-led governance of biological samples and genomic data (49). Additionally, growing IDS networks are working to shape open data principles to better respect the rights of Indigenous peoples, e.g., the establishment of national Data Sovereignty networks in New Zealand (Te Mana Raraunga; https://www.temanararaunga.maori.nz) (79), US (USIDSN; https://usindigenousdata.org) (80), and Australia (Maiamnayri Wingara; https://www.maiamnayriwingara.org) (81); the International Indigenous Data Sovereignty Interest Group at the Research Data Alliance (RDA IDS Group; rd-alliance.org) (82); and the Global Indigenous Data Alliance (GIDA; https://www.gida-global.org) (83). Released under the auspices of RDA, the CARE Principles of Indigenous Data Governance (collective benefit, authority to control, responsibility, ethics) provide the first international framework for the ethical use of Indigenous data (84). The Principles reflect the crucial role of data sharing and complement the existing FAIR (Findable, Accessible, Interoperable, Reusable) principles (85) as a key principle guiding equity and ethics in data open movements.

The need for phenotypic reference data parallels the need for genomic reference data (86). This will require dedicated initiatives for instance for imaging data, such as (3D) facial analysis (87) that can accommodate the same considerations as for (gen)omic data.

## Capacity Building and Empowerment

Engagement and consultation with community members, respect for cultural differences, and transparency around research are key elements for ethical research involving Indigenous peoples (60). These are seen as important steps toward forming research relationships built on trust, respect, and mutual interests, which in turn can enhance research participation. To this end, it is critically important to increase capacity among Indigenous and non-Indigenous peoples alike. Ensuring that non-Indigenous researchers have adequate cultural competency to engage with Indigenous communities in a research project often requires training before its onset. Funding bodies, such as the Australia's National Health and Medical Research Council (NHMRC) and Human Research Ethics Committees (HRECs), require researchers to demonstrate capacity to effectively conduct research with Aboriginal and Torres Strait Islander peoples (70). Recently, Silent Genomes published a document to support and foster cultural safety practices among genetic counselors working with Indigenous families (88). It is also currently developing educational materials to increase communities' genetic literacy (49). Promoting genomic literacy among Indigenous people and communities is an important area to enhance informed decision-making. The Genetic Education for Native Americans (GENA) pioneered such efforts by offering Native Americans workshops on genetics at national conferences. Today, GENA has developed into an educational program to Native Americans on genetic science (89). Although not targeted to Indigenous peoples, the NIH National Human Genome Research Institute (NHGRI; https://www.genome.gov) has developed tools and provides a list of useful educational resources to advance genomic literacy for the general public (90).

The current and projected shortage of medical geneticists and genetic counselors, who historically have provided the majority of genetic services, has created a need for genomic education of health-care professionals across disciplines (91, 92). The African Genomic Medicine Training (AGMT) Initiative, for example, was established to develop knowledge and skills of non-specialist providers in genomic medicine (93). Indigenous people, especially those living in rural and remote areas, have poor access to health services (94) and little to no access to genetic health services. An important aspect for addressing racial and ethnic health-care disparities and improving access to genetic services for underserved patients, and cross-cultural communication, is ensuring a more diverse genetic workforce (95). In a recent survey on the demographics of certified Genetic Counselors in the US, 90% of respondents identified themselves as Caucasian, while only 1% of respondents identified as black or African American. Just over 2% of respondents identified as Hispanic, 0.4% identified as American Indian or Alaskan

Native (96). When compared to the racial/ethnic distribution in medical education and the health-care workforce, Black or African American and American Indian or Alaska Natives are also underrepresented (97). The Minority Genetic Professionals Network (https://minoritygenetics.org) was formed in 2018 as an initial step to develop a more diverse workforce of medical genetic professionals to meet the needs of racial and ethnic minority populations (98). In Australia, the Better Indigenous Genetics (BIG) Project will develop training courses targeted to build capacity of Aboriginal health care workers to collaborate in the provision of genetic health service and primary care for Indigenous people. Songlines and weaving are an educational initiative being developed as a partnership between the Western Australian Register of Developmental Anomalies, Genetic Services of Western Australia, and the Roy Hill Community Foundation to develop culturally appropriate, culturally endorsed, and culturally resonant education information for use in clinical genetic practice (99). The first educational product uses the Aboriginal Song Line narrative to explain basic concepts of genetics, inheritance, and genetic health care. Capacity building in Indigenous rare diseases policy will also be required.

The dynamics of the lack of racial and ethnic diversity in genomics is paralleled in the US science and engineering workforce as according to the most recent data, American Indians or Alaska Natives are underrepresented as are blacks and Hispanics relative to their presence in the overall US workforce (100). Diversification of the research workforce is one way to ensure that genomic research better serves the needs of diverse and under-represented communities (101). Furthermore, the inclusion of Indigenous researchers in genomics can bring greater transparency to the research process, facilitate the dialogue between researchers/universities and Indigenous communities, and foster the development of research skills in genomics within communities, thereby strengthening inclusivity in biomedical research (60, 102). The Summer internship for Indigenous peoples in Genomics (SING; https://www.singconsortium.org) program offers a successful model for training and retaining Indigenous researchers in genomics. Launched first in the US and further expanded to Canada, Aotearoa/New Zealand (www.singaotearoa.net), and most recently Australia, SING is a week-long workshop that aims to train Indigenous students, scholars, community members, and researchers with next-generation genomic and bioinformatics analyses and build capacity for scientific research involving Indigenous communities. SING operates internationally through the expanding network of the SING Consortium and has organized earlier this year the first international conference on Indigenous genomics (103). Capacity building in genomic science for Indigenous communities is also being supported through programs and translational activities associated with Silent Genomes and the Aotearoa Variome (50).

## CONCLUSION

Herein, we outline some challenges and barriers to rare disease diagnosis in Indigenous people, and some examples of initiatives raising awareness and advancing solutions to the related challenges to diagnostic equity. Serving the unmet needs of Indigenous people with undiagnosed rare diseases will require a multifarious and internationally coordinated response that is grounded in engagement, co-design, and trust. Ultimately, improving Indigenous rare disease diagnosis will reduce inequity and deliver discovery, innovation, and solutions that will benefit all.

## AUTHOR CONTRIBUTIONS

GB conceived the study with input from all authors. CD'A wrote the manuscript in consultation with GB. All authors provided critical revision and approved the final manuscript.

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# Sociodemographic Differences in Prenatal Diagnosis of Chromosomal Anomalies: A Population-Based Study

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**Background:** In Europe, about 76% of cases of chromosomal anomalies are prenatally diagnosed. Prenatal diagnosis allows more efficient planning of postnatal treatment and helps parents for an informed decision about the continuation of pregnancy. The main aim of this study was to evaluate whether the sociodemographic maternal characteristics affect the probability of prenatal diagnosis of chromosomal anomalies.

**Methods:** Cases of chromosomal anomalies in the period 2005–2017 came from the population-based registry of congenital anomalies of Tuscany (Italy). Differences in the proportion of cases prenatally diagnosed were investigated through the following maternal characteristics: education, geographic origin and occupation. The association between cases of termination of pregnancy after prenatal diagnosis and maternal characteristics was also analysed. Odds Ratios (OR) adjusted by maternal age were calculated using logistic regression models. Results were provided for all cases of chromosomal anomalies and for Down syndrome cases.

**Results:** A total of 1,419 cases were included in the study. Cases prenatally diagnosed were 1,186 (83.6%). We observed a higher proportion of cases not prenatally diagnosed among cases with low maternal education compared to those with high maternal education (OR = 2.16, $p < 0.001$) and in women from high migratory outflow countries, compared to the Italian ones (OR = 2.85, $p < 0.001$). For prenatally diagnosed Down syndrome cases, we observed a higher proportion of termination of pregnancy for women with low education level (OR = 4.36, $p = 0.023$).

**Conclusions:** In our study evidence of differences in the probability of prenatal diagnosis of chromosomal anomalies associated with maternal education and geographic origin was found. Population-based studies investigating sociodemographic disparities can provide essential information for targeted public health programs. Further studies are recommended to monitor the impact of the increasing availability of non-invasive screening tests.

**Keywords: chromosomal anomalies, down syndrome, prenatal diagnosis, maternal education, maternal geographic origin, maternal occupation, socioeconomic status**



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## INTRODUCTION

Chromosomal anomalies account for about 17% of the major congenital anomalies diagnosed before the age of 1 year in Europe. According to EUROCAT, the European network of population-based registries for the epidemiological surveillance of congenital anomalies, the proportion of chromosomal anomalies prenatally diagnosed is around 76% (1). Prenatal diagnosis may allow more efficient planning of postnatal medical and surgical care of the neonate born with a congenital anomaly. Moreover, prenatal diagnosis constitutes support for parents for an informed decision about the continuation of pregnancy.

The effects of socioeconomic factors on the prevalence and the access to prenatal diagnosis of Down syndrome, the most frequent chromosomal anomaly, have been investigated in a few population-based studies (2–5). The results of these studies suggest the presence of disparities in prenatal diagnosis of Down syndrome among the different ethnic and socioeconomic subgroups. Moreover, differences in the access to prenatal diagnosis of sociodemographic subgroups generate differences in live-birth prevalence across the same subgroups (4).

The Italian healthcare system is structured with two different levels of responsibility and management: a central level and a regional level. The former ensures and guarantees universal access to essential services, regardless of the socioeconomic status (SES); the latter has direct responsibility for management and costs with exclusive competence for the regulation and organisation of services, including those during pregnancy and at birth. However, SES-based disparities persist and affect several dimensions of general health, including access to prenatal diagnosis. In Italy, despite active policies aimed at increasing prenatal testing, little is known (6, 7) about the impact of social changes, both economic and demographic, that have taken place in the last decades.

To meet the needs of this investigation, maternal education, a recognised powerful determinant of health (8), was chosen as a proxy of SES. Since the foreign presence on the national territory is a consolidated phenomenon, the maternal geographic origin was studied as another possible determinant of the access to prenatal diagnosis (9–12). Furthermore, we also explored the possible association with the maternal occupation.

Thus, the main aim of the present study was to evaluate whether the sociodemographic maternal characteristics have a role in the probability of prenatal diagnosis of chromosomal anomalies. Furthermore, we investigated whether these factors were associated with the proportion of termination of pregnancy for foetal anomaly following prenatal diagnosis (TOPFA).

**Abbreviations:** RTDC, Registro Toscano dei Difetti Congeniti; EUROCAT, European network of population-based registries for the epidemiological surveillance of congenital anomalies; TOPFA, termination of pregnancy for foetal anomaly following prenatal diagnosis; HMOC, High Migratory Outflow Countries.

## MATERIALS AND METHODS

The study population consisted of all cases with chromosomal anomalies (live births, foetal deaths with gestational age $\geq$20 weeks and TOPFA) diagnosed during the first year of life for women residing in Tuscany (Italy) in the period 2005–2017. Tuscany is an Italian region with about 3,700,000 inhabitants and 25,000 births yearly. Data on cases of chromosomal anomalies came from the population-based Registry of Congenital Defects of Tuscany (RTDC, from the Italian acronym of "Registro Toscano dei Difetti Congeniti"). RTDC is a full member of EUROCAT (13) and the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) (14). The case collection is based on a widespread network, including all obstetrical and maternity units, paediatric departments, paediatric cardiology departments, paediatric cardiac surgery units, prenatal diagnostic centres, and medical genetics units, which ensures complete geographical coverage. Cases from RTDC are linked with other regional health databases.

Differences in the probability of prenatal diagnosis for chromosomal anomalies were investigated through the following maternal characteristics: education, occupation, and geographic origin. Maternal education was stratified into three levels: low (primary and lower secondary level), medium (upper secondary level), high (tertiary level). Data on maternal education came from RTDC and the regional birth registry. Data on maternal occupation were collected in RTDC using the International Standard Classification of Occupations (ISCO-88 for births up to 2012, ISCO-08 for births from 2013). For our study, we have slightly modified the classification adopted by Zeitlin et al. (15) and we have defined the following 4 groups: manager/professional, technical/clerical/service, unskilled occupation/unemployed, housewives. The geographic origin of the mother was derived from the integrated information reported in the RTDC and the birth registry. The country of origin was classified as Italy, Developed Countries (DC) other than Italy, and High Migratory Outflow Countries (HMOC) (16). Among HMOC the most represented communities in Tuscany are from Romania, Albania, followed by China and Morocco (17). Maternal age was categorised into 3 classes: <35 years, 35–39 years, and 40+ years.

Logistic regression models, adjusted by maternal age, were used to estimate the association of maternal education, occupation, and geographic origin with prenatal diagnosis. For maternal education and occupation, estimates adjusted for geographic origin were also calculated. Adjusted Odds ratios (OR) with 95% confidence interval (95% CI) and $p$-value were calculated. Analyses were performed for all cases with chromosomal anomalies, for cases with Down syndrome only, and cases with a major trisomy. The association between TOPFA and maternal characteristics was analysed in prenatally diagnosed cases using a logistic regression model. The difference in gestational age at diagnosis was evaluated using the Wilcoxon test. Analyses stratified by period (2005–2011 vs. 2012–2017) were also performed. Results with a $p < 0.05$ were defined as statistically significant. Statistical analyses were performed using STATA version 16.0 (StataCorp LP, College Station, TX, USA).

**TABLE 1 |** Characteristics of the study population.

| Maternal characteristics | All chromosomal anomalies | | Down syndrome | |
|---|---|---|---|---|
| | *n*. (%) | Missing | *n*. (%) | Missing |
| **Prenatal diagnosis** | | | | |
| Yes | 1186 (83.6) | | 651 (77.7) | |
| No | 233 (16.4) | | 187 (22.3) | |
| Missing | | – | | – |
| **Age** | | | | |
| <35 | 427 (30.3) | | 212 (25.5) | |
| 35–39 | 557 (39.5) | | 343 (41.2) | |
| ≥40 | 426 (30.2) | | 277 (33.3) | |
| Missing | | 9 (0.6) | | 6 (0.7) |
| **Education** | | | | |
| High | 277 (30.2) | | 166 (29.8) | |
| Medium | 432 (47.2) | | 260 (46.7) | |
| Low | 207 (22.6) | | 131 (23.5) | |
| Missing | | 503 (35.5) | | 281 (33.5) |
| **Occupation** | | | | |
| Manager/Professional | 118 (14.9) | | 69 (14.3) | |
| Technical/Clerical/Service | 495 (62.4) | | 300 (62.2) | |
| Unskilled occupation/ Unemployed | 142 (17.9) | | 92 (19.1) | |
| Housewives | 38 (4.8) | | 21 (4.4) | |
| Missing | | 626 (44.1) | | 356 (42.5) |
| **Geographic origin** | | | | |
| Italian | 1032 (80.7) | | 619 (81.5) | |
| DC | 29 (2.3) | | 19 (2.5) | |
| HMOC | 217 (17.0) | | 122 (16.0) | |
| Missing | | 141 (9.9) | | 78 (9.3) |
| **Study period** | | | | |
| 2005–2011 | 730 (51.4) | | 444 (53.0) | |
| 2012–2017 | 689 (48.6) | | 394 (47.0) | |
| Missing | | – | | – |

*DC, Developed Countries; HMOC, High Migration Outflow Countries.*

**TABLE 2 |** Adjusted Odds Ratio of not having a prenatal diagnosis by maternal characteristics for all chromosomal anomalies.

| | OR[#] | 95% CI | *P* | OR[§] | 95% CI | *P* |
|---|---|---|---|---|---|---|
| **Education** | | | | | | |
| High | Ref. | | | Ref. | | |
| Intermediate | 1.07 | 0.72–1.59 | 0.728 | 1.05 | 0.68–1.63 | 0.811 |
| Low | 2.16 | 1.41–3.32 | 0.000 | 1.89 | 1.16–3.09 | 0.010 |
| **Occupation** | | | | | | |
| Manager/Professional | Ref. | | | Ref. | | |
| Technical/Clerical/Service | 0.68 | 0.36–1.27 | 0.229 | 0.64 | 0.34–1.20 | 0.163 |
| Unskilled occupation/ Unemployed | 1.96 | 1.00–3.84 | 0.051 | 1.32 | 0.65–2.70 | 0.445 |
| Housewives | 0.72 | 0.22–2.34 | 0.583 | 0.46 | 0.13–1.55 | 0.210 |
| **Geographic origin** | | | | | | |
| Italian | Ref. | | | | | |
| DC | 2.68 | 1.11–6.45 | 0.028 | | | |
| HMOC | 2.85 | 2.00–4.07 | 0.000 | | | |

*OR, Odds Ratio.*
[#]*Adjusted by maternal age.*
[§]*Adjusted by maternal age and geographic origin.*
*DC, Developed Countries; HMOC, High Migration Outflow Countries.*

## RESULTS

A total of 1,419 cases with chromosomal anomalies were included in the study. Out of them, 838 were cases of Down syndrome (59.1%). The other more frequent chromosomal anomalies were trisomy 18 (15.2%), trisomy 13 (4.5%), Turner syndrome (5.9%), and Klinefelter syndrome (4.1%). Overall, prenatally diagnosed cases were 1,186 (83.6%). Among them, 122 were liveborn. The proportion of cases with low maternal education was 22.6%. About 18% of the mothers were unemployed or with an unskilled occupation. Mothers from HMOC were 17.0% and from not Italian DC 2.0%. About 30% of the mothers were aged 40 years and older (**Table 1**). Maternal age was strongly associated with prenatal diagnosis ($p < 0.001$).

We observed a higher risk of not having a prenatal diagnosis in cases with low maternal education compared to high maternal education (OR = 2.16, $p < 0.001$) (**Table 2**). The proportion of cases without a prenatal diagnosis was higher also when adjusted for maternal geographic origin (OR = 1.89, $p = 0.01$).

Regarding maternal occupation, we observed a higher proportion of not prenatally diagnosed cases, borderline significant, in the group "unskilled occupation/unemployed" (OR = 1.96, $p = 0.051$). When adjusted by geographic origin the risk was no more significant.

Mothers from HMOC and mothers from DC other than Italy, compared to Italian ones, had a higher proportion of not prenatally diagnosed cases (OR = 2.85, $p < 0.001$ and OR = 2.68, $p = 0.028$, respectively). These results were also confirmed when adjusted by maternal education (OR = 2.96, $p < 0.001$ and OR = 2.94, $p = 0.03$, respectively).

Among all the cases of Down syndrome, 77.7% were prenatally diagnosed. As reported in **Table 3**, a higher risk of not having a prenatal diagnosis was observed in mothers with low education (OR = 2.23, $p = 0.002$), also when adjusted by geographic origin (OR = 1.79, $p = 0.047$).

Concerning maternal occupation, no significantly higher risk was observed in the group "unskilled occupation/unemployed," whereas a lower proportion of cases was observed in the group "technical/clerical/ service" (OR = 0.47, $p = 0.029$).

Mothers from HMOC and DC had a higher risk than Italian mothers of not having a prenatal diagnosis (OR = 3.73, $p < 0.001$ and OR = 2.79, $p = 0.045$, respectively).

Looking at all the major trisomies (i.e. trisomies 13, 18 and 21), the overall number of cases was 1,118, and 82.4% were prenatally diagnosed. The associations with maternal risk factors were confirmed with a higher proportion of not prenatally diagnosed cases in mothers with low education level and mothers from HMOC.

**TABLE 3 |** Adjusted Odds Ratio of not having a prenatal diagnosis by maternal characteristics in Down syndrome.

| | OR[#] | 95% CI | *P* | OR[§] | 95% CI | *P* |
|---|---|---|---|---|---|---|
| **Education** | | | | | | |
| High | Ref. | | | Ref. | | |
| Intermediate | 1.06 | 0.67–1.67 | 0.807 | 0.99 | 0.60–1.64 | 0.963 |
| Low | 2.23 | 1.34–3.71 | 0.002 | 1.79 | 1.01–3.16 | 0.047 |
| **Occupation** | | | | | | |
| Manager/Professional | Ref. | | | Ref. | | |
| Tech/Clerical/Service | 0.47 | 0.24–0.93 | 0.029 | 0.39 | 0.19–0.78 | 0.008 |
| Unskilled occupation/ Unemployed | 1.53 | 0.73–3.21 | 0.258 | 1.03 | 0.47–2.26 | 0.946 |
| Housewives | 0.70 | 0.20–2.47 | 0.574 | 0.43 | 0.11–1.62 | 0.213 |
| **Geographic origin** | | | | | | |
| Italian | Ref. | | | | | |
| DC | 2.79 | 1.02–7.65 | 0.045 | | | |
| HMOC | 3.73 | 2.43–5.73 | 0.000 | | | |

*OR, Odds Ratio.*
[#]*Adjusted by maternal age.*
[§]*Adjusted by maternal age and geographic origin.*
*DC, Developed Countries; HMOC, High Migration Outflow Countries.*

**TABLE 4 |** Percentage and adjusted Odds Ratio of termination of pregnancies after prenatal diagnosis by maternal characteristics in prenatally diagnosed cases for all chromosomal anomalies and for Down syndrome.

| | Chromosomal anomalies | | | Down syndrome | | |
|---|---|---|---|---|---|---|
| | % | OR[#] | *P* | % | OR[#] | *P* |
| **Education** | | | | | | |
| High | 82.4 | Ref. | | 85.6 | Ref. | |
| Medium | 86.4 | 1.58 | 0.062 | 93.2 | 3.24 | 0.005 |
| Low | 85.5 | 1.34 | 0.333 | 95.9 | 4.36 | 0.023 |
| **Occupation** | | | | | | |
| Manager/Professional | 84.5 | Ref. | | 87.0 | Ref. | |
| Tech/Clerical/Service | 90.2 | 1.70 | 0.101 | 96.2 | 3.89 | 0.009 |
| Unskilled occupation/ Unemployed | 86.1 | 1.11 | 0.797 | 90.3 | 1.44 | 0.542 |
| Housewives | 79.4 | 0.98 | 0.965 | 82.4 | 0.76 | 0.719 |
| **Geographic origin** | | | | | | |
| Italian | 90.4 | Ref. | | 94.4 | Ref. | |
| DC | 86.4 | 0.60 | 0.415 | 92.3 | 0.66 | 0.698 |
| HMOC | 78.1 | 0.47 | 0.003 | 83.3 | 0.33 | 0.006 |

*OR, Odds Ratio.*
[#]*Adjusted by maternal age.*

Among all prenatally diagnosed cases, the mean gestational age at prenatal diagnosis was higher in cases with low maternal education than in cases with high maternal education ($14.8 \pm 3.6$ vs. $14.0 \pm 3.1$ weeks, $p = 0.03$). No differences related to maternal geographic origin and occupation were observed.

Cases of TOPFA in all chromosomal anomalies were 1,051 corresponding to 74.1% of all cases and 88.6% of those prenatally diagnosed. Considering cases of all chromosomal anomalies prenatally diagnosed, we did not observe significant differences among classes of maternal education in the proportion of TOPFA (**Table 4**). The results were confirmed when only Italian mothers were considered. TOPFA did not differ among maternal occupation classes. We observed a significantly lower proportion of TOPFA for mothers from HMOC compared to the Italian ones (OR = 0.47, $p = 0.003$).

For cases of Down syndrome, TOPFA were significantly more frequent in mothers with medium and low education (OR = 3.24, $p = 0.005$; OR = 4.36, $p = 0.023$, respectively). Considering only Italian mothers the results were confirmed (OR = 3.68, $p = 0.004$; OR = 10.49, $p = 0.025$, respectively). For mothers from HMOC, we observed a significantly lower proportion of TOPFA than for Italian mothers (OR = 0.33, $p = 0.006$).

## DISCUSSION

In summary, using population-based data from a registry of congenital anomalies, we found that the probability of prenatal diagnosis for chromosomal anomalies was affected by different maternal sociodemographic characteristics. In particular, disparities persisted for women with lower education and for those from countries with a high migratory outflow. The findings referred to all chromosomal anomalies were largely confirmed for Down syndrome only, in agreement with the literature (3–5, 9, 18).

When data on income are not available, various factors have been used to reflect the concept of socioeconomic status. In our study, we have considered and analysed separately maternal education attainment and occupation. Maternal education was our main parameter and the choice was justified by its adequacy in evaluating information provided and in making an informed decision, besides being a good proxy of SES. It proved to be a reliable factor with the greatest impact on the outcome of interest, showing that women with a low level of education were less likely to have a prenatal diagnosis for chromosomal anomalies. Maternal geographic origin also highlighted differences, indicating that HMOC women, compared to the Italian ones, were less likely to have a prenatal diagnosis. These results could be the consequence of lesser access to the screening tests for Down syndrome observed in the same subgroups (i.e., women with low education level and women from HMOC) of the women residing in Tuscany (19).

These two features (education and geographic origin) were intermingled (46% of HMOC women were in the lower education group), but some specific explanations could be found for HMOC women. First, despite HMOC women being resident in Italy, the language barrier can be a key factor in disparities, resulting in lower health literacy (20, 21) and miscommunication between prenatal service providers and pregnant women (22). Second, according to a recent report on welfare and health in Tuscany (17), about 12% of foreign women would be late at the first visit during pregnancy (beyond the twelfth week of gestation), and 9% of them would make less than four visits during the whole period of pregnancy. These indicators are improving, compared

to previous years, but are still at a sub-optimal level and could have an impact on the prenatal diagnosis. However, analysing only the cases from Italian mothers, differences were still present and, among mothers with lower education, the proportion of not prenatally diagnosed cases was higher with a borderline statistical significance (OR = 1.71, $p = 0.058$).

We also assessed maternal occupation which would seem to suggest that pregnant women in the lowest occupational subgroups were more likely not to have a prenatal diagnosis for chromosomal anomalies. Although these observations are to be considered with caution, due to the incompleteness of this variable, they still deserve to be taken into account both because they confirm the results of other studies (4, 5, 15) and because they represent a stimulus to improve data collection for future studies.

In Tuscany, the healthcare system is organized in three large areas (North-West, Centre, and South-East) with care services widespread in the territory, guaranteeing in this way homogeneous access to care. In each of these areas, there are large hospitals with specialized centres and universities. Furthermore, the Tuscany region, through a health performance assessment system, is committed to the continuous improvement of all the health services provided. In this context, the access to prenatal diagnosis is equally offered to all pregnant women residing in the region.

In 2006 the combined test (based on the combination of maternal age, foetal nuchal translucency thickness, maternal serum free β-human chorionic gonadotrophin and pregnancy-associated plasma protein-A) was introduced in Tuscany as a screening test for chromosomal anomalies and offered at a shared cost (the ultrasound scan was free, only the fee for the blood test was required). Over time, the screening test has become more widely used: in 2017, 75% of pregnant women had used the test. Thus, due to this important change in prenatal policy, we performed the analysis by sub-period. We found an increase of prenatal diagnoses (81.9% in 2005–2011 vs. 85.3% in 2012–2017), but differences among the sociodemographic groups were confirmed. In 2019, in Tuscany, the combined test was made free of charge and the NIPT (Non-invasive Prenatal Testing) for the analysis of foetal DNA was introduced. Therefore, it will be important in the future monitoring their impact on the population subgroups.

In our study population, a large proportion of women does not continue the pregnancy after a diagnosis of chromosomal congenital anomalies. Among cases prenatally diagnosed, we did not observe differences in the proportion of TOPFA for levels of maternal education. Conversely, for cases of Down syndrome only, we observed a higher proportion of TOPFA in mothers with low maternal education, in contrast with the less frequent prenatal diagnosis in the same subgroup. These findings confirm that the knowledge of a diagnosis in the prenatal period represents a fundamental element in the decision to continue or to terminate the pregnancy.

Regarding the maternal geographic origin, the proportion of TOPFA was lower in women from HMOC. This result suggests that a different cultural background could play a role in the decision to continue or terminate the pregnancy (23).

Other factors such as previous history termination of pregnancy, gestational age at prenatal diagnosis, number of children of the woman may influence the decision to terminate the pregnancy (24). We have added these variables in the statistical model and the differences in the proportion of TOPFA between the socio-demographic subgroups remained very similar. The results were confirmed also adjusting for gestational age at diagnosis although it was higher in cases with low maternal education.

Prenatal diagnosis has to be considered an important factor in informed decision making and it is recommended that all women, regardless their sociodemographic characteristics, have an equal likelihood to access the prenatal care services (18). Multiple factors influence the decision whether to continue or terminate the pregnancy (24, 25) and ultimately, the parents' decision, whatever it may be, must be respected. Language, cultural traditions and religious beliefs are important aspects that have an impact on the personal decision process. But the nature of this study does not allow us to evaluate them. Other studies including interviews of women following their decision to continue or terminate the pregnancy could provide helpful information and in-depth analysis of their role.

The main strength of the present study is that data are ascertained by a registry based on a regional network allowing the standardized collection of cases diagnosed and validated by health centres. Furthermore, as RTDC is a population-based registry, the cases of all the residing population were collected, reducing the selection bias. Moreover, results from a study using data at the population level represent the most accurate information to detect the presence of sources of health inequalities and, consequently, to help in addressing more efficient public health policy.

The main limitation of the study is the proportion of missing values, especially for maternal education (503 cases, corresponding to 35.5%) and maternal occupation (626, 44.1%). Twenty-eight of the 503 cases with no information on maternal education did not have a prenatal diagnosis (5.6%). However, among them, 61.1% comes from HMOC and we have observed that maternal education was lower in mothers from HMOC. Furthermore, we observed that 447 out of the 503 cases with unknown maternal education, were cases of TOPFA; in addition, we did not observe significant differences in the proportion of TOPFA among the subgroups in cases with known maternal education. Therefore, from these two pieces of information, it is reasonable that the unknown information on maternal education seems not to have generated a bias in our study. Regarding the maternal occupation, the proportion of missing values was even higher than maternal education. Another limitation of these data was the difficulty to define an accurate classification that can be used as a proxy of SES.

Although we have observed some differences in the sociodemographic subgroups suggesting conditions of inequalities, a more accurate and complete data collection on SES variables is needed to better detect possible conditions of disparities.

## CONCLUSIONS

In our study evidence of differences in the probability of prenatal diagnosis of chromosomal anomalies associated with maternal education and geographic origin was found. Our results suggest that, albeit the national healthcare system guarantees universal access to care services, disparities persist in the access to prenatal diagnosis due to socioeconomic status, in particular for women with lower education and women from countries with a high migratory outflow. These findings could give an insight into some aspects concerning the efficacy of the healthcare policies and can further stimulate the counselling activity for an informed choice. Besides, they should be monitored also in light of the recent availability of non-invasive screening tests. Population-based studies investigating sociodemographic disparities as well as healthcare practices can provide essential information for targeted public health programs.

## DATA AVAILABILITY STATEMENT

The data analyzed in this study was subject to the following licenses/restrictions: the owner of data is Regione Toscana that might provide data upon request. Requests to access these datasets should be directed to www.regione.toscana.it.

## AUTHOR CONTRIBUTIONS

MS conceived the study. MS, LM, and AC designed the study. MS and LM performed the data analysis, interpreted the results, and drafted the manuscript. SB, LP, and AP contributed to the design of the study and the interpretation of the results. All authors critically revised the manuscript and approved the final version.

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# Epidemiology of Multiple Congenital Anomalies Before and After Implementation of a Nationwide Prenatal Screening Program in Denmark

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Surveillance of congenital anomalies is important in order to detect negative influences from environment, medication, or lifestyle as early as possible. Since most teratogens are associated with a spectrum of birth defects rather than a single defect, analysis of the epidemiology of multiple congenital anomalies is important to detect an increase due to environmental or medicine exposure. The aim of the study was to describe changes in prevalence, types of anomalies, and outcome of pregnancies for fetuses and infants with multiple congenital anomalies before and after introduction of the new screening program in the County of Funen, Denmark. The study was based on data from the EUROCAT registry of the County of Funen for the period 1990 to 2014 covering 135,057 births. The registry includes information about livebirths, fetal deaths after 20 weeks of gestation and terminations of pregnancy after prenatal diagnosis of fetal anomalies. All cases with two or more major congenital anomalies in different organ systems, where the pattern of anomalies were not recognized as part of a chromosomal or genetic syndrome or a sequence were included in the study. Overall prevalence of multiple congenital anomalies was 19.7 per 10,000 pregnancies. There was no significant change in prevalence over time. The prenatal detection rate increased from 26 to 57% after introduction of the screening program ($p < 0.001$). Proportion of terminations of pregnancy increased from 11 to 30% of all cases and 1-week survival for livebirths increased from 64 to 94%. There was no change in combinations of involved organ systems. The implementation of the new screening program in 2004 has led to an increased prenatal detection rate of multiple congenital anomalies followed by an increased rate of termination of pregnancy for the most severe cases and an increased 1-week survival for liveborn infants with multiple congenital anomalies.

**Keywords: multiple congenital anomalies, EUROCAT, prenatal screening, prevalence, survival**

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## INTRODUCTION

Surveillance of congenital anomalies has become an important public health activity since the thalidomide disaster, aiming to prevent a similar or smaller incidents (1). Surveillance of congenital anomalies is also of significant importance in order to detect negative influences from environment, medication, or lifestyle as early as possible and in this way prevent congenital anomaly clusters due

to new teratogenic exposures (1). Since some teratogens are associated with a spectrum of birth defects rather than a single defect, analysis of the epidemiology of multiple congenital anomalies is important to detect an increase of those due to environmental or medicine exposure (2). Due to the rarity of multiple congenital anomalies, dataset over many years is necessary. Therefore, only very few studies have been dealing with surveillance of congenital anomalies leading to an unknown magnitude of the problem.

In 2004 Denmark replaced its earlier prenatal screening program, which included offering an invasive test to pregnant women over 34 years, with a screening program offered to all pregnant women. The new national screening program includes a nuchal translucency scan and a blood sample at a gestational age between the 11th and 13th week and an anomaly scan between the 18th and 20th week (3). The change was based on a recommendation from a working group set up by the Danish Ministry of Health. The recommendation suggested that by screening all pregnant women with a blood test and an ultrasound, the number of amniocentesis would decrease and simultaneously the prenatal detection rate of congenital anomalies would increase. At that time amniocentesis leads to a miscarriage in about 1 of 100 pregnancies, reducing the numbers was desirable and non-invasive methods should be preferred (3).

Detecting congenital anomalies during pregnancy has many advantages, such as the possibility of referring the mother before birth to a tertiary hospital with the required expertise for delivery, the possibility of giving the parents relevant information and preparation for the life with their infant and in severe cases providing an opportunity to offer termination of the pregnancy.

The aim of this study is to describe the influence of the screening program implemented in Denmark during 2004 on prenatal detection rates, rates of terminations of pregnancy and the impact on survival of liveborn infants with multiple congenital anomalies.

## MATERIALS AND METHODS

EUROCAT is a European network of population-based registries for the epidemiological surveillance of congenital anomalies with more than 1.7 million births surveyed per year in Europe since 1980 (4). This study was based on data from the EUROCAT registry of Funen County, Denmark. The registry includes livebirths, fetal deaths from GA 20 weeks and terminations of pregnancy after prenatal diagnosis of congenital anomalies. Data from 1990 to 2014 was included, covering a population of 135,057 births (live and stillbirths) in the registry area.

Multiple congenital anomaly cases were defined as two or more major structural congenital anomalies in different organ systems. The cases were classified for this study using the EUROCAT Multiple Congenital Anomaly Algorithm (5). Cases with skeletal dysplasias and teratogenic, chromosomal, and genetic syndromes were excluded as well as all cases with anomalies in one organ system only (1). The remaining potential multiple congenital anomaly cases ($n = 331$) were manually evaluated by the authors, excluding cases with anomalies in one organ system only, as well as cases with genetic or chromosomal syndromes which were not picked up by the algorithm.

For the statistical evaluation MATLAB's Statistics and Machine Learning Toolbox was used. Descriptive data is presented in percentage. Trends over time were calculated using logistic regression in single years and presented in five periods (1990–1994, 1995–1999, 2000–2004, 2005–2009, 2010–2014). Logistic regression was also used for comparison of groups before and after introduction of general screening. For the evaluation of the effect of the new screening program, data from before implementation (1990–2004) was compared to data after implementation of the new screening (2005–2014). For all statistical test a significance level of 5% was used.

**TABLE 1 |** Prevalence of multiple congenital anomalies, prenatal detection rates, and outcomes of pregnancy.

| | 1990–2004 | 2005–2014 | |
|---|---|---|---|
| Total births | 84,998 | 50,059 | |
| Cases with MCA | 157 | 108 | |
| Prevalence of MCA per 10,000 births | 18.5 | 21.6 | $p = 0.21$ |
| Prenatal detection rate (N) | 26% (41) | 57% (62) | $p < 0.001$ |
| Median GA at discovery, weeks | 21 | 20 | $p < 0.05$ |
| Number (%) liveborn infants | 127 (81%) | 71 (66%) | $p < 0.01$ |
| Number (%) of liveborn infants alive at 1 week | 81 (64%) | 67 (94%) | $p < 0.001$ |
| Number (%) termination of pregnancy | 17 (11%) | 32 (30%) | $p < 0.001$ |
| Number (%) fetal deaths from GA 20 weeks | 13 (8 %) (13) | 5 (4%) | $p = 0.25$ |

*MCA, multiple congenital anomaly.*

## RESULTS

During the years 1990–2014 there were 265 fetuses and infants with multiple congenital anomalies, giving an overall prevalence of 19.7 per 10,000 births.

Prevalence of multiple congenital anomalies in the 15 years before implementation of the screening program was 18.5 per 10,000 births and increased to 21.6 per 10,000 births in the 10 years after the implementation ($p = 0.21$; **Table 1**). The prevalence in 5-year periods from 1990 to 2014 is presented in **Figure 1**. The observed increase in prevalence during these 25 years was not statistically significant.

The prenatal detection rate increased from 26% before to 57% after implementation of the screening program ($p < 0.001$). Median GA at birth for liveborn infants with multiple congenital anomalies was 38 weeks in 1990–2004 and 39 weeks in 2005–14 ($p = 0.43$). In 1990–2004 infants with a prenatal diagnosis were born almost 2 weeks earlier than infants with a postnatal diagnosis ($p = 0.05$). This difference could not be observed in 2005–14. The introduction of nationwide prenatal screening lead to a mean GA at discovery of multiple anomalies at week 20, which was 3 weeks earlier than it used to be ($p < 0.01$), but the mean gestational age for termination did not change significantly, being 18.9 gestational weeks in the first period and 18.5 in the second period.

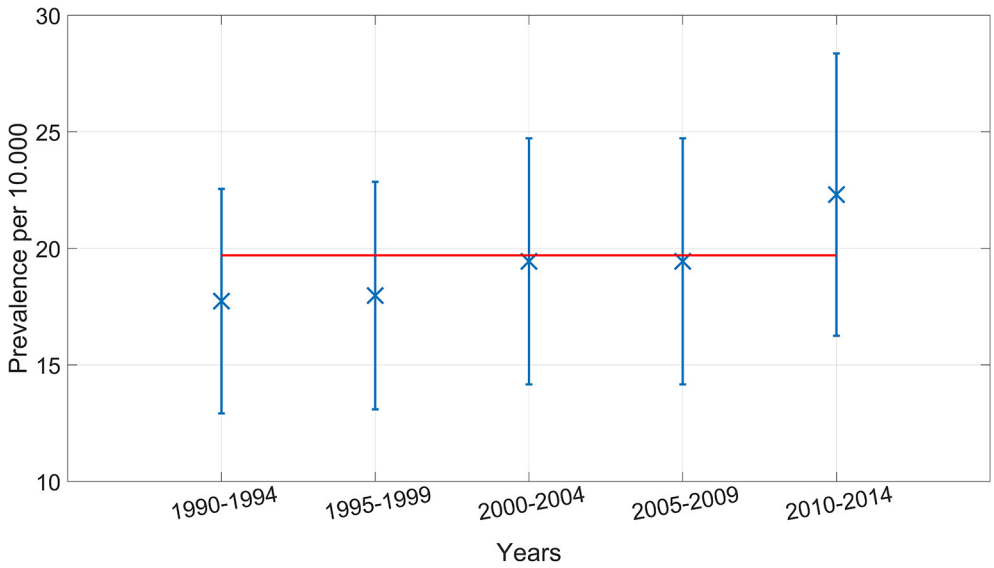


**FIGURE 1 |** Prevalence of fetuses and infants with multiple anomalies per 5 year periods, Funen Denmark 1990–2014.

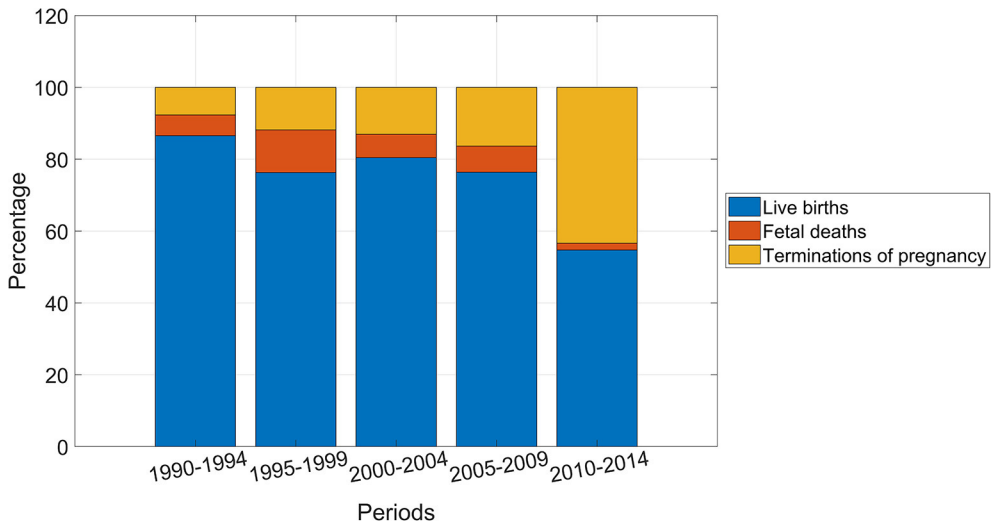


**FIGURE 2 |** Distribution of pregnancy outcome for fetuses and infants with multiple congenital anomalies, Funen, Denmark, 5 year periods 1990–2014.

The distribution of the pregnancy outcomes in 5 year periods is shown in **Figure 2**. Proportion of terminations of pregnancy increased over time and proportion of livebirths decreased (**Table 1**).

First week survival of livebirths was 64% in the 15 years before implementation of the screening program and increased to 94% in the in the 10 years after the implementation ($p < 0.01$).

As shown in **Figure 3** anomalies of the heart were the most common in both time periods (39% of all anomalies before 2005 and 46% in the later years), followed by limb defects. There has been an increase of anomalies of the urinary tract and simultaneous a reduction of anomalies of the digestive system, which is not statistically significant ($p = 0.1$). Anomalies of the eye, the face, the neck, and the abdominal wall were very rare in both periods with less than two cases in each period (not shown in **Figure 3**). The most common combination of anomalies were defects in the digestive system and the heart in both periods followed by defects of the heart and the urinary system with no significant change over time.

## DISCUSSION

Surveillance of multiple congenital anomalies is important in order to detect any increase or clustering, considering that most

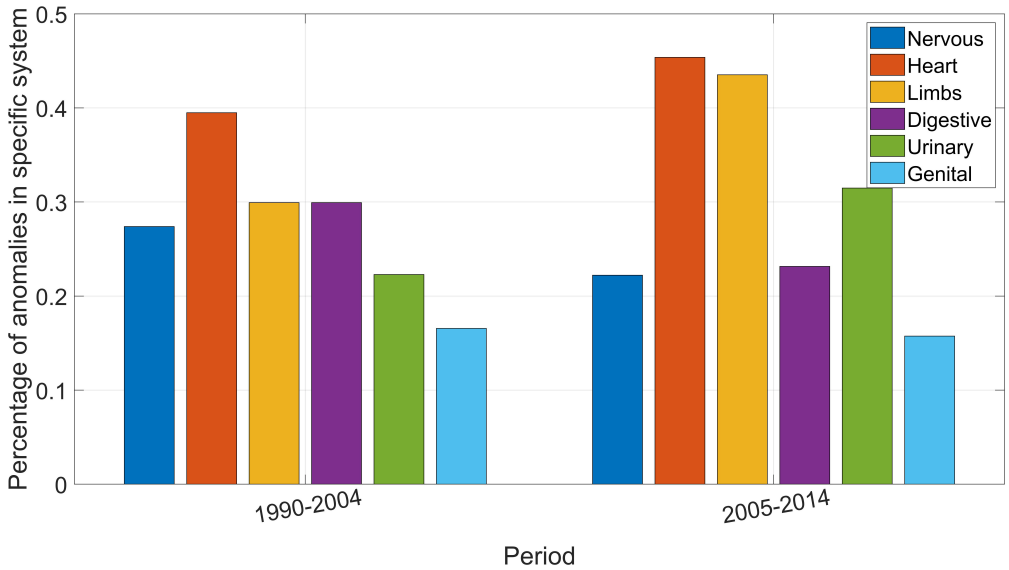


**FIGURE 3 |** Percentage of congenital anomalies in respective organ system.

teratogens are associated with a spectrum of anomalies rather than one specific anomaly.

Our study showed that the prevalence of fetuses and infants with multiple anomalies during the 25 years was 19.7 per 10,000 births. The prevalence published in the literature ranges from 15.1 per 10,000 births (6) to 16.3 per 10,000 births (7) and to 26.3 per 10,000 births (8). The given prevalence for Funen is comparable to these other studies. However, there may be different registration of cases between populations studied and differences in lifestyle/exposure patterns (1).

The study confirmed that the introduction of the nationwide screening program led to an increasing prenatal detection rate of multiple anomalies at an earlier gestational age. It furthermore showed that the prenatal detection has led to an increase in the termination rate, as nearly 30% of fetuses with multiple anomalies diagnosed prenatally were terminated in the recent time period. In the literature a prenatal detection rate between 51 and 58% has been found (6), which is very similar to the detection rate in this study after introduction of the nationwide screening program.

The study showed an increase in the 1-week survival rate, which indicate that the increase in the termination rate was mainly for fetuses with severe anomalies that would not have survived the 1st week. There was an increase of both survival rate and termination of pregnancy rate through all the years, but with an additional upturn seen after 2004. The increase of survival in the first period might be explained by improvements in diagnostic and treatment options in neonatology and intensive care in the early 90's and has also been described worldwide for overall neonatal survival (9). In Western Europe the neonatal mortality rate decreased by 58% from 1990 to 2017. Other published data showed an increase in overall neonatal survival from 96.7% in 1990 to 98% in 2013 (10). The increase in the termination rate might be explained by a more liberal attitude toward termination of pregnancy (11) and improved prenatal detection rates. Interestingly, in our study the gestational age at termination of pregnancy did not decrease significantly, although the gestational age at prenatal detection decreased. It might be due to small numbers in our study with only 17 terminations of pregnancies in the first 15 years of the study. The congenital anomalies detected before the nationwide screening program was introduced, were mainly severe anomalies that were easily detectable, such as anencephaly. There was a very small number of fetal deaths with multiple congenital anomalies in both periods with $<2$ fetal deaths per year.

Prenatal detection of major congenital anomalies requiring surgery in the neonatal period may lead to induced birth at an earlier GA than if spontaneous labor was awaited. In this study we found no significant change in gestational age of live born infants with multiple anomalies before and after introduction of the screening.

The distribution of the organ systems in the fetuses and infants with multiple anomalies was similar in both periods, except for an increase of defects of the urinary tract, and a decrease of defects of the digestive system. The prenatal screening program seems to have diagnosed some urinary anomalies that may have been undiagnosed without the screening program (congenital hydronephrosis and multicystic renal dysplasia). The distribution of organ systems involved is comparable to a study including 19 European countries (6).

## Strengths and Limitations

A major strength of the study is the high rate of participation of the screening program, in 2014 about 95% of all pregnant women participated (12).

Furthermore, the congenital anomalies data has been collected routinely over 25 years from the same data sources which makes the data comparable over time. The data collection has followed

a strict algorithm in all years, enhancing comparability and data quality.

A limitation of the study is the rareness of the diseases, which makes it difficult to give a full comprehension of the influence of the screening procedure and a less accurate statistical analysis. Further, the diagnosis of additional congenital anomalies after the first diagnosis may differ for terminations of pregnancy compared to liveborn infants. Postmortem examinations was not performed for all terminations in the population.

## CONCLUSION

Implementation of a nationwide screening program in Denmark has led to an increasing prenatal detection rate of multiple congenital anomalies and to an increase in the rate of terminations of pregnancy for fetuses with multiple anomalies. Consequently, the 1-week survival rate for liveborn infants with multiple congenital anomalies has increased and most parents were prepared for having a baby with congenital anomalies and prepared for the necessary treatment. There was no significant change in the organ systems involved in the multiple congenital anomalies.

## DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because individual data cannot be shared. Requests to access the datasets should be directed to ester.garne@rsyd.dk.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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POLICY BRIEF
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# Expanded Neonatal Bloodspot Screening Programmes: An Evaluation Framework to Discuss New Conditions With Stakeholders

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Neonatal bloodspot screening (NBS) programmes that screen for rare but serious conditions are expanding worldwide. Fast developments for testing and treatment put pressure on implementation processes. In 2015 the Netherlands embarked on an NBS expansion from 17 to 31 conditions. An evaluation framework was developed based on international NBS frameworks to gain insight in test properties, clinical findings, follow-up and implementation. A stakeholder process took place with implications for the planning of the expanded NBS panel. The evaluation framework progressed into a *go/no go* framework to start national screening, and is currently explored as basis for continuous evaluation of the NBS panel. The framework and stakeholder process may serve as an example for other programmes.

**Keywords: neonatal screening, implementation, stakeholders, decision making, public health policy cycle, evaluation framework**

## INTRODUCTION

Neonatal bloodspot screening (NBS) programmes are important and successful public health initiatives for early recognition of rare, congenital disorders (1). NBS usually focuses on disorders for which early detection enables early intervention that prevents or minimises irreversible health damage. In the Netherlands newborns are screened for 24 conditions as of January 2021 (**Table 1**). NBS can only reach its potential to prevent health damage in babies when the programmes are effectively organised. As we will show in this policy brief, NBS programmes are complex (3), and all steps of the public health policy cycle need to be carefully considered (4, 5): agenda setting; policy advice; policy decision; implementation; and evaluation.

Since its initiation in the 1960s with screening for phenylketonuria (PKU), innovations have accelerated expansion of NBS programmes. An archetypical example is the introduction of tandem mass spectrometry (MS/MS), which facilitated simultaneous biochemical analyses for a significant number of metabolic disorders. The availability of MS/MS led to test-driven expansions in NBS programmes worldwide, resulting in some programmes currently screening more than fifty conditions (1, 6). Innovations both in treatments and test methods continue, enabling screening for additional conditions. When an intervention with substantial health benefit is proven effective for a neonatal condition and a suitable screening test is available, the condition becomes eligible

**TABLE 1 |** Neonatal screening programme in the Netherlands summarised.

| Screening process | Conditions in the current programme (1 February 2021) | Conditions included in the expansion |
|---|---|---|
| All new Dutch parents are offered NBS for their child. Annually, over 99% of ∼170,000 Dutch neonates undergo NBS (2). A few drops of blood are obtained from the heel and collected on a filter paper card between 72 and 168 h post-partum. The cards with the dried bloodspots are sent to one of five Dutch regional screening laboratories and analysed. After analyses, the cards are pseudonymised through an encrypted barcode and stored centrally at the national reference laboratory. The national NBS program has been expanded from 17 to 24 conditions since January 2021. It is expected to be expanded with 8 additional conditions in the coming years. | 1. Alpha-thalassemia (HbH-disease)<br>2. Beta thalassemia major (TM)<br>3. Biotinidase deficiency (BIO)<br>4. Carnitine palmitoyltransferase deficiency type 1 (CPT1)<br>5. Congenital adrenal hyperplasia (CAH)<br>6. Congenital hypothyroidism (CH)<br>7. Cystic fibrosis (CF)<br>8. Galactosemia (GAL)<br>9. Galactokinase deficiency (GALK)<br>10. Glutaric acidemia type I (GA-1)<br>11. HMG-CoA-lyase deficiency (HMG)<br>12. Isovaleric acidemia (IVA)<br>13. Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)<br>14. Multiple CoA Carboxylase deficiency (MCD)<br>15. Maple syrup urine disease (MSUD)<br>16. Medium-chain acyl CoA dehydrogenase deficiency (MCADD)<br>17. 3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC)<br>18. Methylmalonic acidemia (MMA)<br>19. Phenylketonuria (PKU)<br>20. Propionic acidemia (PA)<br>21. Severe combined immune deficiency (SCID)<br>22. Sickle cell disease (SCD)<br>23. Type 1 tyrosinemia (TYR-1)<br>24. Very long-chain acylCoA dehydrogenase deficiency (VLCADD) | 25. Carnitine-acylcarnitine translocase deficiency (CACT)<br>26. Carnitine palmitoyltransferase deficiency type 2 (CPT2)<br>27. Guanidinoacetate methyltransferase deficiency (GAMT)<br>28. Methyl-acetoacetyl-CoA thiolase deficiency, ketothiolase deficiency (BKT)<br>29. Mucopolysaccharidosis type 1 (MPS I)<br>30. Organic cation transporter 2 deficiency (OCTN 2)<br>31. Spinal muscular atrophy (SMA)[a]<br>32. X-linked adrenoleukodystrophy (ALD) |

*NBS, neonatal bloodspot screening; RIVM, National Institute for Public Health and the Environment.*
*[a]Addition of SMA was advised and decided on in 2019–2020.*

for screening. A combination of a suitable test and effective treatment have recently made severe combined immunodeficiency (SCID) and spinal muscular atrophy (SMA) eligible for screening, and they are gradually implemented in NBS programmes.

While significant treatment- and test-driven expansions are seen in several NBS programmes worldwide, other NBS programmes expand at a slower rate (7). This illustrates that even though screening tests and treatments are available, the local context will determine the NBS program put in place (5, 8). Differences in local context include for example available resources, disease prevalence, and (interpretation of) screening criteria. For the latter, the Wilson and Jungner screening principles are used since their publication in 1968 and revision in 2008 (9, 10). However, heterogeneity in applying these principles for decision-making contributes to the different NBS programmes we see today. For example, the Health Council of the Netherlands (GR) translated the 10 principles into 12 criteria, not only focusing on the question whether a screening should be introduced, but also how. It included elements on practical aspects, such as quality control after a screening is introduced, but also other important aspects, such as cost-effectiveness (11, 12).

To structure the implementation of fourteen new conditions to the Dutch NBS program, an evaluation framework was developed based on international NBS frameworks. This evaluation framework focused on the translation from policy to practice. It included aspects for insight on how to implement the expanded screening programme, specifically for test properties, clinical findings, and follow-up. To implement screening for each condition in the expanded NBS programme in a timely but responsible manner, a stakeholder process was employed, which we will summarise here. While the process and framework summarised can be used by public health professionals in other countries as a starting point for the implementation process, local contexts for other countries and for example approaches to agenda setting are not discussed. As NBS programmes are expected to continue to expand, policy processes that support timely and responsible decision-making and implementation are paramount.

## NBS POLICY IN THE NETHERLANDS

Various governmental bodies are involved in the (re)assessment and implementation of NBS. The three main parties that

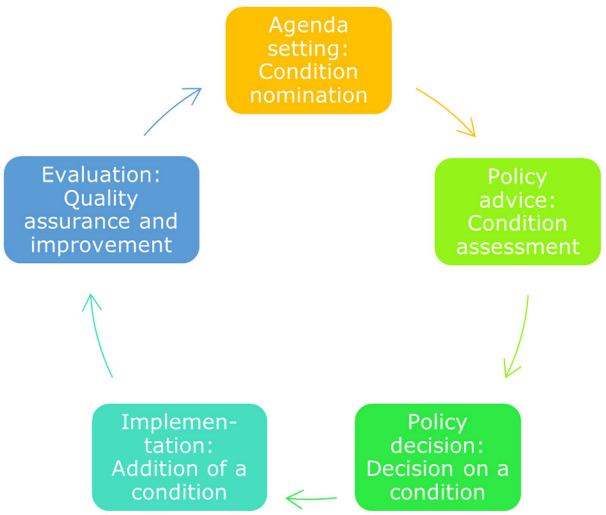


**FIGURE 1 |** The public health policy cycle. The public health policy cycle consists of five phases. These five phases translate to specific activities for NBS, from nomination of a condition to its quality assurance after implementation (5).

are discussed here are the (1) Ministry of Health, Welfare and Sport (VWS) (2) GR, and (3) Centre for Population Screening of the National Institute for Public Health and the Environment (RIVM-CvB):

1. The Ministry of VWS bears political responsibility for NBS and defines the NBS-policy, including the legal and policy framework. It is also responsible for the programme's funding and facilitation of the co-ordination of the programme, which is delegated to RIVM-CvB.
2. The GR is an independent national scientific advisory body. Pursuant to the Public Health Act, the GR has the task of advising ministers and parliament on public health and research into health and healthcare. Ministers ask the GR for advice to underpin policy decisions.
3. RIVM-CvB co-ordinates the national NBS programme. RIVM-CvB directs NBS and manages the implementation, to ensure that the legal and policy frameworks, the public values and clinical care are aligned.

Below we will summarise the policy cycle for public health screening programmes applied to NBS (**Figure 1**). Then we will report on the policy process that was followed to implement the expanded NBS panel in phases.

## Agenda Setting

In the Netherlands, the agenda for NBS is set on a national level by the Ministry of VWS. When evaluation of the NBS programme as a whole or for a specific condition is on their agenda, the Ministry asks scientific advice from the GR (see Policy Advice).

## Policy Advice

To develop a policy advice, the GR summarises scientific evidence related to the questions from the Ministry. It has a number of permanent committees, which advise on specific areas and take care of consistency in those advices over time. Since 2019 one of these committees is the pre- and neonatal screening committee. Before 2019 NBS was an *ad hoc* committee when evaluation was needed. The advice the GR provides is always based on published, peer-reviewed evidence, reviewed by experts and supplemented with expert opinions when needed.

The GR has advised the Ministry of VWS on NBS expansions upon request in 2005 (advice to add 14 conditions), 2011 (advice to add cystic fibrosis), 2015 (advice to add 14 conditions) and 2019 (advice to add SMA) (12–15). Depending on the GR advice and the Ministry's initial decision, the Ministry requests a feasibility advice from RIVM-CvB for a (group of) condition(s). The advice of RIVM-CvB focusses on the practical feasibility of the programme.

## Policy Decision

Based on the GR and RIVM-CvB advice, respectively through a scientific report (GR) and a feasibility study (RIVM-CvB), the Ministry takes a decision to change the national screening programme or not. In 2015, the GR advised to add fourteen conditions to the NBS programme and in 2017 RIVM-CvB advised a phased implementation of these conditions over a period of 5 years. The Ministry followed this advice, and initiated the implementation phase. This was different from the expansion advised on in 2005 when the Minister of Health, Welfare and Sport (VWS) decided to implement screening on all proposed conditions by 2007. This relatively quick expansion led to some suboptimal results, such as high numbers of false positives.

## Implementation

After the decision of the Ministry of VWS to start implementation, RIVM-CvB embarks on realising the implementation steps evaluated in the feasibility study. RIVM-CvB assures programme quality by setting requirements and monitoring them, such as programme organisation, guidelines, and accreditation requirements. An important advisory committee for RIVM-CvB is the NBS Programme Committee. This committee contains experts from relevant professional and patient organisations. For each condition in the expansion, a final decision to start screening is made by the Ministry, based upon a final advice by the RIVM-CvB. This final decision is based on a positive assessment using the *go/no go* framework, which was developed after the stakeholder process in 2016 and is discussed in the section Stakeholder process. This framework includes all the requirements that have to be in place before starting screening.

## Evaluation

Evaluation of the NBS policy takes place on different levels. Shortly after the introduction of a new condition to the programme, the performance parameters of screening are carefully monitored by RIVM-CvB. RIVM-CvB informs the Ministry of VWS on the outcomes, such as false positives.

RIVM-CvB also alerts and advises the Ministry and other governmental parties about longer term developments and major changes, including innovations, that are important for NBS and

that require measures and/or policy changes. The continuous evaluation of the whole NBS programme to complete the policy cycle is carried out on some parameters, but is also under development to monitor an increasingly complex programme through long-term follow-up.

# STAKEHOLDER PROCESS

## Insight in the Practical Feasibility

In 2015 the GR published an advice to expand the NBS programme from 17 to 31 conditions. The Ministry of VWS followed this advice and assigned RIVM-CvB to study the feasibility of adding these fourteen conditions. To initiate the feasibility study, the GR advice was complemented with additional information from grey literature and expert opinion. To structure the additional information, an evaluation framework was developed including aspects, such as Dutch prevalence numbers, test characteristics, and consensus in clinical follow-up in Dutch hospitals.

International policy frameworks were investigated before drawing up the evaluation framework for the feasibility of implementing a new condition. An internet search was conducted and international contacts were approached, resulting in the identification of six frameworks based on previous work (16). These were the frameworks from Australia, Canada (Ontario), Denmark, New Zealand, United Kingdom, and United States of America. The identified frameworks are used for the initiation or cessation of screening, and are therefore very comprehensive.

Since the aim of our evaluation framework was primarily to gain insight into the practical feasibility of adding a condition to the NBS programme, for example cost-effectiveness is not included in the *go/no go* framework, because the GR evaluates this aspect in their role in the decision making process. We focused on characteristics related to the execution of screening, such as availability of a test method in the Netherlands. These aspects are part of the feasibility study by RIVM-CvB. The items were the core of the discussion within the expert groups (**Figure 2**). We used the evaluation framework to gather information and to structure the discussion, so the expert groups could reach a standardised decision with regard to the (group of) condition(s) to be added to the NBS programme.

## Conducting Expert Group Meetings

RIVM-CvB facilitated a stakeholder process to gain insight in the practical feasibility of screening. First, RIVM established *ad hoc* experts groups for each (group of) condition(s) and organised expert meetings. The expert groups met once for each (group of) condition(s) between June 2016 and October 2016 at RIVM. Prior to all expert meetings an elaborate background document was prepared with information from academic literature, policy reports, conference presentations and information from the project team's (inter)national network. When relevant, experts were asked to prepare a short presentation for the group. Discussion points for the different meetings were based on the information gathered for the evaluation framework (**Figure 2**).

During each meeting, the experts advised RIVM on potential knowledge gaps and appropriate follow-up (research) to overcome these gaps. They gave an estimation of a feasible timeframe for implementation taking this follow-up (research) into account. For most conditions follow-up research was advised by the experts, mostly validation studies in the Dutch context. For example, studies were proposed to evaluate cut-off values relevant for the Dutch population and screening setting, and clinical outcomes. Furthermore, additional condition-specific research was suggested for X-linked adrenoleukodystrophy (ALD), organic cation transporter 2 deficiency (OCTN-2), and guanidinoacetate methyltransferase deficiency (GAMT). For ALD a pilot study was recommended, since only boys are to be screened which poses significant challenges to the programme (17). For OCTN-2 a study into the predictive value was advised, especially since symptom-free mothers are often detected instead of sick newborns (18). Additional information on the test method was considered necessary for GAMT, as it would be the first fully in-house test method applied in the Netherlands for all neonatal samples.

## Outcomes of the Stakeholder Process

The expert meetings resulted in advisory documents for each condition, with information summarised for each topic from the evaluation framework and suggestions for follow-up (research). Where uncertainties existed, pilot studies and other research projects were advised to resolve the unanswered questions, to be funded by the Organisation for Health Research and Development (ZonMw). The additional information from both the Dutch experts and international experience led to a shift in the previously suggested phasing by the Ministry: three conditions were expected to be implemented sooner, and one later. These outcomes were provided to and discussed with a large group of stakeholders at a meeting held in February 2017. The members of the expert groups, the programme committee, the working group on finance, Youth Health Centre, Dutch Midwives Association, Dutch General Practitioner Association, umbrella association for care organisations, GR and the Ministry of VWS were all invited to this meeting. Finally, the feasibility study for the 14 conditions was compiled and presented to the Ministry of VWS in July 2017.

## From Evaluation to *go/no go* Framework

After the decision of the Ministry of VWS to initiate the implementation of fourteen conditions to the NBS programme, the evaluation framework used in the stakeholder process was developed into a *go/no go* framework for each of the conditions. The combined criteria make a framework which the RIVM-CvB uses to advise the Ministry of VWS on how to add each of the conditions to the programme, and covers topics, such as the test method, the follow-up in healthcare, but also communication and education, costs and legal requirements (**Supplementary Table 1**). The *go/no go* framework is used to prevent overlooking aspects that are of importance when deciding on the start of screening for a new condition.

A condition-specific implementation plan is based upon the framework, as each criterium is scored green, orange

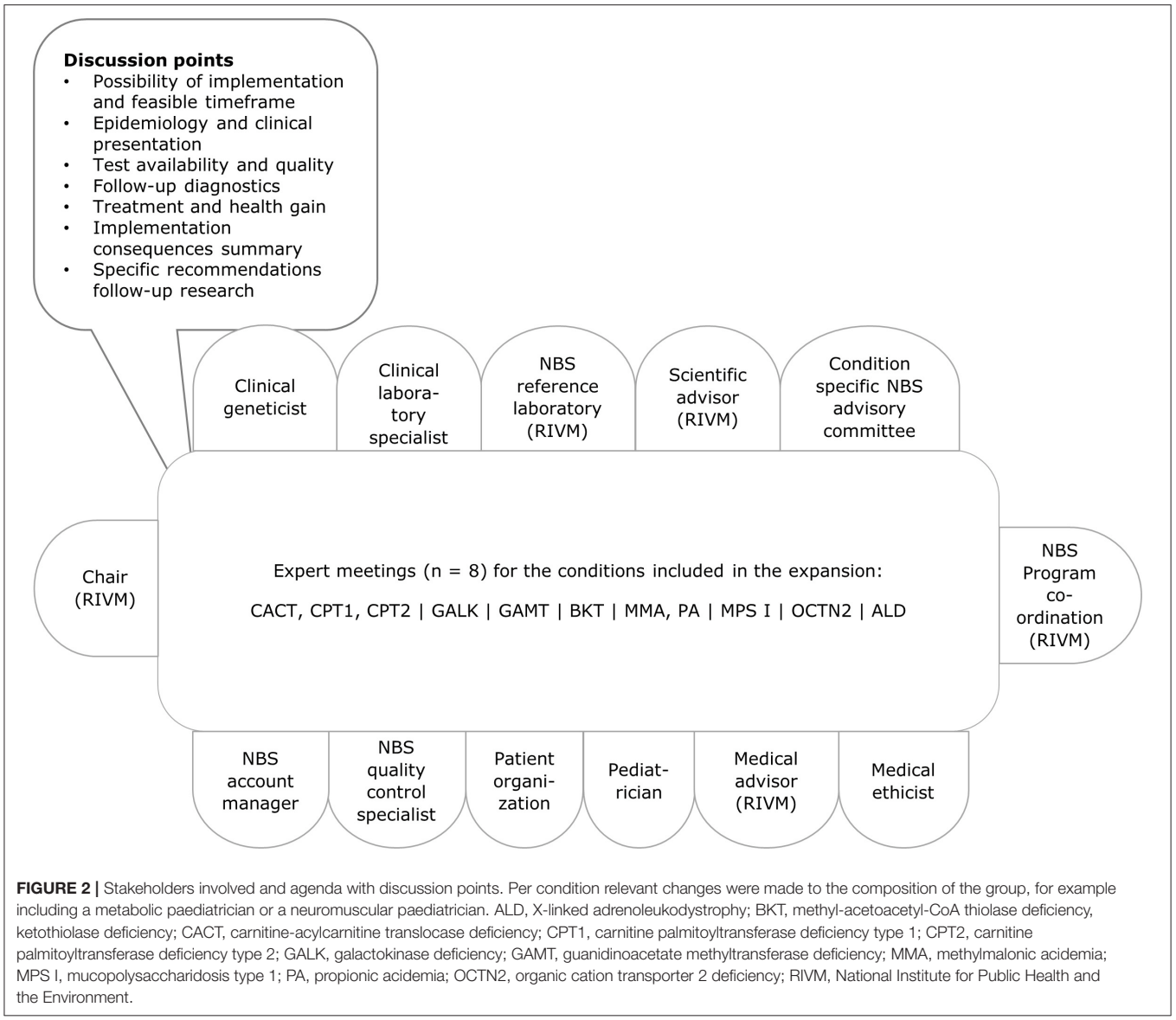


**FIGURE 2 |** Stakeholders involved and agenda with discussion points. Per condition relevant changes were made to the composition of the group, for example including a metabolic paediatrician or a neuromuscular paediatrician. ALD, X-linked adrenoleukodystrophy; BKT, methyl-acetoacetyl-CoA thiolase deficiency, ketothiolase deficiency; CACT, carnitine-acylcarnitine translocase deficiency; CPT1, carnitine palmitoyltransferase deficiency type 1; CPT2, carnitine palmitoyltransferase deficiency type 2; GALK, galactokinase deficiency; GAMT, guanidinoacetate methyltransferase deficiency; MMA, methylmalonic acidemia; MPS I, mucopolysaccharidosis type 1; PA, propionic acidemia; OCTN2, organic cation transporter 2 deficiency; RIVM, National Institute for Public Health and the Environment.

or red to indicate the readiness of implementation on that item (**Supplementary Table 1**). For each condition the implementation plan is discussed in multidisciplinary project teams, similar to the expert groups during the stakeholder process, and the NBS Programme Committee. The RIVM-CvB provides an advice on the *go* or *no go* for screening to the Ministry of VWS in which input of the discussion with stakeholders is used.

## Using the *go/no go* Framework

The *go/no go* framework has been used for eight conditions (BKT, CACT, CPT1, CPT2, GALK, MMA, MPS I, and PA, **Table 1**). It was useful to identify and address the critical points for implementation. It appeared to be challenging to decide on cut-off values for the conditions, due to the rare nature of conditions in NBS, and availability of patient samples. Moreover, the clinical follow-up when a condition will be included in NBS is not always straightforward, this can pose a challenge to reach consensus or lead to a "no go" recommendation.

Applying the framework in different countries, could be challenging if the stakeholder network and support from government is different from the Dutch situation. Nonetheless, involving stakeholders as much as possible early on is highly recommended.

# ACTIONABLE RECOMMENDATIONS AND CONCLUSIONS

## Stakeholder Process

Using the knowledge and expertise of the stakeholders in the NBS programme is of crucial importance to the programme. This is true both for a situation in which the programme is

expanded with new disorders and in evaluating the existing programme. Bringing the stakeholders together and regularly discussing relevant topics leads to new insights, for example concerning the timing of an addition to the program or appropriate clinical follow-up.

Employing a stakeholder process as part of the policy process provided suggestions for additional research and an updated timeline for the implementation of the expansion of the NBS programme. Furthermore, it facilitated support for the upcoming expansion and offered a network of (new) experts while preparing the implementation. The role of a government agency to facilitate the stakeholder process is important to offer a neutral presentation of perspectives and to include a broad range of stakeholders. Stakeholders to include in the NBS programme are for example patients and patient organisations, parents, paediatricians, internal medicine specialists, laboratory experts, scientific researchers and advisors, clinical geneticists, medical ethicists, and medical advisors.

## Roles and Responsibilities of GR, VWS, RIVM

After a policy advice of the GR, based on peer-reviewed publications and expert opinion, the Minister of VWS decides whether a certain condition should be added to the national NBS programme. Subsequently RIVM investigates how this could be implemented, and which uncertainties should be solved or choices that need to be made before the start of screening. Shortly after the condition has been added to the NBS programme, RIVM monitors and evaluates initial results and whether these meet the expectations. It feeds back the results to GR, VWS, and other stakeholders, both short-term and long-term, to make it possible to complete the policy cycle.

As a lesson learned from the previous expansion (2005–2007), for the ongoing expansion extra focus was put on the practical feasibility of screening for the new conditions, such as the potential number of false positives. During the stakeholder process that was employed for the expansion, the applied evaluation framework proved very useful in structuring the discussion. Furthermore, the stakeholder process inspired another framework to assess the *go/no go* for each condition before the actual implementation. Stepping through the policy cycle together with a broad representation of stakeholders, and using this additional implementation step has facilitated a more robust implementation process.

## Conclusions

Sustainability of NBS programmes is an important topic, as the programmes become increasingly complex due to expansions. To further support the sustainability of NBS programmes, closing the public health policy cycle with evaluation and changes to a programme when needed requires more attention. As the framework presented here is based on international examples, and some of these frameworks are designed to be applied for the initiation or cessation of screening for a condition, large parts of this framework could also be applicable to evaluate a condition that is currently included in a NBS programme. Recently, New Zealand applied their screening framework to evaluate OCTN-2 and decided to discontinue screening for it (18). In the Netherlands, the Ministry of VWS has recently asked the GR to evaluate the current screening panel on its sustainability. The applicability of the *go/no go* framework will also be explored in this evaluation.

Test- and treatment driven expansions of NBS programmes occur globally. After decisions to add a certain condition to a screening programme, the complex aspects of how to implement each step of the screening process for each condition require a systematic and sustainable approach. We suggest a *go/no go* framework used in a stakeholder process with broad representation of NBS stakeholders. Furthermore, the international NBS community could benefit from not only sharing experiences on implementation of conditions, but also on other aspects from the policy cycle, such as agenda setting in collaboration with the local government.

## AUTHOR CONTRIBUTIONS

MJ contributed to the conception and design of the work, data collection, analysis and interpretation, drafted the work, and revised it. AK contributed to the design of the work, collection and interpretation of data, and critically revised the work. EB contributed to the design of the work and critically revised it. WR contributed to the conception of the work, data collection, and critically revised the work. MC contributed to the conception and design of the work, interpretation of data, and critically revised the work. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.635353/full#supplementary-material

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# Ophthalmic Manifestation and Pathological Features in a Cohort of Patients With Linear Nevus Sebaceous Syndrome and Encephalocraniocutaneous Lipomatosis

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**Purpose:** This study aimed to figure out the association between ophthalmic and pathological features in patients with Linear Nevus Sebaceous Syndrome (LNSS) and in patients with Oculoectodermal Syndrome—Encephalocraniocutaneous Lipomatosis (OES-ECCL).

**Methods:** It is a retrospective, non-consecutive, observational case series. Twenty-seven patients (12 with LNSS and 15 with OES-ECCL, 41 eyes) referred to the Department of Ophthalmology of the Shanghai Ninth People's Hospital between 2000 and 2020 were included. The mean age of the study population for the first-time consult was 5.7 years, ranging from 3 months to 34 years. Clinical notes, pathological records, and imaging findings were reviewed in all the patients.

**Results:** Fourteen (51.9%) cases showed bilateral ocular involvement. Epibulbar choristomas were seen in all the patients. All the lesions involved the conjunctiva and cornea simultaneously. Multiple lesions were observed in 12 eyes. Of the 14 excised lesions, 11 were found to be complex choristomas. Further, 24 (89%) patients had eyelid coloboma. Also, 13 patients (48%) were diagnosed with strabismus, and 12 patients (44%) had abnormal fundus imaging, including optic nerve hypoplasia.

**Conclusions:** LNSS and OES-ECCL shared common ophthalmic features, including epibulbar choristomas with distinctive characteristics, eyelid coloboma, strabismus, and optic nerve hypoplasia. The complex choristoma was found to be associated with the diseases. These specific patterns can be diagnostic clues to distinguish them from other syndromes, such as craniofacial defects, and to remind ophthalmologists that such patients require additional dermatological and neurological examinations and referral. Moreover, a thorough evaluation of ocular conditions is imperative for early interventions.

**Keywords: encephalocraniocutaneous lipomatosis, linear nevus sebaceous syndrome, oculoectodermal syndrome, ophthalmic symptoms, pathological features**

## INTRODUCTION

Linear Nevus Sebaceous Syndrome (LNSS) and Oculoectodermal Syndrome—Encephalocraniocutaneous Lipomatosis (OES-ECCL) are unique developmental mosaic RASpathies of phacomatoses (neurocutaneous disorders), encompassing both distinctive features and a broad spectrum of manifestations (1–3). Nevus sebaceous is a hallmark of LNSS, characterized as papillomatous hyperplasia of the epidermis (4). LNSS was first reported by Schimmelpenning in 1957 (5). And in 1962 Feuerstein and Mims reported it as a triad of linear naevus sebaceous with epilepsy and developmental delay (6). Several names have been applied since so far, such as epidermal nevus syndrome, Schimmelpenning Feufstein-Mims syndrome, Solomon syndrome and Jadassohn's syndrome. In 1975, Solomon and Esterly reviewed 60 cases and broadened the spectrum of multiorgan involvement: ophthalmological, neurologic, skeletal, cardiovascular, and urologic comorbidities (4). The incidence of ocular Involvements is up to around 60%, and clinical presentations vary from each other, of which the main abnormalities are strabismus, colobomas and choristomas. Complex choristoma is the most characteristic but not the specific ocular feature of the syndrome. Other rare conditions include iris and chorioretinal colobomas, generalized retinal degeneration, antimongoloid lid fissures, asymmetry of orbital bones, circumscribed choroidal hemangioma, etc. (7, 8).

Encephalocraniocutaneous lipomatosis (ECCL), also called Haberland syndrome or Fishman's syndrome, was first reported by Haberland in 1970 (9). There have been roughly 75 cases reported till now. The most characteristic anomalies are nevus psiloliparus (hairless fatty tissue nevus of the scalp), epibulbar choristomas and ipsilateral central nervous system (CNS) anomalies (10). The clinical phenotype of CNS anomalies comprises cranial lipomas, arachnoid cysts, seizures and intellectual disability (11, 12). The ocular manifestations had many similarities in ECCL and LNSS, which include choristomas, coloboma and periocular skin tag. Choristomas, with or without other associated ocular anomalies are the most common clinical features, which can be seen in approximately 80% of the patients. Lipodermoids are usually mild and won't affect the optic axis as well as visual development. However, dermolipomas always present with other atypical ophthalmic malformations, including anterior segment anomalies, cornea anomalies, ocular coloboma, globe calcifications, etc. The pattern of eye findings in ECCL patients was found to be very consistent and recognizable (13).

Oculoectodermal syndrome was first described in 1993 by Toriello et al. (14). Less than 20 cases have been recorded since then (2, 13, 15). Classic clinical features include the association of epibulbar dermoids and aplasia cutis congenita (16). Nevertheless the epibulbar dermoids are not the distinct findings, which can also occur in Goldenhar syndrome. Additional ocular involvements include strabismus, microcornea, nystagmus, etc. The clinical manifestations of the overlap between OES and ECCL are observable with regard to ocular and dermatological symptoms. Evidence showed that the OES is a mild variant of ECCL, differing primarily by lack of intracranial anomalies on brain imaging (17).

The great overlap in clinical manifestations makes it hard to distinguish them from other neurocutaneous disorders.The incidence of ophthalmic anomalies of LNSS and OES-ECCL was around 60–80%, which was relatively high (4, 7, 13, 16, 18). Several ophthalmic conditions have been reported as a part of the case review, which were found to be common between the syndromes, including ocular colobomas and choristomas. Besides, various rare ocular symptoms can also be seen (8, 11, 16, 18). Moreover, the common symptoms are not specific and also can be seen in other diseases as craniofacial defects (4, 13, 18, 19), which makes, leading pediatricians and ophthalmologists to be easily confused with the conditions and overlook the extraocular symptoms. However, few studies explored the association between ocular manifestations in LNSS and OES-ECCL owing to the low incidence of diseases; even few ophthalmologists had the experience of dealing with multiple patients. Whether each of the syndromes has specific ocular manifestations for differential diagnosis or great clinical ophthalmic overlap, like the pattern of skin or neurological symptoms, was still unclear.

This study was performed to share the experience of 27 patients (41 eyes) with LNSS and OES-ECCL. Attempts were made to find distinct clinical patterns and illustrate the common ground and variants among the syndromes by reviewing all the ocular manifestations and pathological findings.

## METHOD

This study was conducted in compliance with the tenets of the Declaration of Helsinki and approved by the ethics committee of the Shanghai Ninth People's Hospital (approvals ID: S9H9-2019-T359-1). The study was a retrospective, non-consecutive, observational case series. All the 27 patients (41 eyes) diagnosed with LNSS or OES-ECCL, including LNSS (12 patients, 20 eyes) and OES-ECCL (15 patients, 21 eyes; OES 10 patients, 14 eyes; ECCL five patients, seven eyes), were referred to the Department of Ophthalmology of the Shanghai Ninth People's Hospital between January 2000 and January 2020. Clinical notes, pathological records, and imaging findings were reviewed

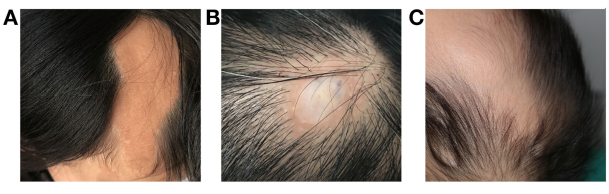


**FIGURE 1 |** Dermatological manifestations of patients with LNSS an OES-ECCL. Sebaceous nevus in a patient with LNSS (patient 4) **(A)**. Nevus psiloliparus (ECCL, patient 25) **(B)** and congenital alopecia (OES, patient 13) **(C)**.

**Abbreviations:** LNSS, linear nevus sebaceous syndrome; OES, oculoectodermal syndrome; ECCL, encephalocraniocutaneous lipomatosis.

in detail. A full ophthalmologic evaluation was performed with special attention to the eyelid coloboma, epibulbar choristomas, eyelid tags, retinal hypoplasia, and strabismus. The assessment included measurement of a slit-lamp biomicroscopic examination, fundus examination, ultrasound biomicroscopy, B-scan ultrasonography, optical coherence tomography, and three-dimensional orbital computed tomography scan for bony defects. One patient underwent targeted next-generation sequencing of skin lesions. Eleven patients underwent excision of epibulbar choristomas. Pathologic diagnoses were confirmed by two pathologists. All patients were photographed. Informed consent forms were signed by the patients and guardians of the children.

# RESULTS

## Study Populations

A total of 27 patients with neurocutaneous syndromes were identified from the clinical database of the Shanghai Ninth People's Hospital, of which 12 were diagnosed with LNSS and 15 with OES-ECCL (10 with OES and five with ECCL) (**Figure 1**). The mean age of the study population for the first-time consultation was 5.7 years, ranging from 3 months to 34 years. No marked sexual dominance was found in patients with a ratio of 1:1 in female vs. male participants. Patient 3 showed a low-level heterozygous mutation in the KRAS gene (c.35C>T; p.G12D, 5 %) (20). Systemic symptoms are listed in **Table 1**.

**TABLE 1 |** Basic information and systemic manifestations of the patients.

| Diagnosis | | Patient no. | Sex | Age | Skin | | | Neurology | | | Other systemic involvement |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Nevus sebaceous | Nevus psiloliparus | Aplasia cutis congenita | Intellectual disability | Seizure | Other | |
| LNSS | | 1 | F | 8 years | + | | | | | | |
| | | 2 | M | 4 months | + | | | | | | |
| | | 3* | F | 1 year | + | | | + | | | |
| | | 4 | F | 1 year | + | | | | | | G, H, and I |
| | | 5 | M | 7 years | + | | | | | | |
| | | 6 | M | 5 years | + | | | + | | | |
| | | 7 | F | 32 years | + | | | | | | |
| | | 8 | F | 6 years | + | | | + | + | | |
| | | 9 | F | 3 years | + | | | | | | |
| | | 10 | F | 11 years | + | | | | | | |
| | | 11 | M | 3 years | + | | | | | | |
| | | 12 | M | 1 year | + | | | + | | A | J, K, L, M, and N |
| OES-ECCL | OES | 13 | M | 7 months | | | + | | | | |
| | | 14 | F | 5 years | | | + | | | | |
| | | 15 | M | 3 years | | | + | | | | O |
| | | 16 | M | 7 years | | | + | | | | P |
| | | 17 | F | 1 year | | | + | | | | |
| | | 18 | F | 6 years | | | + | | | | |
| | | 19 | F | 1 year | | | + | | | | |
| | | 20 | F | 12 years | | | + | | | | |
| | | 21 | F | 34 years | | | + | | | | |
| | | 22 | M | 10 months | | | + | | | | |
| | ECCL | 23 | M | 1 year | | | | | | B | G |
| | | 24 | M | 1 year | | + | | | + | | |
| | | 25 | M | 4 years | | + | | | + | A and C | |
| | | 26 | M | 5 months | | + | | + | + | A, C, D, and E | O and Q |
| | | 27 | M | 3 months | | + | | | | C, E, and F | |

*A, Ventricular dilation; B, pontine lipoma; C, arachnoid membrane tumor; D, pachygyria; E, cerebral hemorrhage; F, hypoxic ischemic encephalopathy; G, cleft palate; H, anotia; I, atresia aural; J, atrial septal defect; K, patent ductus arteriosus; L, pulmonary arterial hypertension; M, neonate hyperbilirubinemia; N, hydrocele of tunica vaginalis; O, temporal bone defect; P, hypoesthesia of limb (right); Q, atrial septal aneurysm.*

**Patient showed a KRAS somatic mosaic mutation.*

**TABLE 2 |** Ocular manifestations of patients with LNSS and OES-ECCL.

| Diagnosis | | Patient no. | Bilateral | Choristoma | Ptosis | Eyelid coloboma | Strabismus | Eyelid tag | Optic nerve abnormalities | Symblepharon | Other symptoms |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LNSS | | 1 | + | + | + | + | + | + | + | | a |
| | | 2 | | + | | | | | + | | b |
| | | 3 | + | + | + | + | + | + | + | + | |
| | | 4 | + | + | + | + | | + | | | c |
| | | 5 | + | + | + | + | + | | | | d |
| | | 6 | + | + | + | + | | | | | |
| | | 7 | | + | + | + | | | | | |
| | | 8 | | + | + | + | + | + | + | | d, e, and f |
| | | 9 | | + | | + | | + | + | | |
| | | 10 | + | + | + | + | + | + | | + | |
| | | 11 | + | + | + | + | + | + | + | | e and g |
| | | 12 | + | + | + | + | + | + | + | + | e |
| OES-ECCL | OES | 13 | | + | | + | + | + | | | |
| | | 14 | | + | | | | | + | | |
| | | 15 | + | + | | + | | | | | e |
| | | 16 | + | + | + | + | + | | | | h |
| | | 17 | + | + | + | + | | | | + | h |
| | | 18 | | + | | + | | + | | | |
| | | 19 | + | + | | + | + | | | | i |
| | | 20 | | + | | + | | + | | | e |
| | | 21 | | + | | + | | | | | |
| | | 22 | | + | + | + | + | + | + | | |
| | ECCL | 23 | + | + | | + | | + | + | | j |
| | | 24 | | + | | + | + | | + | | b, k, and h |
| | | 25 | + | + | + | + | + | + | + | + | l |
| | | 26 | | + | | | | + | | | |
| | | 27 | | + | | + | | + | | | |

*a, Retinal anomalies; b, corneal neovascularization; c, vitreous opacity; d, microphthalmia; e, trichiasis; f, entropion; g, thinning of the retina; h, cornea opacity; i, lower eyelid retraction; j, coloboma chorioideae; k, A-R syndrome; l, globe calcification.*

## Ophthalmic Manifestations

The ocular symptoms are listed in **Table 2**. Among all the participants, 14 (51.9%) showed bilateral ocular involvement. Choristomas, as benign epibulbar tumors, were seen in all the patients (41/41 eyes). Multiple lesions (**Figure 2B**) were seen in 12 eyes. All the lesions, including eight limbus lesions, covered both the conjunctiva and the cornea simultaneously. More interestingly, one of the lesions spanned the bulbar conjunctiva and the outer canthus. Except for isolated limbus lesions, the distribution of the bulbar conjunctiva choristomas (38 eyes) included two choristomas in $\leq$1 quadrant, 10 in more than 1 but $\leq$2 quadrants, 16 in more than 2 but $\leq$3 quadrants (42%), and 10 in more than 3 quadrants (26%). The most common lesion location was superotemporal quadrant (38/38 eyes, 100%) and infratemporal quadrant (28/38 eyes, 74%). Five of the patients had symblepharon secondary to choristomas.

Eyelid anomalies were the second common conditions. Further, 24 children (89%) had eyelid coloboma (**Figures 2A,C**), of which 15 (63%) had an accessory skin tag (**Figure 2A**). The defects mostly affected the middle and inner one third of the upper eyelid. Also, 14 patients (51.9%) had ptosis. Lid abnormality led to trichiasis in five patients (18.5%). Moreover, 13 patients (16 eyes, 48%) had strabismus. Esotropia was found in eight eyes, catatropia in five eyes, esocataphoria in one eye, exocataphoria in one eye, and exotropia in one eye. Moreover, 12 patients (44%) had abnormal fundus imaging showing optic nerve abnormalities. Optic nerve hypoplasia (**Figures 2D,E**) was found in nine patients.

Other rare malformations included corneal opacity, lower eyelid retraction, coloboma chorioideae, thinning retina, and enlarged C/D ratio. The severity of skin and eye findings was not always consistent. The prevalence of symptoms is presented in **Figure 3**.

## Pathology

Lesions on conjunctiva varied in gross appearance, ranging from small, flat pterygium-like lesions to large raised masses. However, unclear boundaries and

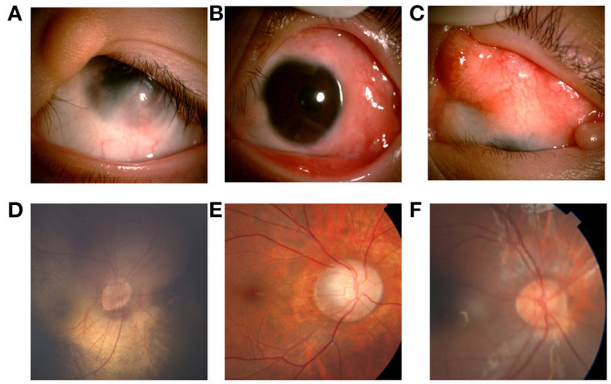


**FIGURE 2 |** Ocular manifestations of patients with LNSS an OES-ECCL. Single flat epibulbar choristoma involving the cornea with unclear boundaries and upper eyelid coloboma with skin tag (LNSS, patient 9) **(A)**. Mutiple epibulbar choristomas involving the cornea, and esotropia (LNSS, patient 1) **(B)**. Large, raised epibulbar choristoma, and upper eyelid coloboma (ECCL, patient 25) **(C)**. The oval optic disc, inferior temporal optic disc coloboma, and the vessels crawl out the edge of the disc, which is similar to the pattern of morning glory disc. The patient with corneal choristoma was examined under anesthesia (LNSS, patient 3) **(D)**. The boundary of the optic disk is not clear with a lighter color, and the exposed scleral ring can be seen around the disk (ECCL, patient 25) **(E)**. Congenital coloboma of the optic disk (OES, patient 14) **(F)**.

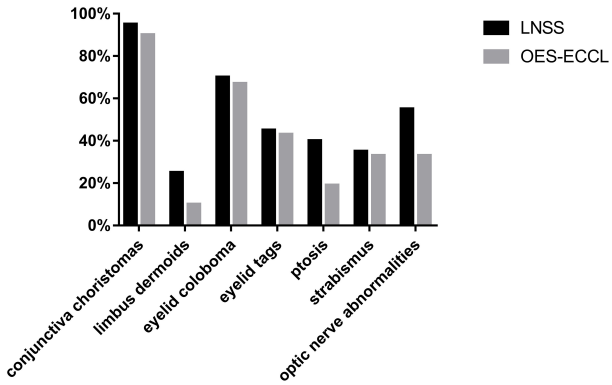


**FIGURE 3 |** The prevalence of main ocular manifestations in patients with LNSS and OES-ECCL.

the involvement of the cornea were common among most lesions.

Twelve patients with choristomas underwent surgical removal. The histopathological findings are summarized in **Table 3**. Two patients had dermolipomas, another one was diagnosed with limubs dermoid, whereas the remaining (78.5%) had complex choristomas (**Figure 4**). Complex choristomas composed of fibrous connective tissue, smooth muscle, and glandular tissue, such as lacrimal tissue and sebaceous glands, accounted for eight cases. Hyaline cartilaginous tissues were found in five cases.

## DISCUSSION

In this study, the largest case series was used to study the association between ocular manifestations in LNSS and OES-ECCL. No obvious difference was found with regard to the ocular symptoms between the two groups, which was in contrast with the pattern of neurological and dermatological symptoms (**Figure 3**). Besides, shared features included epibulbar choristomas, eyelid colobomas, periocular skin tags, and optic nerve anomalies. The ocular symptoms might also follow a certain pattern according to clinical experience; patients with epibulbar choristomas involving both conjunctiva and cornea, combined with eyelid coloboma, might lead to the suspicion of having LNSS or OES-ECCL. Of these, patients with ECCL were more likely to have eyelid skin tags (74%) on account of the pathogenesis. The complex choristomas were both closely associated with LNSS (six in seven lesions) and OES-ECCL (five in seven lesions).

Epibulbar choristomas (except for limbus dermoids) were mostly observed in the present cohort (38/41 eyes, 92.7%). Epibulbar choristomas are relatively rare benign tumors. They are masses of histologically normal tissue in an abnormal location, mostly located on the limbus or solely on the superior temporal quadrant of conjunctiva, with clear boundaries (19, 21). In this cohort, a type of epibulbar choristomas with unique features was identified, which were large masses involving both the cornea and the conjunctiva, with unclear boundaries. The unique characteristics may distinguish them from dermoids, lipodermoids, and prolapsed orbital fat. Most of them (26/38 eyes) filled in more than two quadrants of the bulbar conjunctiva in this study, and all covered the supratemporal quadrant. All masses were reported without growth in size by the parents.

For associated ocular anomalies, eyelid coloboma is the most recorded anomaly with a featured location of the upper inner one-third eyelid. Periocular skin tags, located on the site of eyelid coloboma, are also common among patients. Strabismus was one of the common conditions with the foremost diagnosis of esotropia. The etiology of strabismus in children is complicated. The possible causes include congenital abnormalities of extraocular muscle development, nuclei abnormalities that dominate eye movements, abnormal vision development, and restrictive diseases. Patients with strabismus also have large choristomas, mostly combined with ptosis, optic nerve abnormalities, and symblepharon, attributable to multiple causes. Large-sized choristomas and secondary symblepharon may cause abnormal eye positions and restricted eye movement. At the same time, strabismus of visual deprivation occurs when patients have abnormal development of the optic nerve, severe ptosis, and blocked visual axis. When dealing with particular patients, the first step is to remove the restrictive factors and the obstructions that affect the development of the optic axis, and then re-evaluate the strabismus and the fundus. The principle of the treatment is to deal with amblyopia to improve vision.

Fundus anomalies are hard to describe in a predictable pattern due to significant clinical variability (22). However, coloboma is the most frequent malformation involving the optic

**TABLE 3 |** Histopathologic features of epibulbar complex choristoma in 12 surgically resected cases.

| Diagnosis | | Patients | Lesion | Location (quadrant) | Fibrous connective tissue | Hair follicle tissue | Adipose tissue | Lacrimal gland | Epidermal tissue | Sebaceous gland | Smooth muscle bundles | Cartilage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LNSS | | 4 | Complex choristoma OD | Temporal with eyelid involvement | + | + | | | | + | | |
| | | | Complex choristoma OS | Temporal | + | | + | + | + | | | + |
| | | 7 | Complex choristoma | Upper | + | | + | | | | + | + |
| | | 9 | Complex choristoma | Upper | + | + | + | + | + | + | + | |
| | | 11 | Complex choristoma | Circumferential | + | + | + | + | + | + | + | |
| | | | dermoid | limbus | + | + | | | | + | | |
| | | 12 | Complex choristoma | Upper | + | | | | | + | + | + |
| OES-ECCL | OES | 14 | Complex choristoma | Upper | + | | + | + | | | | + |
| | | 16 | Dermolipoma | Upper | + | | + | | | | | |
| | | 19 | Complex choristoma | Upper with temporal | + | | + | + | + | | + | |
| | | 22 | Complex choristoma | Temporal | + | + | + | | + | + | | + |
| | ECCL | 24 | Complex choristoma | Temporal | + | + | + | + | | + | | |
| | | 25 | Complex choristoma | Circumferential | + | | + | | | | | + |
| | | 26 | Dermolipoma | Upper | + | | + | | | | | |

disk (**Figure 2F**) (13, 23). Optic disk hypoplasia was found in most cases, appearing typically as a white, decentered excavation of the optic disk, or with marginal hypoplasia, accompanied by surrounding retina-choroidal atrophy. Congenital disk conformation anomalies can be related to all levels of vision, ranging from 20/200 to finger counting (24). Thus, fundus assessments should be emphasized for routine screening for patients as early as possible.

Regarding the histological diagnosis, complex choristomas (11/14 lesions) are associated with the subtypes of a neurocutaneous syndrome. Complex choristomas contain cells from two or more different tissue types, such as bone, cartilage, nerve, muscle, sebaceous secretions, or lacrimal tissues. Complex choristomas can be sporadic cases or can be associated with Goldenhar syndrome and LNSS (25–28). For patient 24, the first biopsy of the nasal part showed a dermolipoma, and the result of the second biopsy of the temporal part disclosed a complex choristoma with immature hair follicle tissue, sebaceous gland, and hyperplasia of adipose tissue. Thus, it is suggested that biopsy at more than one site should be taken into consideration for large epibulbar masses associated with congenital neurocutaneous syndromes.

LNSS and OES-ECCL are the results of postzygotic KRAS, HRAS, NRAS, and FGFR1 mutations (2, 29). The genetic testing of somatic mutations in KRAS and FGFR1 genes could provide a tool for early diagnosis. In DNA from the skin lesion of patient 3, mosaicism of KRAS c.35C>T; p.G12D was found. The mutation identified in this study was also reported in a patient with rhabdomyosarcoma (19, 30) which supports the conclusion of emerging mutation overlap between mosaic developmental disorders and tumorigenesis (29). Even with relatively large sample sizes, the rarity of the diseases might limit the analysis. Also, fundus characteristics were hardly summarized thoroughly because the evaluation was impossible for most of the patients with corneal anomalies. The RAS-MAPK pathway regulates important cellular processes, including DNA synthesis. Therefore, further functional studies of the mutation spot should be carried out (29). Despite these limitations, the study provided a fresh perspective on the association between ocular manifestations in LNSS and OES-ECCL. Unlike the cutaneous or neural symptoms, these types of diseases have no distinct clinical patterns of ocular anomalies. In other words, a differential diagnosis cannot be made based solely on ophthalmic examinations. However, these diseases have common but unique ophthalmic features, such as distinct epibulbar choristomas, or combinations of choristomas with eyelid colobomas. These specific patterns can be diagnostic clues to distinguish them from other syndromes,

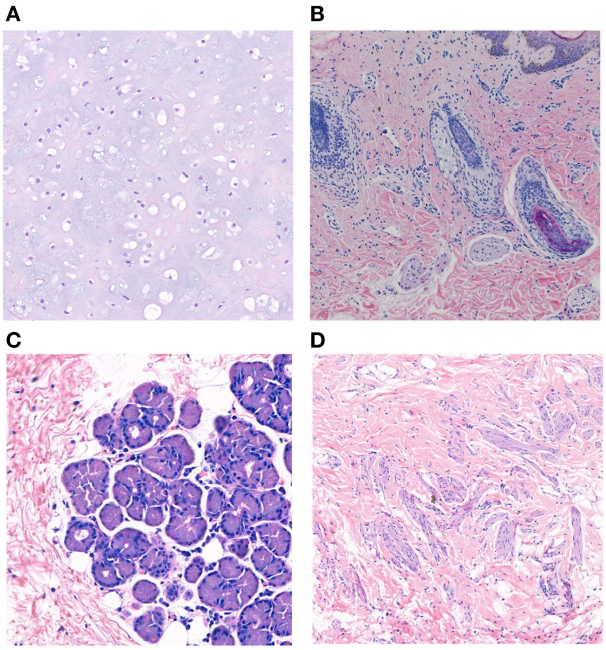


**FIGURE 4 |** Histopathological studies of complex choristoma cases. The complex choristoma was composed of cartilaginous tissue (patient 7) **(A)**. Immature hair follicle tissues combined with sebaceous glands were found in the biopsy of conjunctival lesions (patient 24) **(B)**. The complex choristoma contained lacrimal gland tissue (patient 9) **(C)**. The large complex choristoma contained both fibrous connective tissue and smooth muscle (patient 11) **(D)** (hematoxylin-eosin-stained, ×20 original magnification).

such as craniofacial defects and Goldenhar syndrome, and to remind ophthalmologists that such patients require additional dermatological and neurological examinations and referral. The ocular anomalies can be primary clinical presentations, requiring more attention.

The ophthalmic referral is vital as early as possible for patients who may only have a suspected diagnosis. Eyelid coloboma is with high incidence in these two diseases. It is proved that early surgical treatment of eyelid coloboma can not only improve the transparency of the cornea and the homeostasis of the ocular surface but also protect the visual development. Timely treatment requires a multidisciplinary physician collaboration. For pediatricians, understanding the characteristics of eye complications for timely referral can improve the patient's appearance and protect visual development, which reduces the burden on the children's family and improves their quality of life. Updating research on these rare congenital neurocutaneous syndromes' clinical features and experience is a win-win scenario for both patients and doctors.

## CONCLUSIONS

The ocular involvement of LNSS and OES-ECCL has a great clinical overlap, including epibulbar choristomas, eyelid coloboma, strabismus, and optic nerve hypoplasia. The distinctive characteristic is a large epibulbar complex choristoma involving the cornea and conjunctiva, with unclear boundaries. The unique clinical patterns can serve as diagnostic clues to differ LNSS and OES-ECCL from other syndromes. A thorough evaluation of ocular conditions is imperative for early interventions.

## DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai Ninth People's Hospital and complied with the tenets of the Declaration of Helsinki for clinical research (IRB: S9H9-2019-T359-1). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

YY, YF, and YZ had full access to all of the clinical data in the study and take responsibility for the integrity of the data and the accuracy of the case series. YY and YF: study concept and design. YY, HZ, SZ, YZ, and YF: acquisition, analysis of data. YY: drafting of the manuscript. YY, YZ, and YF: critical revision of the manuscript for important intellectual content. YF: obtained funding, administrative, technical, or material support. YZ and YF: study supervision. All authors have read and approved the manuscript and agree to be accountable for all aspects of the work.

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# Prevalence of Growth Restriction at Birth for Newborns With Congenital Heart Defects: A Population-Based Prospective Cohort Study EPICARD

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**Background and Objectives:** Congenital heart defects (CHD) and growth restriction at birth are two major causes of childhood and adult morbidity and mortality. The aim of this study was to assess the overall risk of growth restriction at birth, as measured by its imperfect proxy small (< 10th percentile) for gestational age (SGA), for newborns with CHD.

**Methods:** Using data from a population-based cohort of children born with CHD, we assessed the risk of growth restriction at birth using SGA and severe SGA (3rd percentile). To compare the odds of SGA and severe SGA across five specific major CHD, we used ordinal logistic regression using isolated, minor (non-operated) ventricular septal defect (VSD) as the control group.

**Results:** The overall proportion of SGA for "isolated" CHD (i.e., those not associated with other anomalies) was 13% (95% CI, 12–15%), which is 30% higher than what would be expected in the general population (i.e., 10%). The risk of severe SGA was 5% (95% CI, 4–6%) as compared with the expected 3% in the general population. There were substantial differences in the risk of overall SGA and more so severe SGA across the different CHD. The highest risk of SGA occurred for Tetralogy of Fallot (adjusted OR 2.7, 95% CI, 1.3–5.8) and operated VSD (adjusted OR 2.1, 95% CI, 1.1–3.8) as compared with the control group of minor (non-operated) VSD.

**Conclusion:** The overall risks of both SGA and severe SGA were higher in isolated CHD than what would be expected in the general population with substantial differences across the subtypes of CHD. These results may provide a clue for understanding the underlying mechanisms of the relation between alterations in fetal circulation associated with different types of CHD and their effects on fetal growth.

**Keywords: small for gestational age, congenital heart defects, population-based cohort, prevalence, ordinal logistic regression**

## INTRODUCTION

Congenital heart defects (CHD) are the most frequent group of congenital anomalies with a prevalence of about 1% of all births (1, 2). Newborns with CHD are at a higher risk of growth restriction at birth (3–5). The latter may be an independent risk factor for adverse outcomes in newborns with CHD (5).

By far most of the previous studies that investigated the relation between CHD and growth restriction were hospital-based and population-based studies remain rare (3). Some of the literature has the shortcoming of including CHD associated with chromosomal or other anomalies without separate analyses of "isolated" CHD (not associated with chromosomal or other anomalies). Hence, the effects associated with the CHD *per se* are not always clear. Moreover, the specific effects of different types of CHD on the risk of growth restriction has not been adequately studied. Such an analysis may provide clues about the possible underlying mechanisms of the associations between CHD and growth restriction.

We used data from a population-based, prospective cohort study of more than 2,000 newborns with CHD to: (i) Assess the overall risk of growth restriction at birth for newborns with isolated CHD and to: (ii) Compare the risk and severity of growth restriction for five major types of CHD.

## MATERIALS AND METHODS

### Data Source

The EPICARD study was a population based prospective cohort of children born with CHD in the Greater Paris area (Paris and its surrounding suburbs) of France carried out between 2005 and 2008.

All cases (live births, terminations of pregnancy for fetal anomaly, TOPFA and fetal deaths) diagnosed prenatally or up to 1 year of age were eligible for inclusion. Diagnoses of CHD and associated comorbidities (i.e., genetic, extra cardiac anomalies and/or syndromes) were confirmed by specialized pediatric cardiologists. Detailed description of the EPICARD cohort has been provided elsewhere (6).

From the EPICARD cohort of all live births ($N = 2{,}348$), we excluded 112 multiple pregnancies and ten subjects with missing data on birthweight and/or gestational age. Newborns with chromosomal anomalies ($n = 142$) or anomalies of other systems and/or genetic syndromes ($n = 295$) were also excluded from the study population. Our final study population comprised 1,789 singleton newborns with isolated CHD and known birthweight and gestational age (**Figure 1**).

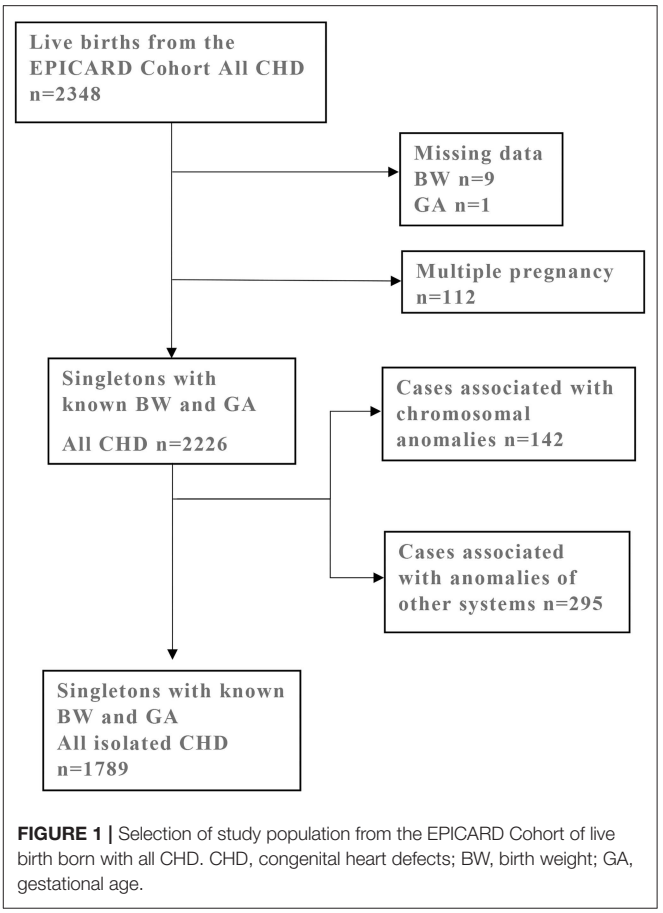


**FIGURE 1 |** Selection of study population from the EPICARD Cohort of live birth born with all CHD. CHD, congenital heart defects; BW, birth weight; GA, gestational age.

### Outcome and Predictor Variables

The outcome variable, Small for Gestational Age (SGA) was defined as sex- and gestational age-specific birthweight <10th percentile based on the EPOPé population-based growth curves (7). We defined severe SGA using the cut-off birthweight <3rd percentile and Intermediate SGA as birthweights between the 3rd < 10th percentiles. Not -SGA was defined based on birthweight ≥ 10th percentile (8).

The main predictor variable of interest was type of CHD. We also took into account a set of potentially confounding variables including maternal diabetes, hypertension, smoking, maternal age, geographic origin, parity, prenatal diagnosis, infertility treatments, sex and preterm (< 37 weeks) delivery.

### Statistical Analysis

As the outcome variable comprised ordered outcomes (severe SGA/intermediate SGA/Not SGA) we used ordinal logistic regression for the statistical analysis. The proportional odds assumption for the ordinal logit models was tested and the models were found to be consistent with a proportional odds model.

The proportional odds model considers the cumulative probability of an individual event and all other events that are ordered before it (**Box 1**) (9, 10). Whereas, binary logistic regression uses the logit (log odds) function, ordinal logistic regression uses the logit transformation of the cumulative odds. In the proportional odds logit model, the slopes that correspond to the model coefficients are parallel to one another and the odds for each cut-off category differ only with regards to the intercept (**Box 1**). The $\chi^2$-test for the proportional odds assumption

**Abbreviations:** CHD, Congenital Heart defects; VSD, Ventricular Septal Defects; CoA, Coarctation of the Aorta; ToF, Tetralogy of Fallot; TGA, Transposition of the Great Arteries; FUH, Functionally Univentricular Heart; SGA, Small for Gestational Age; TOPFA, Termination of Pregnancy for Fetal anomaly.

**BOX 1** | Ordinal logistic regression (9, 10).

*Cumulative Odds* $(Y \leq j) = \frac{P(Y \leq j)}{1-P(Y \leq j)}$ , $j = 1,\ldots,k$

and in *logit* form :

*logit* $(Y \leq j) = \ln\left(\frac{P(Y \leq j)}{1-P(Y \leq j)}\right)$ , $j = 1,\ldots,k$

$$logit\,(Y \leq j) = \alpha_j + X'\beta$$

Where:

$P(Y \leq j) = P_1 + P_2 + \ldots + P_j$ is the cumulative probability of the event

$\alpha_j$: intercept parameters

$\beta = (\beta_1, \beta_2, \ldots, \beta_3)$: a vector of unknown regression coefficients.

suggested that this was a reasonable assumption in the case of our models.

The statistical significance level was set at $\alpha = 0.05$ and all analyses were done using Stata v15.1 software (StataCorp LP, College Station, TX, USA).

Ethics approval was obtained from the CNIL (Commission nationale de l'informatique et des libertés) (6).

## RESULTS

**Table 1** shows characteristics of the study population. Overall, 1,789 newborns with isolated CHD (not associated with chromosomal or other anomalies) were included in the study population. Of those, 47% were boys and 11% born preterm. Approximately 3% of women reported smoking during pregnancy, 1% had diabetes and 1% reported illicit drug use. Maternal age was 35 years or older for one quarter of women. One half of women were of French origin and 19% of North African origin. Approximately 7% of the study population were born after infertility treatments and 17% had a prenatal diagnosis of the CHD.

**Table 2** shows the proportions of SGA and severe SGA for isolated CHD and isolated specific CHD. The prevalence of SGA for isolated CHD was 13% (95%CI 12–15%) and 5% (95%CI 4–6%) for severe SGA. For specific CHD, SGA, ranged from 10% (95% CI 9–12%) for minor non-operated VSD to 26% (95% CI 16–40%) for Tetralogy of Fallot (ToF). Severe SGA proportions for specific CHD ranged from 4% (95% CI 3–5%) for non-operated VSD to 17% (95% CI 9–31%) for the ToF.

**Table 3** shows the results of the ordinal logistic regression analysis for the five different types of CHD. There were substantial differences in the odds of both intermediate SGA and severe SGA across the five specific CHD.

In particular, the odds of overall and severe SGA were substantially higher for operated VSD and for ToF as compared with minor non-operated VSD; the adjusted odds ratios from the ordinal logit model were 2.1 (95% CI, 1.1–3.8) and 2.7 (95% CI, 1.3–5.8) for operated VSD and ToF, respectively.

**TABLE 1** | Characteristics of the study population: EPICARD Study.

| | *N* | % | 95% CI |
|---|---|---|---|
| Sex | | | |
| Male | 847 | 47 | 45–50 |
| Preterm birth (< 37 weeks) | | | |
| Yes | 192 | 11 | 9–12 |
| Smoking during pregnancy | | | |
| Yes | 50 | 3 | 2–4 |
| Maternal diabetes | | | |
| Yes | 25 | 1 | 1–2 |
| Maternal illicit drug use | | | |
| Yes | 10 | 1 | 0–1 |
| Maternal age | | | |
| < 29 | 675 | 38 | 36–40 |
| 30-34 | 650 | 37 | 34–39 |
| 35-39 | 343 | 19 | 18–21 |
| > 40 | 111 | 6 | 5–7 |
| Parity | | | |
| 0 | 638 | 36 | 34–38 |
| 1 | 545 | 31 | 29–33 |
| > 2 | 595 | 33 | 31–36 |
| Maternal geographic origin | | | |
| France | 907 | 51 | 49–53 |
| North Africa | 330 | 19 | 17–20 |
| Sub Saharan Africa | 217 | 12 | 11–14 |
| Other | 329 | 18 | 17–20 |
| Maternal high blood pressure | | | |
| Yes | 22 | 1 | 1–2 |
| Prenatal diagnosis of CHD | | | |
| Yes | 313 | 17 | 16–19 |
| Assisted reproductive technologies | | | |
| Yes | 124 | 7 | 6–8 |
| Small for gestational age | | | |
| Normal | 1,554 | 87 | 85–88 |
| < 10th percentile | 235 | 13 | 12–15 |
| 3rd−10th percentile | 142 | 8 | 7–9 |
| < 3rd percentile | 93 | 5 | 4–6 |
| Birth weight (gr) | *Mean* | *SD* | |
| | 3,175 | 618.71 | 3,147–3,204 |
| Total number of patients | 1,789 | | |

## DISCUSSION

Using population-based data from a large prospective cohort of children born with isolated CHD, we found that the overall prevalence of SGA was 13% and that of severe SGA 5%, both of which are higher than the expected proportions in the general population, 10 and 3%, respectively, based on the EPOPé population-based growth curves in France (7, 11).

We also found important differences in the probability of SGA and of severe SGA across the different types of CHD. In particular, VSD, which required surgery and Tetralogy of Fallot were associated with two- to three-folds higher odds of both intermediate and severe SGA, whereas minor VSD that did not require surgery was not associated with any significant increase in the risk of SGA as compared with the expected proportions in the general population. Whereas, newborns with SGA as a whole may include those who are constitutionally small, our findings

**TABLE 2 |** Proportions of SGA (<10th percentile), intermediate SGA (≥ 3rd percentile <10th percentile), and severe SGA (<3rd percentile) for all isolated CHD, major isolated CHD, and isolated specific CHD.

| | | SGA | | | Intermediate SGA | | | Severe SGA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | ***N*** | **%** | **95% CI** | ***N*** | **%** | **95% CI** | ***N*** | **%** | **95% CI** |
| All isolated CHD | 1,789 | 235 | 13 | 12–15 | 142 | 8 | 7–9 | 93 | 5 | 4–6 |
| All isolated major CHD | 493 | 78 | 16 | 13–19 | 51 | 10 | 8–13 | 27 | 6 | 4–8 |
| Specific isolated CHD | | | | | | | | | | |
| ToF | 53 | 14 | 26 | 16–40 | 6 | 11 | 5–24 | 8 | 15 | 8–28 |
| TGA | 78 | 9 | 12 | 6–21 | 8 | 10 | 5–19 | 1 | 1 | 0–9 |
| CoA | 71 | 12 | 17 | 10–28 | 6 | 9 | 4–18 | 6 | 9 | 4–18 |
| FUH | 36 | 7 | 19 | 9–36 | 3 | 8 | 3–24 | 4 | 11 | 4–27 |
| Operated VSD | 128 | 27 | 21 | 15–29 | 16 | 13 | 8–20 | 11 | 9 | 5–15 |
| Non-Operated VSD | 1,063 | 113 | 11 | 9–13 | 69 | 6 | 5–8 | 44 | 4 | 3–6 |

*CHD, congenital heart defects; CoA, coarctation of the aorta; FUH, functionally univentricular heart; SGA, small for gestational age; TGA, transposition of the great arteries; ToF, Tetralogy of Fallot; VSD, ventricular septal defects.*

**TABLE 3 |** Odds ratios of SGA (severe and intermediate vs. normal) for different types of isolated CHD by ordinal logistic regression.

| | **Crude odds ratio** | **95% CI** | **Adjusted odds ratio**** | **95% CI** |
|---|---|---|---|---|
| Minor ventricular septal defect (VSD) | Reference | | Reference | |
| Operated ventricular septal defect (VSD) | 2.8 | 1.6–4.8 | 2.0 | 1.1–3.8 |
| Univentricular heart (UVH) | 2.2 | 0.9–5.1 | 2.0 | 0.7–5.5 |
| Tetralogy of Fallot (ToF) | 3.3 | 1.7–6.2 | 2.7 | 1.3–5.8 |
| Transposition of great arteries (TGA) | 1.1 | 0.5–2.2 | 1.1 | 0.5–2.5 |
| Coarctation of the aorta (CoA) | 1.8 | 0.9–3.4 | 1.4 | 0.6–3.0 |

***Adjusted on diabetes, maternal high blood pressure, maternal smoking during pregnancy, maternal geographic origin, parity, prenatal diagnosis, assisted reproductive therapy, gender and prematurity.*

were similar when we looked at severe SGA (birthweight <3rd percentile) and the latter is considered, by definition, to represent growth restriction at birth (12).

Our findings on the overall proportion of SGA for isolated CHD are comparable with a previous systematic review (3). However, previous data summarized in this systematic review did not allow estimates for severe SGA or for comparison of proportions of SGA across different types of CHD. Variations in the proportions of SGA and severe SGA for different CHD as reported in our study may provide insights into the pathophysiological mechanisms that link CHD with growth restriction at birth.

Two potential mechanisms may explain the relation between CHD and growth restriction at birth in general and in the case of differences across various types of CHD in particular. These include altered fetal hemodynamics and placental anomalies.

Matthieson et al. studied placental weight z scores in a Danish cohort of 7,569 children with CHD. They found that ToF and major VSD had lower placental weight which was in turn correlated with reduced birth weight and head circumference z scores (13).

Jones et al. found increased placental leptin secretion in children born with hypoplastic left heart syndrome and SGA (14). They argued that placental insufficiency results in SGA through reduced angiogenesis, which in turn reduces the surface area for gaseous exchange.

There may also be common etiological factors that cause both CHD and placental anomalies. Nitric oxide synthase (NOS) deficiency may be the common etiological factor that results in both CHD and fetal growth restriction. Liu et al. found that NOS was important in fetal heart development with deficiencies resulting in CHD (15). Other studies have shown that endothelial NOS may play an important role in fetal growth (16, 17).

Another possible mechanism is that alterations in blood flow circulation, result in differential perfusion and/or oxygen supply in the fetal body, which may in turn cause growth restriction in certain types of CHD but not necessarily others.

Wallenstein et al. found that CHD are associated with growth restriction and based on previous works by Rizzo et al. and Lutin et al., they argued that decreased ventricular output results in SGA but this may only occur in CHD with altered ventricular function (4, 18, 19). Using cardiovascular magnetic resonance, Al Nafsi et al. found that superior vena cava blood flow varied in left sided CHD compared to controls without CHD (20). Story et al. also found differences in the proportion of SGA in specific CHD, notably 13% SGA in TGA, 17% SGA in CoA and 26% SGA in ToF (results similar to those in our study) (5). The authors hypothesized that growth restriction for ToF was due to decreased fetal blood flow and hence reduced oxygenation. This may only be true however, in case of fetal heart failure or when the arterial duct is absent or closed. They also reported that newborns with CoA have decreased overall birthweight and

length but normal head circumference and a greater head volume to birthweight ratio. The latter may be due to decreased caudal blood flow (without a decrease in oxygen saturation.

Donfrio et al. used Doppler ultra sound to demonstrate that decreased blood oxygenation due to abnormal fetal hemodynamics results in enhanced cerebral blood perfusion (brain sparing effect) as an adaptive compensatory mechanism in single ventricle defects (hypolastic left and right syndromes) (21). However, enhanced cerebral perfusion occurs at the expense of fetal liver, renal, pancreatic and mesenteric circulation, which in turn results in decreased production of insulin growth factor, angiotensin and other endocrine hormones essential for fetal growth (22). These hormones may affect the fetal growth directly as is the case of insulin growth factor or indirectly via placental function (e.g., renin-angiotensin system), inducing an inflammatory response or through other biomolecular pathways (23, 24).

Alternatively, abnormal fetal hemodynamics may affect the placenta resulting in SGA either through elevated fetoplacental vascular resistance due to placental ischemia or by fetoplacental endothelial dysregulation which plays a key role in tempering inflammatory regulators and nutrient exchange (25). Nevertheless, in general, the underlying pathophysiological mechanisms of the associations (or lack thereof) between CHD and the risk of growth restriction are complex and most likely involve multifactorial causal pathways and compensatory mechanisms that are not completely understood. Moreover, in addition to the placental and fetal hemodynamic mechanisms, there are also genetic and epigenetics factors related to the risk of growth restriction with CHD (26–28).

Our study has certain limits. Our study was not designed and cannot disentangle the possible mechanisms that may explain our empirical findings. Indeed, investigation of the possible underlying mechanisms of the relation between CHD and fetal growth were beyond the scope or ambition of our study.

In addition, even if data were from a large, population-based of newborns with CHD, the number of cases for individual specific CHD was relatively small, which resulted in reduced precision in our estimates, particularly for the fortunately less common severe SGA outcomes. However, by using the ordinal logit model, which allowed looking at both severe and intermediate SGA outcomes in the same model, we were able to increase the statistical power of our study, including for severe SGA outcome (9, 10).

## Conclusion

Congenital heart defects are associated with a higher risk of growth restriction at birth, including a higher risk of severe growth restriction.

The risk of growth restriction was substantially higher for certain types of CHD, notably operated ventricular septal defects and the Tetralogy of Fallot. The underlying mechanisms of the relation between CHD and growth restriction at birth may be hypoxia and alternations in blood perfusion in the fetus. In addition, placenta is likely to play an important role in the causal links between CHD and fetal growth restriction. Future studies are needed to disentangle the underlying mechanisms, including genetic and epigenetic factors that may explain the higher risk of growth restriction for newborns with congenital heart defects.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CNIL (French National Committee of information and Liberty). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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## AUTHOR CONTRIBUTIONS

BK conceived the study. AG conducted the statistical analyses and wrote the first draft of the manuscript. DB, FG, and BK contributed to the conceptualization of ideas and made suggestions about the required analyses. All authors contributed to the interpretation of findings and revisions of the article.

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# Petroleum and Chlorinated Solvents in Meconium and the Risk of Hypospadias: A Pilot Study

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**Background:** Hypospadias is a male congenital malformation that occurs in ∼2 of 1,000 births. The association between hypospadias and fetal exposure to environmental chemicals has been studied, but the results are inconsistent. Although several petroleum and chlorinated solvents are suspected to have teratogenic effects, their role in the occurrence of hypospadias has been little studied and never using biomarkers of exposure. We aimed to evaluate the association between fetal exposure to petroleum and chlorinated solvents measured in meconium and the occurrence of hypospadias.

**Methods:** We conducted a pilot case-control study in the maternity of the University Hospital of Rennes (France). Eleven cases of hypospadias and 46 controls were recruited between October 2012 and January 2014. Data from hospital records and maternal self-reported questionnaires, including socio-demographic characteristics and occupational and non-occupational exposure to chemicals, were collected. Meconium samples were collected using a standardized protocol. Levels of petroleum solvents (toluene, benzene, ethylbenzene, and p, m, and o xylene), certain metabolites (mandelic acid, hippuric acid, methylhippuric acid, S-phenylmercapturic acid, S-benzylmercapturic acid, and phenylglyoxylic acid), and two chlorinated solvents (trichloroethylene and tetrachloroethylene) were measured in meconium by gas and liquid chromatography, both coupled to tandem mass spectrometry. Associations between the concentration of each chemical and the occurrence of hypospadias were analyzed using exact logistic regressions adjusted for maternal age, educational level, pre-pregnancy body mass index, and alcohol, and tobacco consumption during pregnancy. Results are presented with odds ratios (ORs) and their 95% confidence intervals (CIs).

**Results:** Quantification rates for petroleum and chlorinated solvents or metabolites ranged from 2.2% (for methylhippuric acid) to 77.1% (for trichloroethylene) of the meconium samples. We found a significant association between the quantification of phenylglyoxylic acid (metabolite of styrene and ethylbenzene) in the meconium and a higher risk of hypospadias (OR = 14.2, 95% CI [2.5–138.7]). The risk of hypospadias was non-significantly elevated for most of the other solvents and metabolites.

**Conclusion:** This exploratory study, on a limited number of cases, suggests an association between petroleum solvents and hypospadias. Additional studies are needed to confirm these results and identify the determinants for the presence of these solvents in meconium.

**Keywords: hypospadias, BTEX, petroleum solvents, chlorinated solvents, meconium, volatile organic compound**

## INTRODUCTION

Hypospadias is a male congenital malformation defined by a displaced urethral meatus on the ventral surface of the penis. The prevalence of hypospadias is ∼2 in 1,000 total births (male and female) according to EUROCAT and the International Clearinghouse for Birth Defects and Surveillance and Research data (1, 2). However, the geographical variation of its prevalence is large, depending on how it is defined and ascertained, ranging from 0.2 to 3.9 per 1,000 births (2). The etiology remains largely unknown and is likely to be multifactorial, probably involving both genetic and environmental factors (3).

Among the most widespread chemicals, organic solvents are present in a large range of products used at home or at work, including paints, varnishes, inks, and cleaning agents and may be present as volatile organic compounds (VOCs) for some. There are three major organic solvent chemical families: oxygenated, petroleum, and chlorinated solvents. Their main routes of exposure are dermal and respiratory. Although several studies have suggested an association between *in utero* exposure to organic solvents and hypospadias (4–11), only a few have focused on the subtypes (7, 10, 11). The studies of Cordier et al. (10) and Warembourg et al. (11) suggested an association between the presence of metabolites of urinary glycol ethers, an oxygenated solvent chemical family, during pregnancy and hypospadias. Another study suggested a non-significant elevated risk of hypospadias associated with tetrachloroethylene-contaminated drinking water (estimated using water distribution system modeling software) (7). Certain petroleum solvents, such as "BTEX" (benzene, toluene, ethylbenzene, and *o,m,p*-xylene), are suspected to act as endocrine disruptors (12) that may affect the hormonal environment during pregnancy and thus the genital development of the fetus. However, they have never been studied in relation to the occurrence of hypospadias in newborns.

Exposure to chlorinated and petroleum solvents could be potentially assessed using biomarkers in urine or serum. However, their relatively short half-lives, especially that of petroleum solvents, limit the assessment of exposure throughout pregnancy, especially during the specific windows of susceptibility. Meconium is a useful matrix to assess fetal exposure (13, 14). It is the first feces to be expelled by the neonate after birth. It is composed of water, lipids, and proteins derived from swallowed amniotic fluid and secretions and desquamated epithelial cells from the fetal intestine (13). It starts being produced from around the 12th to 13th week of gestation, accumulates in the colon, and is passed by the neonate within the first 24–48 h after birth (14). It may therefore reflect cumulative fetal exposure, even to toxicants with a short half-life (parent molecules or metabolites). Its collection is non-invasive and simple and it provides unequivocal evidence that the chemical has reached the fetus (15). It has already been used to detect fetal exposure to various toxicants, such as illicit drugs (16), alcohol, certain pesticides (17–19), and various non-persistent organic pollutants (20).

The aim of this exploratory study was to evaluate the association between fetal petroleum and chlorinated solvent levels measured in meconium and the risk of hypospadias.

## MATERIALS AND METHODS

### Population

We conducted a pilot case-control study on a subsample of the PENEW (Pregnancy Environment and NEWborn) project, led by the Brittany Registry of Congenital Anomalies, to study the impact of environmental chemical exposure (solvents and pesticides) on congenital malformations in Brittany (France) between October 2012 and December 2018. Cases in the PENEW project consisted of neonates or fetuses affected by any congenital malformation diagnosed at birth in one of the 13 participating maternity wards in Brittany. For each case, the controls were the two following livebirths of the same gender without any birth defects, born in the same maternity and not hospitalized in a neonatal unit. For both cases and controls, exclusion criteria were residence of the mother outside of Brittany and refusal to participate. All cases and controls were ascertained by a pediatrician in charge of the Brittany congenital malformation registry and trained according to EUROCAT classification guidelines (21).

For this study, we selected hypospadias cases and controls that were recruited at birth in the maternity ward of the University Hospital in Rennes during the first 15 months of enrolment (between October 7, 2012 and January 6, 2014).

During the inclusion period, ∼5,000 livebirths were recorded in the maternity ward. Cases ($N = 11$) consisted of male live births diagnosed with hypospadias on the first day of life by a pediatrician. They represented 69% (11/16) of the eligible children born in the maternity during the same period and diagnosed with hypospadias within the first day of life (before expelling the meconium). Hypospadias was defined as an abnormal location of the urethral orifice on the ventral surface of the penis and coded using the International Classification Disease 10 (ICD 10), following EUROCAT guidelines (21). This classification is based on the location of the ectopic meatus, i.e., balanic (Q540), penile (Q541), penoscrotal (Q542), or perineal (Q543). Hypospadias associated with chromosomal and genetic conditions was excluded. All cases were confirmed by

a pediatric surgeon and included in the Brittany registry of congenital anomalies.

Controls ($N = 47$) consisted of male live birth controls of all PENEW cases (excluding those of syndromic and chromosomic cases) with available meconium analysis ($N = 46$) and delivered during the same time period. This enabled us to obtain a ratio of one case to four controls to maximize the power of the study.

## Data Collection

During their stay in the maternity ward, the mothers completed a questionnaire after delivery that provided information on their medical history, tobacco exposure during pregnancy (maternal consumption and passive smoking), alcohol consumption, medication during pregnancy, food habits, and occupational and domestic exposure to a large number of environmental pollutants.

Medical data, including obstetrical history, the health of the newborn, and a description of the malformation (for cases), came from hospital medical records, including clinical examination by the pediatrician before discharge and follow-up by pediatric surgeons.

## Indirect Assessment of Exposure to Solvents

We assessed maternal occupational exposure to solvents during pregnancy using two job-exposure matrices (JEMs) constructed by Santé Publique France (French national public health agency), one concerning petroleum solvents (22) and the other concerning chlorinated solvents (23). Each allowed the assessment of exposure to the family of solvents in a single group (petroleum and chlorinated solvents) and to subgroups of solvents within each family: benzene, other aliphatic mineral spirits, motor gasoline, white-spirits, and other light aromatic mixtures, gasoil, fuels, and kerosene for petroleum solvents and chloroform, methylene chloride, tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane for chlorinated solvents.

In addition, information about the domestic use of products containing solvents during pregnancy was collected from the questionnaire: frequency of damp cleaning (<5 times/month, between 5 and 20 times/month, more than 20 times/month), home renovation activities (yes/no), and other chemicals used during leisure activities (yes/no) during pregnancy.

## Meconium Collection

Three meconium samples were collected into dedicated stool-culture containers by healthcare staff within the first 2 days of life following a standardized procedure for both cases and controls. Only water and cotton pads were used to clean the diaper area, excluding any use of cream or lotions. The healthcare staff was instructed to use gloves (without talc) instead of hydroalcoholic gel during collection to prevent any contamination of the samples. Samples were immediately frozen and initially stored in a dedicated freezer at −20°C, in the maternity ward, and then transferred to The French National Institute for Industrial Environment and Risk (INERIS) laboratory and stored at −80°C until analysis.

Possible contamination of the diapers by BTEX or chlorinated solvents that could have led to the contamination of the meconium samples was assessed by analyzing the three references of diapers used in the maternity ward during the study period. Details of the chemical analysis, including quality assurance and quality control (QA/QC), are provided in **Supplementary Material 1**. Under these conditions, no BTEX or chlorinated solvents were quantifiable in the three references, except toluene, which was detected at slightly above the limit of quantification for only one.

## Chemical Analysis of Meconium Samples

Meconium samples were analyzed by the INERIS laboratory. A specific analytic method based on headspace (HS) solid phase microextraction (SPME) gas chromatography coupled with mass spectrometry (GC/MS) was developed to detect and quantify aromatic hydrocarbons "BTEX" (benzene, toluene, ethylbenzene, and *o,m,p*-xylene) and two chlorinated solvents (trichloroethylene and tetrachloroethylene). This method has been described elsewhere (24). The limits of quantification (LOQ) ranged from 0.08 ng/g of meconium (tetrachloroethylene) to 0.12 ng/g (benzene, toluene) (24). Metabolites of BTEX [o-cresol, hippuric acid, mandelic acid (MA), 2-methylhippuric acid (MHA), S-phenylmercapturic acid (s-PMA), and benzylmercapturic acid (s-BMA)], and one metabolite of styrene and ethylbenzene [phenylglyoxylic acid (PGA)] were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) according to a previously described method (25). The limits of quantification ranged from 4.8 ng/g (MHA) to 174.7 ng/g (o-cresol) (25). Analyses were blindly performed for cases and controls.

## Statistical Analysis

Concentrations of chemicals were classified into quartiles. For association analyses, two categorizations were considered with different cut-offs according to the frequency of quantification among controls. The cut-off was the third quartile if the quantification frequency was above 50% (i.e., for toluene, benzene, and trichloroethylene) or the limit of quantification if the quantification frequency was below 50% (i.e., for other solvents).

Correlations between solvents and metabolites were studied using chi-square or Fisher exact tests, Wilcoxon tests, or Spearman rank correlations, depending on the level of quantification. We explored the relationship between solvent or metabolite concentrations and certain potential sources: tobacco consumption during pregnancy, occupational exposure to chlorinated and petroleum solvents, self-reported domestic uses of products that may contain solvents (for damp cleaning, home renovation, and leisure activities) using chi-square or Fisher exact tests.

We used an exact logistic regression model to study the risk of hypospadias related to BTEX or chlorinated solvents to reduce the small sample bias, which generally leads to overestimation of the true value (26). Associations were tested independently for each chemical and only for those detected in more than 10% of samples. Potential confounders included in

the adjustment set of variables were chosen a priori: maternal age (<25, 25–35, ≥35 years), education level (high school or less, post-secondary school), body-mass index (<25, ≥25 m/kg²), tobacco consumption during pregnancy (yes/no), and alcohol consumption during pregnancy (yes/no). Results are presented as odds ratios (ORs) with their 95% confidence intervals (CIs).

We did not correct *p*-values for multiple testing and accepted the possibility of false discoveries, as our study was a pilot study. Nevertheless, and, as recommended (27, 28), all comparisons were planned a priori and all results are reported.

All statistical analyses were performed using SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA) and LogXact 10 (Cytel).

## RESULTS

The characteristics of the cases and controls are presented in **Table 1**. Mothers of cases were significantly less educated and more affected by hypertension than those of controls. There were no other significant differences between cases and controls in terms of other maternal characteristics or environmental exposure.

Among the 11 cases of hypospadias, four were balanic (Q540), five penile (Q541), and two penoscrotal (Q542). Only penoscrotal cases were associated with another malformation (one of esophageal atresia and one of multicystic renal dysplasia).

The frequency of quantification and distribution (in quartiles) of chemical concentrations in the meconium of controls are presented in **Table 2**. Solvents were quantified from 2.2% of samples for MHA and S-PMA to 63.9% for benzene and 77.1% for trichloroethylene.

There were strong correlations between the levels of several solvents and metabolites (**Supplementary Material 2**). Such correlations were expected, as these solvents share common major sources of exposure, such as indoor and outdoor air pollution for petroleum solvents.

Among the mothers who declared working during pregnancy, only one was classified as potentially exposed at work to chlorinated solvents (probably to chloroform but not trichloroethylene or tetrachloroethylene) and another was classified as potentially exposed at work to petroleum solvents by

**TABLE 1 |** Characteristics of the 11 cases of hypospadias and 46 controls.

| | | Controls (*n* = 46) | | Cases (*n* = 11) | | *p*-value |
|---|---|---|---|---|---|---|
| | | *n* | % | *n* | % | |
| Age | <25 | 3 | 6.5 | 1 | 9.1 | 0.91 |
| | 25–35 | 37 | 80.4 | 9 | 81.8 | |
| | ≥35 | 6 | 13.0 | 1 | 9.1 | |
| | mean (± SD) | 30.2 (±3.6) | | 29.3 (± 4.2) | | 0.62 |
| Educational level | High school or less | 10 | 21.7 | 7 | 63.6 | 0.006 |
| | Postsecondary school | 36 | 78.3 | 4 | 36.7 | |
| Work during pregnancy | No | 29 | 63.0 | 6 | 55.5 | 0.73 |
| | Yes | 17 | 37.0 | 5 | 45.5 | |
| BMI (kg/m²) | <25 | 33 | 71.7 | 7 | 63.6 | 0.60 |
| | ≥25 | 13 | 28.3 | 4 | 36.4 | |
| | mean (± SD) | 23.5 (± 4.2) | | 24.3 (± 5.0) | | 0.66 |
| Tobacco consumption during pregnancy | No | 31 | 67.4 | 7 | 63.6 | 0.81 |
| | Yes | 15 | 32.6 | 4 | 36.4 | |
| Passive smoking during pregnancy | No | 16 | 38.1 | 4 | 36.4 | 0.92 |
| | Yes | 26 | 61.9 | 7 | 63.6 | |
| | missing | 4 | – | 0 | | |
| Alcohol consumption during pregnancy | No | 40 | 87.0 | 11 | 100.0 | 0.20 |
| | Yes | 6 | 13.0 | 0 | 0.0 | |
| Vitamin Supplementation (beginning pregnancy) | No | 25 | 55.6 | 6 | 60.0 | 0.80 |
| | Yes | 20 | 44.4 | 4 | 40.0 | |
| | missing | 1 | – | 1 | – | |
| Diabetes during pregnancy | No | 44 | 95.7 | 10 | 90.9 | 0.53 |
| | Yes | 2 | 4.3 | 1 | 9.1 | |
| Hypertension (before and during pregnancy) | No | 46 | 100.0 | 8 | 72.7 | 0.003 |
| | Yes | 0 | 0.0 | 3 | 27.3 | |
| SGA 10th percentile (AUDIPOG curves) | No | 38 | 82.6 | 8 | 72.7 | 0.43 |
| | Yes | 8 | 17.4 | 3 | 27.3 | |

*BMI, body-mass index; SGA, small for gestational age.*

**TABLE 2 |** Distribution of chemical concentrations in meconium samples (BTEX and chlorinated solvents and metabolites) among controls ($n = 46$).

| | *N* | LOQ (ng/g) | *N* (%) >LOQ | P25 (ng/g) Q1 | P50 (ng/g) Q2 | P75 (ng/g) Q3 | Maximum (ng/g) |
|---|---|---|---|---|---|---|---|
| Toluene | 35 | 0.120 | 22 (62.9) | <LOQ | 3.78 | 94.9 | 538 |
| Benzene | 46 | 0.120 | 33 (63.9) | <LOQ | 0.300 | 1.07 | 41.0 |
| Ethylbenzene | 46 | 0.120 | 20 (43.5) | <LOQ | <LOQ | 0.540 | 3.17 |
| p-xylene | 46 | 0.100 | 14 (30.5) | <LOQ | <LOQ | 0.120 | 0.920 |
| m-xylene | 46 | 0.120 | 15 (32.6) | <LOQ | <LOQ | 0.320 | 1.67 |
| o-xylene | 46 | 0.120 | 6 (13.0) | <LOQ | <LOQ | <LOQ | 2.54 |
| Phenylglyoxylic acid (PGA) | 46 | 29.9 | 3 (6.5) | <LOQ | <LOQ | <LOQ | 403 |
| O_cresol | 46 | 175 | 6 (13.0) | <LOQ | <LOQ | <LOQ | 960 |
| Mandelic acid | 46 | 72.2 | 5 (9.8) | <LOQ | <LOQ | <LOQ | 122 |
| Hippuric acid | 46 | 17.4 | 17 (37.0) | <LOQ | <LOQ | 29.9 | 1330 |
| Methylhippuric acid (MHA) | 46 | 4.82 | 1 (2.2) | <LOQ | <LOQ | <LOQ | 113 |
| S-phenylmercapturic acid (S-PMA) | 46 | 51.6 | 1(2.2) | <LOQ | <LOQ | <LOQ | 87.3 |
| S-benzylmercapturic acid (S-BMA) | 46 | 10.4 | 3 (6.5) | <LOQ | <LOQ | <LOQ | 20.2 |
| Trichloroethylene | 46 | 0.100 | 34 (77.1) | <LOQ | 0.210 | 0.350 | 0.900 |
| Tetrachloroethylene | 46 | 0.0800 | 22 (47.8) | <LOQ | <LOQ | 0.230 | 0.380 |

*LOQ, limit of quantification.*
*Q1, 1st quartile; Q2, 2nd quartile; Q3, 3rd quartile.*

the JEMs (probably to white spirit but not benzene). Hippuric acid was the only detected metabolite associated with smoking during pregnancy (**Supplementary Material 2**, **Table 1**). In terms of self-reported non-occupational exposure, the frequency of damp cleaning was associated with higher quantification rates of ethylbenzene, p-xylene, and o-xylene. Home renovation was associated with higher quantification rates of m-xylene, hippuric acid, and trichloroethylene (**Supplementary Material 2**, **Table 1**). Other self-reported exposure to chemicals during leisure-time activities was not associated with any changes in the levels of solvents or their metabolites measured in the meconium samples (**Supplementary Material 2**, **Table 1**).

The associations between the levels of chemical exposure and the risk of hypospadias are presented in **Table 3**. We found a significant association between the levels above the limit of quantification for PGA in meconium samples and a higher risk of hypospadias (OR = 14.2, 95% CI [2.5–138.7]). The risk of hypospadias was elevated for most of the other compounds and metabolites but was not statistically significant.

## DISCUSSION

This pilot study shows that several petroleum and chlorinated solvents and their metabolites can be quantified in meconium samples and suggests an association between quantification of PGA in meconium samples and a higher risk of hypospadias. The risk of hypospadias was elevated for most of the other detected solvents and metabolites but was not statistically significant.

This study had several strengths, including the direct assessment of fetal exposure via the analysis of biomarkers in meconium samples, which reflects cumulative exposure starting from the 12th to 13th week of gestation until birth (13, 14, 29). Interestingly, the cumulative exposure traced in this matrix covered the window of susceptibility for the development of hypospadias: human male urethral development starts from the indifferent stage of genital development at 6–8 weeks gestation, with progression of the urethral meatus from the scrotal folds to its terminal position on the glans, ending at 16–17 weeks (30, 31). The analytical methods were specifically developed and validated for this study (24, 25). Other strengths of our study were the ascertainment and validation of cases by pediatricians and surgeons, which avoided a misclassification bias, and a large number of potential confounders. Although maternal hypertension was associated with hypospadias, we decided a priori to not adjust for it, as it can be considered as an intermediate factor.

This study also had several limitations, mainly due to the small number of cases, leading to imprecision in the estimates (i.e., wide 95% confidence intervals). The low number of cases was due to the necessity of early inclusion after birth, and therefore the early diagnosis of hypospadias, to collect meconium before its being expelled. Certain forms of balanic hypospadias are hidden by an overlapping or tight prepuce and can be difficult to diagnose at birth; the diagnosis is sometimes made later during the stay at the maternity ward by the pediatrician, or even after discharge, by a surgeon. This constraint may have led to the selection of major forms. However, the repartition of anatomical subtypes of hypospadias (balanic, penile, and penoscrotal) was no different between the included and non-included cases born in the same maternity ward during the same period.

This pilot study is the first to focus on "BTEX-Styrene" (BTEX-S) or chlorinated solvent exposure in relation to the occurrence of hypospadias based on measurements in meconium. We found that petroleum solvents could be quantified in 15% to 65% of samples and most of their metabolites in <20%, except for hippuric acid. The levels observed in the present study are difficult to compare, as, to our knowledge, no other study has measured these compounds in meconium. Thus, comparison

**TABLE 3 |** Association between the levels of BTEX and chlorinated solvents and their metabolites in the meconium and the occurrence of hypospadias ($n = 11$ cases and 46 controls).

| | | Cases ($N = 11$) | Controls ($N = 46$) | OR [95% CI] | OR* [95% CI] |
|---|---|---|---|---|---|
| Toluene[#] | <Q3 | 5 | 26 | Ref | Ref |
| | ≥Q3 | 2 | 9 | 0.87 [0.11–10.64] | 2.91 [0.12–202.10] |
| Benzene | <Q3 | 8 | 34 | Ref | Ref |
| | ≥Q3 | 3 | 12 | 0.94 [0.18–6.41] | 2.67 [0.28–34.34] |
| Ethylbenzene | <LOQ | 4 | 26 | Ref | Ref |
| | ≥LOQ | 7 | 20 | 2.27 [0.58–8.86] | 1.81 [0.46–7.61] |
| p-Xylene | <LOQ | 6 | 32 | Ref | Ref |
| | ≥LOQ | 5 | 14 | 1.90 [0.50–7.29] | 2.38 [0.53–11.17] |
| m-Xylene | <LOQ | 4 | 31 | Ref | Ref |
| | ≥LOQ | 7 | 15 | 3.61 [0.92–14.30] | 3.17 [0.76–14.00] |
| o-Xylene | <LOQ | 8 | 40 | Ref | Ref |
| | ≥LOQ | 3 | 6 | 2.50 [0.51–12.39] | 2.77 [0.43–17.55] |
| Phenylglyoxylic acid (PGA) | <LOQ | 6 | 43 | Ref | Ref |
| | ≥LOQ | 5 | 3 | 11.94 [2.25–63.25] | 14.17 [2.45–138.66] |
| o-Cresol | <LOQ | 9 | 40 | Ref | Ref |
| | ≥LOQ | 2 | 6 | 1.48 [0.26–8.58] | 2.12 [0.31–12.48] |
| Mandelic acid | <LOQ | 11 | 41 | Ref | Ref |
| | ≥LOQ | 0 | 5 | – | – |
| Hippuric Acid | <LOQ | 7 | 29 | Ref | Ref |
| | ≥LOQ | 4 | 17 | 0.97 [0.24–3.82] | 2.25 [0.42–14.57] |
| Methylhippuric acid (MHA) | <LOQ | 11 | 45 | Ref | Ref |
| | ≥LOQ | 0 | 1 | – | – |
| S-phenylmercapturic acid (S-PMA) | <LOQ | 11 | 45 | Ref | Ref |
| | ≥LOQ | 0 | 1 | – | – |
| S-benzylmercapturic acid (S-BMA) | <LOQ | 10 | 43 | Ref | Ref |
| | ≥LOQ | 1 | 3 | 0.70 [0.05–40.25] | 0.62 [0.03–12.56] |
| Trichloroethylene | <Q3 | 7 | 34 | Ref | Ref |
| | ≥Q3 | 4 | 12 | 0.62 [0.13–3.43] | 1.41 [0.22–8.04] |
| Tetrachloroethylene | <LOQ | 6 | 24 | Ref | Ref |
| | ≥LOQ | 5 | 22 | 0.91 [0.24–3.40] | 1.25 [0.32–5.04] |

[#]*Toluene measurements were available for seven cases and 35 controls.*

*OR, Odds ratio; 95% CI, 95% confidence interval.*

[*]*Adjusted for maternal age, educational level, body-mass index, and alcohol and tobacco consumption during pregnancy.*

*LOQ, limit of quantification (LOQ = 0.12 mg/g for ethylbenzene, m-xylene, o-xylene; 0.10 mg/g for p-xylene; 29.92 mg/g for PGA; 174.69 mg/g for O-cresol; 17.36 mg/g for hippuric acid; and 0.08 mg/g for tetrachloroethylene).*

*Q3, third quartile (Q3 = 91.9 ng/g for toluene, 1.07 ng/g for benzene, and 0.35 ng/g for trichloroethylene).*

with other biomonitoring studies using other matrices are difficult to interpret, even if BTEX-S have been widely detected in the general population in blood samples (32) and their metabolites in blood and urine samples (33–35). For example, NHANES biomonitoring studies of urine among children in 2005–2012 (35) detected PGA in more than 90% of samples, suggesting that exposure to PGA is indeed widespread compared to the 6.5% of quantification in our study. However, little is known about the fetal pharmacokinetics and metabolism of PGA and we cannot currently infer external exposure associated with the potential increased risk suggested by our results. In non-occupational settings, the major source of exposure to BTEX-S comes from exposure to tobacco (35, 36), as well as indoor and outdoor air pollution (37–39). In addition, BTEX-S have been reported to be present in food (40, 41), although this source of exposure contributed less than other sources, except for styrene, for which food appeared to contribute to the same extent as airborne exposure (42). In our study, the levels of BTEX, their metabolites, and chlorinated solvents were not associated with occupational exposure or to tobacco (maternal consumption or passive exposure), except for hippuric acid (for maternal consumption only). The relationship between the presence of BTEX or their metabolites in the meconium and airborne or food sources could not be investigated in our study. However, the domestic use of solvents through cleaning habits (damp cleaning) or renovation activities was associated with more frequent detection of ethylbenzene, p-xylene, and o-xylene (damp cleaning), and m-xylene, hippuric acid, and trichloroethylene (home renovation). Home renovation has already been found to be associated with BTEX-S levels in a study on predictors of indoor BTEX concentrations in Canadian homes (43). Finally, although the French Agency for Food,

Environmental and Occupational Health and Safety (ANSES) has reported that several brands of diapers contain BTEX-S, such as o-, m-, and p-xylene, toluene, and styrene (44), the analyses we conducted on the diapers used in our maternity ward before the study did not show any contamination by BTEX-S. We consequently did not consider the diapers themselves to be a possible source of these compounds. PGA, the only metabolite significantly associated with a higher risk of hypospadias in our study, has no known sources other than the metabolization of styrene and ethylbenzene (45).

Our study is the first to focus on the potential association of maternal exposure to BTEX and chlorinated solvents and the risk of hypospadias using objective measures for fetal assessment. Animal data have suggested that BTEX (46–48), styrene (49, 50), and chlorinated solvents (51, 52) can be teratogens. However, their association with male genital malformations has been rarely investigated. Although Bolden et al. suggested that BTEX-S may have endocrine disrupting properties at exposure levels below reference concentrations, the evidence is scarce (12, 52, 53). However, an experimental study in rats showed that styrene, one of the principal sources of PGA, has estrogenic properties that can affect genital development of the offspring, such as shortened anogenital distance, lower testis weight, and disruption of hormonal levels (41). In addition, ethylbenzene, the other precursor of PGA, was found to be associated with urinary tract malformations in the mouse in one study, but this was not observed in other developmental studies (54).

Our pilot study reports the feasibility of measuring petroleum solvents and chlorinated solvents and their metabolites in the meconium and suggests an association between petroleum solvents, especially PGA, and the occurrence of hypospadias. Although such an association is biologically plausible, we recognize that it may be due to a marker of co-exposure not investigated in our study, residual confounding or simply a chance finding. Given the small sample size, these results must be interpreted with caution and additional studies are needed to replicate them. If confirmed, these results may be of particular importance for the prevention of hypospadias.

## DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of regulatory restrictions. Requests to access the datasets should be directed to Violaine Benoit, violaine.benoit@chu-rennes.fr.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by all required ethics committee in France: Person Protection Committee (Comité de Protection des Personnes Ouest V): reference 11/22-811 (06/09/2011); ANSM (AFSSAPS): ID-RCB: 2010—A01445-34—Ref. AFSSAPS: B110827-40—Authorization 01/07/2011; CCTIRS: n° 11.590—Approval on 20/11/2011; CNIL: n° 911526—Approval DR-2012-332 on 03/07/2012. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin and the mothers.

## AUTHOR CONTRIBUTIONS

FR, SC, PP, LM, and RG contributed conception and design of the study. FR contributed to acquisition and interpretation of data. AB organized the database and contributed to the statistical analyses. MM-M performed the chemical analysis. RG performed the statistical analysis. FR and RG wrote the first draft of the manuscript. FR, RG, and FM wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.640064/full#supplementary-material

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# Prevention of Neural Tube Defects in Europe: A Public Health Failure

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**Objective:** Thirty years ago it was demonstrated that folic acid taken before pregnancy and in early pregnancy reduced the risk of a neural tube defect (NTD). Despite Public Health Initiatives across Europe recommending that women take 0.4 mg folic acid before becoming pregnant and during the first trimester, the prevalence of NTD pregnancies has not materially decreased in the EU since 1998, in contrast to the dramatic fall observed in the USA. This study aimed to estimate the number of NTD pregnancies that would have been prevented if flour had been fortified with folic acid in Europe from 1998 as it had been in the USA.

**Design and Setting:** The number of NTD pregnancies from 1998 to 2017 that would have been prevented if folic acid fortification had been implemented in the 28 countries who were members of the European Union in 2019 was predicted was predicted using data on NTD prevalence from 35 EUROCAT congenital anomaly registries and literature searches for population serum folate levels and folic acid supplementation.

**Results:** From 1998 to 2017 an estimated 95,213 NTD pregnancies occurred amongst 104 million births in the 28 countries in the EU, a prevalence of 0.92 per 1,000 births. The median serum folate level in Europe over this time period was estimated to be 14.1 μg/L. There is a lack of information about women taking folic acid supplements before becoming pregnant and during the first trimester of pregnancy, with one meta-analysis indicating that around 25% of women did so. An estimated 14,600 NTD pregnancies may have been prevented if the European countries had implemented fortification at the level adopted by the USA in 1998 and 25% of women took folic acid supplements. An estimated 19,500 NTD pregnancies would have been prevented if no women took folic acid supplements.

**Conclusions:** This study suggests that failure to implement mandatory folic acid fortification in the 28 European countries has caused, and continues to cause, neural tube defects to occur in almost 1,000 pregnancies every year.

**Keywords: neural tube defects, prevention, folic acid, fortification, Europe**

## INTRODUCTION

Thirty years ago (in 1991) the Medical Research Council Vitamin Study randomized controlled trial showed that taking 4 mg folic acid before conception reduced the risk of a pregnancy with spina bifida, anencephaly or encephalocele (neural tube defects) by an estimated 72% (1). Dietary modification alone is not likely to achieve a high enough daily intake of folate to provide a reasonable reduction in risk; taking folic acid supplements or eating food fortified with folic acid is necessary.

Mandatory fortification of flour with folic acid has been introduced in 78 countries starting with the United States of America (USA) in 1998, over 20 years ago (2, 3). Despite evidence that such fortification is effective (2, 4–10), mandatory folic acid fortification of flour has not been introduced in Europe. Women in Europe are currently advised to take 0.4 mg folic acid (in the form of tablets or capsules) before pregnancy and in the first trimester to reduce their risk of a neural tube defect (NTD) pregnancy (11).The prevalence of NTDs has not decreased in Europe, with an estimated prevalence of 0.91 per 1,000 births from 1991 to 2011 (12) in comparison to the prevalence in Canada where the prevalence of NTDs decreased from 1.58 per 1,000 births before fortification to 0.86 per 1,000 births after fortification (6) and in the USA where the prevalence of spina bifida and anencephaly reduced from 0.76 per 1,000 births pre-fortification to 0.56 per 1,000 births after fortification (4).

Daly et al. demonstrated that there is a log linear association between a woman's serum folate level and her risk of having an NTD pregnancy (13). A meta-analysis by Wald et al. subsequently estimated that serum folate concentrations increase by 0.94 ng/mL for every 0.1 mg/day increase in folic acid intake in women aged 20–35 years (14). Wald combined these two results to predict the reduction in risk resulting from additional folic acid intake according to background serum folate concentration (14). This means that the risk reduction in NTD pregnancies resulting from the adoption of fortification can be predicted if the prevalence of NTDs in a country and the population serum folate levels in the country are known.

The aim of this study was to estimate the potential effect there would have been on the prevalence of NTDs over 20 years from 1998 to 2017 if fortification of flour with folic acid had been implemented in 1998 in Europe, as was done in the USA using data from European Congenital Anomaly Registries on the prevalence of NTDs and data from a literature search on population serum folate levels. An earlier study quantified the numbers of pregnancies with an NTD that could have been prevented in the UK and this study provides the relevant estimates for Europe.

## METHODS

### Membership of Europe

In 2017 there were 28 countries belonging to the EU, 10 of which joined in 2004, two in 2007 and one in 2013. When estimating the numbers of NTDs that could be prevented in Europe from 1998, we have used two groups of countries—the 15 members in the EU in 1998 (EU15) and the 28 who were members of the EU in 2019 (EU28). In addition, we provide information on Norway, Switzerland and Ukraine, who are not members of the EU, but who have congenital anomaly registries that are members of EUROCAT (the European network of Congenital Anomaly Registries).

## Prevalence of Neural Tube Defect Pregnancies

EUROCAT currently surveys births from 39 registries in 21 countries in Europe (15, 16). The EUROCAT coverage varies over time according to which registries are able to provide data, with the maximum coverage being over 1.7 million births per year (39% of births in Europe). Details on the data and their collection are available at https://eu-rd-platform.jrc.ec.europa.eu/eurocat_en. Registries use multiple sources of information to ascertain cases including maternity, neonatal, and pediatric records; fetal medicine, cytogenetic, pathology, and medical genetics records; specialist services including pediatric cardiology; and hospital discharge and child health records. Anomalies were coded according to the International Classification of Disease (ICD) version 10 (17) (ICD-10) or ICD-9 with the British Pediatric Association (BPA) code extension for higher specificity and categorized by CA/syndrome subgroup (the system and the individual disorder), following the EUROCAT guidelines (18). NTDs include anencephalus, encephalocele and spina bifida. Cases were coded as having encephalocele only if there was no mention of anencephalus. Similarly, cases were classified as having spina bifida only if there was no mention of anencephalus or encephalocele. All cases with a known genetic condition were excluded.

Data about cases with an NTD [live births, late fetal deaths (≥20 weeks' gestation) and terminations of pregnancy for fetal anomaly at any gestation (TOPFA)] from 1st January 1998 to 31st December 2017 were obtained from the EUROCAT website (https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence), as this website allows bespoke prevalence tables to be obtained interactively. Data were not available for all countries in Europe and for many countries the data available only covers births in particular regions and particular years (see **Table 1**). EUROCAT collects individual case data from full member registries, but only aggregate data from associate registries. Data from associate registries were only used if data were not available from a full registry in the same country. **Table 1** shows which registries provided data to EUROCAT. Nine countries currently in the EU do not have any congenital anomaly registries that are members of EUROCAT. PubMed was searched for any additional information on the prevalence of NTDs in these countries from 1998 to 2017 using the terms "neural tube defect" and each individual country separately. Two studies were identified providing the prevalence for the Slovak Republic (20) and for Slovenia (21). For Luxembourg, the prevalence was assumed to be the same as that in Switzerland, for Greece and Cyprus the prevalence was assumed to be the same as that in Italy, for Estonia the prevalence was assumed to be the same as that in Finland, for Romania, the prevalence was assumed to be the same as that in Poland and for Latvia and Lithuania the prevalence was assumed to be the same as the average prevalence in Poland, Finland and Sweden.

The annual prevalence of NTD pregnancies was estimated within each registry over the time period during which the registry was active. For countries in which there were several registries, the overall prevalence was estimated by calculating the total number of cases divided by the population births covered by the registries for each year. For European countries having registries located outside the geographical limits of Europe, only mainland registries were included (for example for France the registry on Isle de Reunion was not included). Previous studies have shown that the overall prevalence of NTD pregnancies in Europe has not significantly changed over this period (12, 22). Therefore, for years in which there was no registry reporting the prevalence, the average prevalence over the 3 previous years (where available) or the following 3 years (where available) was used.

## Population Serum Folate Levels

Any information from studies measuring serum folate levels in women of childbearing age was searched for in PubMed using the terms "folate" or "folic acid" and each individual European country separately. In addition, the above search was repeated using Google Scholar as this search engine also allows searching of country of authors. If several studies were available from a single country the largest study in the most recent years was used (see **Table 2**) (23–43). Serum folate has a log Gaussian distribution and therefore the median or geometric mean and centile values should be reported. Many studies presented the arithmetic mean and standard deviation (SD). The correct median and SD (on a log scale) were estimated by simulating different log Gaussian distributions and calculating the observed means and SDs until these calculated means and SDs matched the published values and then the median and SD (on a log scale) from the simulated distribution were used instead of the published values. If only the median was available and not the SD, the median SD from all the studies was used. As the same increase in folic acid will result in a greater risk reduction in women with lower serum folate compared with women with higher serum folate levels, we divided the serum folate levels in each country into quintiles and used the quintile medians to predict the risk reduction in each quintile of women separately. We then summed the quintiles to obtain the risk reduction in the whole population. We compared these estimates to the risk reduction using just the median folate level of the whole population. To determine how sensitive our estimates were to the reporting of serum folate levels in the different countries we also calculated the median serum folate level across all countries and used that value as the median serum folate level within each country.

## Folic Acid Supplementation

Recent studies have found that the proportion of women taking folic acid supplements at the correct time for prevention of NTDs (which is 4 weeks before pregnancy and the first 8 to 12 weeks of pregnancy) varies from around 10.4% (44, 45) up to 35% (46). An older meta-analysis of 34 studies found that around 25% of women took folic acid supplements periconceptually (47). Two estimates of the number of NTDs cases prevented were calculated; (i) Assuming that 25% of women took folic acid supplementation at the correct time and (ii) Ignoring any reductions in risk due to folic acid supplementation.

**TABLE 1 |** Number and prevalence of neural tube defect (NTD) pregnancies in EUROCAT registries reported from 1998 to 2017 and the proportion of the population covered over the whole 20 year period.

| Country | EU member | | Registry | First year | Last year | Total number of NTD pregnancies* | Prevalence of NTD pregnancies per 1,000 births | Coverage of all 20 years national births data available† (%) |
|---|---|---|---|---|---|---|---|---|
| | 2017 | 1998 | | | | | | |
| Belgium | Y | Y | Antwerp | 1998 | 2016 | 271 | 0.73 | 15 |
| Belgium | Y | Y | Hainaut | 1998 | 2017 | 194 | 0.79 | 10 |
| Denmark | Y | Y | Odense | 1998 | 2015 | 105 | 1.14 | 7 |
| France | Y | Y | Auvergne | 2002 | 2015 | 170 | 1.05 | 1 |
| France | Y | Y | Brittany | 2011 | 2017 | 321 | 1.31 | 2 |
| France | Y | Y | Paris | 1998 | 2017 | 634 | 1.13 | 3 |
| Italy | Y | Y | Emilia Romagna | 1998 | 2017 | 391 | 0.57 | 6 |
| Italy | Y | Y | Tuscany | 1998 | 2017 | 317 | 0.56 | 5 |
| Ireland | Y | Y | Cork and Kerry | 1998 | 2017 | 210 | 1.16 | 14 |
| Ireland | Y | Y | Dublin | 1998 | 2012 | 255 | 0.70 | 28 |
| Ireland | Y | Y | SE Ireland | 1998 | 2016 | 137 | 1.05 | 10 |
| Netherlands | Y | Y | North Netherlands | 1998 | 2017 | 291 | 0.81 | 10 |
| UK | Y | Y | East Midlands and South Yorkshire | 1998 | 2017 | 1,288 | 1.13 | 8 |
| UK | Y | Y | Northern England | 2000 | 2017 | 775 | 1.35 | 4 |
| UK | Y | Y | South West England | 2005 | 2017 | 717 | 1.12 | 4 |
| UK | Y | Y | Thames Valley | 1998 | 2017 | 448 | 1.04 | 3 |
| UK | Y | Y | Wales | 1998 | 2017 | 942 | 1.41 | 4 |
| UK | Y | Y | Wessex | 1998 | 2017 | 684 | 1.20 | 4 |
| Portugal | Y | Y | S. Portugal | 1998 | 2017 | 156 | 0.43 | 18 |
| Spain‡ | Y | Y | Basque | 1998 | 2016 | 374 | 1.02 | 4 |
| Spain‡ | Y | Y | Valencian Region | 2007 | 2016 | 293 | 0.60 | 6 |
| Germany | Y | Y | Mainz | 1998 | 2014 | 89 | 1.63 | 0 |
| Germany | Y | Y | Saxony-Anhalt | 1998 | 2017 | 308 | 0.92 | 2 |
| Austria | Y | Y | Styria | 1998 | 2014 | 129 | 0.73 | 11 |
| Finland | Y | Y | Finland (A) | 1998 | 2014 | 899 | 0.91 | 86 |
| Sweden | Y | Y | Sweden (A) | 2007 | 2016 | 803 | 0.73 | 53 |
| Croatia | Y | N | Zagreb | 1998 | 2017 | 60 | 0.49 | 15 |
| Malta | Y | N | Malta | 1998 | 2016 | 73 | 0.92 | 96 |
| Poland | Y | N | Wielkopolska | 1999 | 2017 | 517 | 0.73 | 9 |
| Bulgaria | Y | N | Sofia (A) | 1998 | 1999 | 40 | 2.07 | 1 |
| Hungary | Y | N | Hungary (A) | 1998 | 2012 | 953 | 0.66 | 76 |
| Czech Rep | Y | N | Czech Republic (A) | 2000 | 2010 | 848 | 0.74 | 55 |
| Switzerland | N | N | Vaud | 1998 | 2017 | 135 | 0.87 | 10 |
| Norway | N | N | Norway | 1999 | 2016 | 907 | 0.84 | 92 |
| Ukraine | N | N | Ukraine | 2005 | 2016 | 650 | 1.80 | 4 |

*(A) Associate registry.*

**Includes NTDs occurring in live births, fetal deaths (stillbirths and later miscarriages ≥ 20 gestational age) and terminations of pregnancy for fetal anomaly excluding cases with known genetic conditions.*

*† This figure is the total number of births covered by the registry in the years reported divided by the total numbers of births occurring in the countries over the whole 20-year period.*

*‡ The prevalence is confirmed by the data from hospitals recording terminations of pregnancy for fetal anomalies in the Spanish Collaborative Study of Congenital Malformations (ECEMC)—an associate registry in EUROCAT which covers 16% of all births and includes hospitals from Valencian and Basque regions (19).*

## Calculation of Number of Neural Tube Defects Prevented

In the USA, mandatory fortification with 140 μg of folic acid per 100 g of enriched cereal grain product was fully implemented in 1998 and has been estimated to provide 200 μg of folic acid per day to women of childbearing age (48). Wald et al. estimated that an increase in folic acid intake of 200 μg per day would increase the average serum folate concentration by 1.88 ng/ml or 4.26 nmol/L in women of childbearing age (14). We therefore estimated that the average serum folate level would have increased by 4.26 nmol/L in each country if fortification had occurred. The predicted reduction in prevalence if fortification had occurred is given by the formula: reduction in prevalence = (new serum folate/original serum folate)$^{-0.81}$ from Wald et

**TABLE 2 |** Estimated median folate levels and standard deviations (on a log scale) in European countries.

| Country | EU member | | Median (nmol/L) | Standard deviation (log scale) | Source of information (reference) |
|---|---|---|---|---|---|
| | 2017 | 1998 | | | |
| Austria | Y | Y | 11.5 | 0.77 | (23) |
| Belgium | Y | Y | 13.8 | 0.41 | (24) |
| Denmark | Y | Y | 8.6 | 0.29 | (25) |
| Finland | Y | Y | 12.4 | 0.55 | (26) |
| France | Y | Y | 14.8 | 0.58 | (27) |
| Germany | Y | Y | 14.3 | 0.41 | (28) |
| Greece | Y | Y | 17.4 | 0.69 | (29) |
| Ireland | Y | Y | 16.2 | 0.53 | (30) |
| Italy | Y | Y | 10.4 | 0.45 | (31) |
| Luxembourg | Y | Y | | | Values from Switzerland used |
| Netherlands | Y | Y | 7.3 | 0.48 | (32, 33) |
| Portugal | Y | Y | 15.2 | 0.42 | (34) |
| Spain | Y | Y | 16.5 | 0.51 | (35) |
| Sweden | Y | Y | 15.0 | 0.40 | (36) |
| United Kingdom | Y | Y | 16.2 | 0.54 | (30) |
| Bulgaria | Y | N | | | Values from Poland used |
| Croatia | Y | N | 9.1 | | (37) |
| Cyprus | Y | N | | | Values from Greece used |
| Czech Republic | Y | N | 14.0 | 0.38 | (38) |
| Estonia | Y | N | 12.4 | 0.60 | (39) |
| Hungary | Y | N | 19.0 | 0.45 | (38) |
| Latvia | Y | N | | | Values from Poland used |
| Lithuania | Y | N | | | Values from Poland used |
| Malta | Y | N | | | Values from Greece used |
| Poland | Y | N | 14.3 | 0.52 | (40) |
| Romania | Y | N | | | Values from Poland used |
| Slovak Republic | Y | N | 16.0 | 0.35 | (41) |
| Slovenia | Y | N | | | Values from Poland used |
| Norway | N | N | 7.3 | 0.50 | (42) |
| Switzerland | N | N | 14.3 | 0.67 | (43) |
| Ukraine | N | N | [3.2] | | Values estimated from prevalence of NTDs |
| Estimated median in countries in EU in 1998 | | | 14.1 | | Median weighted by numbers of births in each population. |
| Estimated median in countries in EU in 2019 | | | 14.1 | | Median weighted by numbers of births in each population. |

al. (14). For the scenario of 25% of women taking folic acid supplements, the effect of fortification is assumed to apply to only 75% of all births.

The total number of NTD diagnoses prevented from occurring annually in each country was estimated by multiplying the reduction in prevalence in the country by the total number of births in the country in that year. The numbers of births were obtained from Eurostat if they were not available from the EUROCAT registries (https://ec.europa.eu/eurostat/databrowser/view/TPS00204/default/table).

## Patient and Public Involvement

This study was not funded and therefore there were no funds or time allocated for PPI so we were unable to involve patients. We will invite patients to help us develop our dissemination strategy.

# RESULTS

From 1998 to 2017 an estimated 95,213 NTD pregnancies occurred amongst 104 million births in the 28 countries in the EU in 2019, a prevalence of 0.92 per 1,000 births. The median serum folate level in Europe over this time period was estimated to be 14.1 nmol/L.

**Table 3** shows that 14,600 NTD pregnancies could have been averted if the 28 countries in the EU in 2019 had implemented fortification at the level adopted by the USA in 1998, assuming that at least 25% of women in these countries had taken folic acid supplements before becoming pregnant and would therefore not have received a benefit from fortification. An estimated 19,500 NTD pregnancies could have been averted if it is assumed that no supplementation is occurring as then all women in the population are assumed to benefit from fortification. As it

**TABLE 3 |** NTD: numbers of pregnancies and the prevalence if fortification had occurred from 1998 to 2017 according to European country.

| Country | EU member | | | Assuming no supplementation occurred | | | Assuming 25% of women took supplements (47) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2017 | 1998 | Prevalence per 1,000 births[†] | Prevalence per 1,000 births with fortification | Percentage reduction due to fortification (%) | Estimated NTD pregnancies prevented | Prevalence per 1,000 births with fortification | Percentage reduction due to fortification (%) | Estimated NTD pregnancies prevented |
| Austria | Y | Y | 0.71 | 0.53 | 25 | 279 | 0.57 | 19 | 209 |
| Belgium | Y | Y | 0.75 | 0.60 | 20 | 369 | 0.63 | 15 | 276 |
| Denmark | Y | Y | 1.12 | 0.80 | 28 | 394 | 0.88 | 21 | 296 |
| Finland | Y | Y | 0.90 | 0.70 | 23 | 234 | 0.75 | 17 | 175 |
| France | Y | Y | 1.16 | 0.93 | 20 | 3,735 | 0.99 | 15 | 2,801 |
| Germany | Y | Y | 1.02 | 0.82 | 20 | 2,893 | 0.87 | 15 | 2,170 |
| Greece[b] | Y | Y | 0.57 | 0.46 | 18 | 214 | 0.49 | 14 | 160 |
| Ireland | Y | Y | 0.93 | 0.75 | 18 | 222 | 0.80 | 14 | 166 |
| Italy | Y | Y | 0.57 | 0.43 | 25 | 1,526 | 0.46 | 19 | 1,145 |
| Luxembourg[a] | Y | Y | 0.87 | 0.70 | 20 | 20 | 0.74 | 15 | 15 |
| Netherlands | Y | Y | 0.82 | 0.55 | 32 | 962 | 0.62 | 24 | 721 |
| Portugal | Y | Y | 0.43 | 0.35 | 19 | 168 | 0.37 | 14 | 126 |
| Spain | Y | Y | 0.85 | 0.69 | 18 | 1,332 | 0.73 | 14 | 999 |
| Sweden | Y | Y | 0.79 | 0.64 | 19 | 315 | 0.67 | 14 | 237 |
| United Kingdom | Y | Y | 1.22 | 0.99 | 18 | 3,344 | 1.05 | 14 | 2,508 |
| Bulgaria | Y | N | 1.63 | 1.26 | 22 | 510 | 1.35 | 17 | 382 |
| Croatia | Y | N | 0.49 | 0.36 | 28 | 112 | 0.39 | 21 | 84 |
| Cyprus[b] | Y | N | 0.57 | 0.47 | 17 | 25 | 0.49 | 13 | 18 |
| Czech Republic | Y | N | 0.73 | 0.59 | 20 | 306 | 0.62 | 15 | 230 |
| Estonia[c] | Y | N | 0.90 | 0.70 | 23 | 58 | 0.75 | 17 | 43 |
| Hungary | Y | N | 0.64 | 0.54 | 16 | 193 | 0.56 | 12 | 145 |
| Latvia[d] | Y | N | 0.82 | 0.64 | 22 | 77 | 0.68 | 17 | 58 |
| Lithuania[d] | Y | N | 0.82 | 0.64 | 22 | 115 | 0.69 | 17 | 86 |
| Malta | Y | N | 0.92 | 0.76 | 17 | 13 | 0.80 | 13 | 10 |
| Poland | Y | N | 0.77 | 0.61 | 20 | 1,173 | 0.65 | 15 | 879 |
| Romania[e] | Y | N | 0.77 | 0.61 | 22 | 746 | 0.65 | 17 | 559 |
| Slovak Republic[f] | Y | N | 0.53 | 0.43 | 18 | 106 | 0.46 | 13 | 80 |
| Slovenia[g] | Y | N | 0.74 | 0.57 | 22 | 65 | 0.62 | 17 | 49 |
| Norway | N | N | 0.84 | 0.57 | 32 | 312 | 0.63 | 24 | 234 |
| Switzerland | N | N | 0.87 | 0.69 | 21 | 286 | 0.73 | 16 | 214 |
| Ukraine | N | N | 1.80 | 0.91 | 49 | 8,047 | 1.13 | 37 | 6,035 |
| Countries in EU in 1998 | | | 0.95 | | 21 | 16,007 | | 15 | 12,005 |
| Countries in EU in 2019 | | | 0.92 | | 21 | 19,504 | | 15 | 14,628 |

[a] *For Luxembourg the prevalence was assumed to be the same as that in Switzerland.*
[b] *For Greece and Cyprus the prevalence was assumed to be the same as that in Italy.*
[c] *For Estonia the prevalence was assumed to be the same as that in Finland.*
[d] *For Latvia and Lithuania the prevalence was assumed to be the same as the mean of Poland, Finland and Sweden.*
[e] *For Romania the prevalence was assumed to be the same as that in Poland.*
[f] *Prevalence in Slovak Republic from Behunova et al. (20).*
[g] *Prevalence in Slovenia from Kokalj et al. (21).*
[†] *The prevalence may differ slightly from that in* ***Table 1*** *as in* ***Table 3*** *it is the prevalence over the whole time period from 1998 to 2017 and in* ***Table 1*** *it is the prevalence for the years of available data.*

is likely that fewer than 25% of women in Europe did take supplements at the correct time and also that these women would have received some benefit from fortification the true estimate of the number of NTDs prevented lies between 14,600 and 19,500; a 15–21% reduction. This is equivalent to saying fortification could prevent almost 1,000 pregnancies with an NTD occurring every year in the 28 countries in the EU. If only those countries in the EU in 1998 had implemented fortification then the number of NTD pregnancies that would have been prevented lies between 12,000 and 16,000 (again 15% to 21% reduction).

The two different methods of estimating the effect of fortification used (that is using the median value or splitting the distribution into quintiles) gave similar answers to the

nearest percentage point reduction in prevalence and therefore for simplicity the median method was used. Assuming that all countries had the same medium serum folate level (of 14.1 nmol/L) resulted in the reductions in NTD pregnancies being 15 and 20% (with and without supplementation, respectively). This would be expected as if the serum folate is assumed to be 10 nmol/L for example the predicted reduction in NTD pregnancies will be large, but if it is assumed to be 14.1 nmol/L the predicted reduction will be smaller. In other words, all those countries with serum folate below the median will have smaller reductions predicted if it is assumed their serum folate is the level of the median value. Similarly all those countries with serum folate levels above the median will have greater reductions predicted if it is assumed their serum folate is the level of the median. These effects are likely to be of a similar magnitude and hence the overall estimate remains similar.

## DISCUSSION

This study suggests that failure to implement folic acid fortification has resulted in an estimated 15,000–20,000 pregnancies affected with an NTD in the EU from 1998 to 2017. In Chile, the level of flour fortification adopted was higher than that in the USA (2.2 μg/100 g flour vs. 1.4 μg/100 g). If this level of fortification had been adopted in the EU, the reduction in affected pregnancies would have been twice as large. Given the known benefits, there is no reason not to adopt the level of fortification introduced in Chile in all countries in the EU.

This paper has used robust methods to estimate the numbers of NTD-affected pregnancies that could have been prevented by fortification. The method was compared to an earlier study in the UK, which predicted that 2014 affected pregnancies could have been prevented in the UK from 1998 to 2012 (49). This compares with 2,508 in this study from 1998 to 2017 (given in **Table 3**) which is consistent with the earlier study.

A weakness of the study is that there is very little information on population serum folate levels in all the countries across the 20-year study period. Measurements of serum folate in women of childbearing age were obtained for 21 countries, but assumptions about serum folate needed to be made for the other countries (see **Table 2**). In addition, most serum folate values related to one point in time only and often from only a small sample of people as in Portugal with data only from a small sample of women and men. For Ukraine (not included in the European estimates but included in the study for completeness), there were no population estimates of serum folate. The prevalence of NTD pregnancies in Ukraine is extremely high and therefore this prevalence was used to estimate the serum folate levels—they would be expected to be considerably lower than any other European country. Serum folate levels were often analyzed in papers assuming serum folate had a Gaussian distribution, which is not a correct assumption and the median and SD (on a log scale) had to be estimated. However, the sensitivity analysis did demonstrate that assuming the folate levels were the same in all countries decreased the estimated numbers of NTDs prevented by only 1%, demonstrating the robustness of the estimation.

A further weakness in this study is that as there were no known NTD prevalence figures for several European countries, with assumptions needing to be made. These assumptions may not be correct. In addition, full member registries in EUROCAT submit individual case data which are extensively analyzed to ensure consistent coding within all EUROCAT registries. Associate member registries only submit aggregate data which limits the amount of data quality checks that can be performed on them. Even in those countries with EUROCAT registries, the registries may only cover a small proportion of all births in a country requiring generalization about the whole population to be made. For Spain the prevalence was obtained from two full member registries and confirmed by the prevalence in the associate registry—The Spanish Collaborative Study of Congenital Malformations (ECEMC) which has a greater coverage of 16% of births over the same time period (19).

Information on whether the mothers who had had an affected pregnancy took folic acid supplements before and during the first trimester of pregnancy would have been very informative, but unfortunately information on folic acid consumption is not well recorded in the EUROCAT registries, partly because such supplements are available as over the counter medications and do not require a prescription.

In many European countries preconceptual folic acid supplementation is not covered by the national health systems, which means that this important preventive measure is subject to individual's being able to afford it, which increases inequity in society.

The main obstacle for folic acid fortification was the concern that it may result in a proportion of the population consuming too much folate. In 2000, the European Commission Scientific Committee on Food report stated that "Although there is no conclusive evidence in humans, the Committee concludes that the risk of progression of the neurological symptoms in vitamin B12 deficient patients as a result of folic acid supplementation cannot be excluded and should be considered the most serious adverse effect" (50). However, a recent paper demonstrated that the evidence used by the Food and Nutrition Board at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences) in the USA to develop the Dietary Reference Intake for folate was scientifically flawed and that there is no level of folate which increases the risk of progression of the neurological symptoms in vitamin B12 deficient patients (3).

This study suggests that the lack of adoption of an extremely cheap and effective intervention will continue to result in an estimated additional 1,000 affected pregnancies each year. We therefore suggest that all flour and other non-wheat products such as "gluten free" be fortified in the

European countries with at least 2.2 μg Folic acid/100 g flour or equivalent.

## DATA AVAILABILITY STATEMENT

All prevalence data analyzed in this study were obtained from the EUROCAT website (https://eu-rd-platform.jrc.ec.europa.eu/eurocat_en) and **Table 2** provides all the data extracted from the referenced papers.

## ETHICS STATEMENT

Local procedures regarding ethics approval for the registries' activities and their collaborations with EUROCAT are available on the EUROCAT website (https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-members/registries_en). The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

## AUTHOR CONTRIBUTIONS

JM: drafting/revising the manuscript for content, study concept or design, analysis of interpretation of data, acquisition of data, and statistical analysis. M-CA, EB, IB, LB-B, PB, CC-C, EDH, EG, MG, MH, BK, NL, AK-O, SK-K, KK, AL-B, EL, MO'M, IP, AP, JR, AR, FR, GS, AS, SS, DT, CV-D, HdW, DW, WW, and EB-S: drafting/revising the manuscript for content, acquisition of data. All authors contributed to the article and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.647038/full#supplementary-material

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# The Improved Prognosis of Hypoplastic Left Heart: A Population-Based Register Study of 343 Cases in England and Wales

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**Background:** Hypoplastic Left Heart Syndrome (HLHS) is a severe congenital heart defect (CHD) characterised by the underdevelopment of the left side of the heart with varying levels of hypoplasia of the left atrium, mitral valve, left ventricle, aortic valve and aortic arch. In the UK, age 12 survival for cases born between 1991 and 1993 was 21%. UK survival estimates corresponding to cases born between 2000 and 2015 were improved at 56%, but survival was examined up to age five only. Contemporary long-term survival estimates play a crucial role in counselling parents following diagnosis. The aim of this study was to report survival estimates up to age 15 for children born with HLHS or hypoplastic left ventricle with additional CHD in England and Wales between 1998 and 2012.

**Methods:** Cases of HLHS notified to four congenital anomaly registers in England and Wales during 1998–2012, matched to Office for National Statistics mortality information, were included. Kaplan-Meier survival estimates to age 15 were reported. Cox regression models were fitted to examine risk factors for mortality.

**Results:** There were 244 cases of HLHS and 99 cases of hypoplastic left ventricle co-occurring with other CHD, with traced survival status. Kaplan-Meier survival estimates for HLHS were 84.4% at age 1 week, 76.2% at 1 month, 63.5% at age 1 year, 58.6% at age 5 years, 54.6% at age 10 years, and 32.6% to age 15 years. The Kaplan-Meier survival estimates for cases of hypoplastic left ventricle co-occurring with additional CHD were 90.9% at age 1 week, 84.9% at 1 month, 73.7% at age 1 year, 67.7% to age 5 years, 59.2% to age 10 years, and 40.3% to age 15 years. Preterm birth ($p = 0.007$), low birth weight ($p = 0.005$), and female sex ($p = 0.01$) were associated with mortality.

**Conclusions:** We have shown that prognosis associated with HLHS in the twenty first century exceeds that of many previous population-based studies, likely due to improvements in intensive care technologies and advances in surgical techniques over the last few decades.

**Keywords: congenital heart, hypoplastic left heart, survival, epidemiology, register, congenital anomalies**

## INTRODUCTION

Hypoplastic Left Heart Syndrome (HLHS) is a severe congenital heart defect (CHD) characterised by the underdevelopment of the left side of the heart with varying levels of hypoplasia of the left atrium, mitral valve, left ventricle, aortic valve and aortic arch (1). Survival was not possible until the 1980s when the Norwood procedure was introduced (2, 3). Since then, improvements in antenatal screening, preoperative management, resuscitation, and surgical techniques such as right ventricle-to-pulmonary artery conduits and the hybrid procedure for neonates too unstable (e.g., very preterm or with other comorbidities) to undergo the Norwood procedure, have further improved the prognosis for HLHS patients (4, 5). However, the impact of right ventricle-to-pulmonary artery conduits and the hybrid procedure on longer term prognosis remains under debate (6). While not the standard approach, survival in children and adults with HLHS may also have improved due to increased frequency and improved survival associated with heart transplants (7).

Today the standard treatment for individuals with HLHS is to undergo the Norwood stage I procedure (reconstruction of the left track and modified Blalock Taussig shunt or Sano technique) within the first 2 weeks of life, the Norwood stage II procedure (Hemi fontan, Glenn, or partial cavo-pulmonary bypass) procedure between 4 and 6 months of age and finally the Norwood stage III (Fontan final phase, full cavo-pulmonary bypass) procedure between 18 months and 4 years of age (8). According to the UK's National CHD audit, 30 day post-operative survival for the Norwood, Glenn and Fontan procedures exceeds 90% (9). However, when they occur with a hypoplastic left ventricle, other forms of CHD such as atrioventricular septal defect (AVSD), common arterial trunk (CAT), and double outlet right ventricle (DORV) may also be surgically treated with the Fontan procedure, but not necessarily after a Norwood stage I surgery as is the case for HLHS (10, 11). These forms of CHD with a hypoplastic left ventricle, often known as functional univentricular hearts, have better prognosis than HLHS and so the post-surgical survival rates for HLHS specifically is likely to be lower (12). A recent UK study reported 5 year survival for cases of HLHS born between 2000 and 2015 was 56.3%, although this study did not ascertain those who did not undergo any interventional treatment (13). Outside of the UK, recent age five survival rates range between 18.8% in New Zealand to 72% in Denmark (14, 15). Longer-term estimates of survival from birth are important for clinicians counselling parents when HLHS is diagnosed prenatally, as they may aid decision making regarding continuation of pregnancy. Given that individuals with palliated CHD will require follow-up throughout their lives, contemporary survival estimates are also important for commissioners whose role is to ensure services are in place for the growing population of adults with CHD.

The primary aim of this study was to report contemporary survival estimates up to age 15 for children born with HLHS or hypoplastic left ventricle in addition to other CHD subtypes in England and Wales, and to examine risk factors for mortality among these cases.

## MATERIALS AND METHODS

### Data

The British and Irish Network of Congenital Anomaly Research Database (BINOCARD) was a collaborative network of regional population-based congenital anomaly registers (16), which was superseded in England by a national system, the National Congenital Anomaly and Rare Diseases Registration Service (NCARDRS) in 2015 (17). Each regional register prospectively collected data on congenital anomalies occurring in the pregnancies of women residing in their geographically defined populations (**Figure 1**). Data were obtained from three registers in England and the register for Wales, covering ∼28% of the birth population of England and Wales. The Northern Congenital Abnormality Survey (NorCAS), established in 1985, covered 32,000 births per year in the North East of England and North Cumbria; the East Midlands and South Yorkshire Congenital Anomaly Register (EMSYCAR), established in 1997 covered 74,000 births per year; the South West Congenital Anomaly Register (SWCAR), established in 2002, covered 50,000 births per year in South West England; and the Congenital Anomaly Register and Information Service (CARIS), established in 1998, covers 35,000 births per year in Wales. Each register recorded up to eight congenital anomalies for each case. Anomalies were coded using the WHO International Classification of Disease (ICD) version ten, consistent with the


**FIGURE 1 |** Map showing the area covered by the congenital anomaly registers.

European Surveillance of Congenital Anomalies (EUROCAT) guidelines (18). To ensure a high case ascertainment, congenital anomalies were notified to each register from a variety of sources including prenatal ultrasound departments, foetal medicine units, cytogenetic laboratories, regional cardiology centres, pathology departments, and paediatric surgery departments. CHD diagnoses were confirmed at surgery, by echocardiography or cardiac catheterisation, or at post-mortem.

The register data were linked to death registrations via NHS Digital in December 2017, using the "fuzzy matching" of infant name, sex, date of birth, and postcode at delivery. These identifier variables were removed or pseudo-anonymised (e.g., date of birth was replaced with year of birth only) following the linkage before the data were accessed by the researcher (KEB). While NHS Digital were able to provide the exact age of cases resulting in mortality, it was not possible to provide the exact age at case censorship for survivors as this would have allowed date of birth to be identified by the researcher (due to the linkage date being known). Therefore, age at censorship was estimated by subtracting the number of days between the midpoint of year of birth and December 2017. For each case with an English postcode at birth, NHS Digital assigned the English Index of Multiple Deprivation (IMD) score and rank. IMD is a measure of area-level socioeconomic deprivation calculated from income, employment, health, education, access to services, social environment, housing stress, living environment and crime (19, 20). IMD 2004, 2007, 2010 were used according to the year of birth of the case. It was not possible for NHS Digital to provide the Welsh IMD and so analysis of IMD was restricted to cases notified to the English registers only (80.8% of cases).

England and Wales population survival estimates at ages 1 month and 1 year were taken from the Office for National Statistics' online vital statistics for comparison with cases of HLHS and hypoplastic left ventricle with additional CHD (21).

## Case Inclusion

Cases coded as hypoplastic left heart (ICD 10: Q234) that were live born during 1st January 1998–31st December 2012 were included. The EUROCAT uses the same ICD 10 code (Q234) to describe both HLHS and severe hypoplastic left ventricle co-occurring with other CHD subtypes such as AVSD, CAT, and DORV (18). Given that these two forms of CHD are likely to have different survival rates, they were classed as two separate groups. HLHS was defined as cases coded with ICD Q234 but no other CHD codes except for those that commonly occur as part of HLHS: aortic valve stenosis/ atresia, mitral valve atresia/ stenosis, mitral regurgitation, aortic regurgitation, patent ductus arteriosus, atrial septal defect, and coarctation of aorta. We defined the remainder of cases as hypoplastic left ventricle with additional CHD, i.e., those cases coded as ICD Q234 in addition to any other CHD subtypes (Q20–26), excluding those listed above. Cases that occurred with extra-cardiac anomalies (ECA's, i.e., chromosomal anomalies or structural congenital anomalies of another organ system) or that occurred with additional CHD subtypes, were included. Twenty-eight (7.5%) cases were untraced, and these were excluded following descriptive statistics.

## Ethical Approval

The BINOCARD has approval from the Confidentiality Advisory Group of the Health Research Authority (PIAG 2-08I/2012), to hold data without consent and ethics committee approval (09/H0405/48) to undertake studies involving their data. The data linkage aspect of this study was given a favourable ethical opinion by the Newcastle & North Tyneside 2 Local Research Ethics Committee (13/NE/0188) and the Confidentiality Advisory Group of the Health Research Authority [5-08(b) 2013].

## Statistical Analysis

The associations between untraced survival status and demographic (categorical) variables were examined using $\chi^2$ tests of association or Fisher's exact tests. Kaplan-Meier survival estimates were estimated at ages 1 week, 1 month, 1, 5, 10, and 15 years and according to period of birth (categorised as 1998–2005 and 2006–2012). Survival estimates were not reported (numerically or graphically) where the number of cases at risk was <5, given that this can produce unstable estimates.

Hazard ratios (HRs) were estimated using univariable Cox regression models for the following predictors: extra-cardiac anomalies [none (i.e., isolated CHD), structural (e.g., digestive system congenital anomalies), chromosomal (e.g., Down syndrome)], maternal age at delivery (<20, 20–34, ≥35 years), birth weight (low: <2,500 g, average: 2,500–3,999 g, high: ≥4,000 g), gestational age at birth (preterm: <37 weeks, term: 37–41 weeks, post-term ≥42 weeks), infant sex (male, female) and IMD at birth [quintile 1 (most deprived), quintile 2, quintile 3 quintile 4, quintile 5 (least deprived)], plurality (singleton or multiple).

The proportional hazards assumption was checked using log-log plots, Schoenfield residulas, and by comparing hazard ratios for different categorisations of survival time. $P < 0.05$ was considered statistically significant and analyses were performed in Stata 14.

# RESULTS

There were 371 live born cases included in the study, giving a live birth prevalence of 1.5 (1.4, 1.7) per 10,000 live births. Survival status was traced for 343 (92.5%) cases. Untraced cases were more common in cases born earlier in the study period ($p < 0.001$) and in cases born with a low or high birth weight ($p = 0.002$). The proportion of traced cases also varied by register ($p = 0.001$), with 82.5% of CARIS cases traced up to 100% of SWCAR cases. There was no association between traced status and gestational age at delivery ($p = 0.17$), maternal age at delivery ($p = 0.35$), infant sex ($p = 0.90$), plurality ($p = 0.14$), ECAs ($p = 0.45$), or IMD ($P = 0.48$). The 28 untraced cases were excluded from further analysis.

## Case Characteristics

There were 244 cases defined as HLHS and 99 defined as hypoplastic left ventricle with additional CHD. Cases of HLHS were most commonly: isolated CHD ($n = 220$, 90.2%), born at >37 weeks gestation ($n = 195$, 83.7% of cases with available gestational age), with average birth weight ($n = 171$, 71.8%), to mothers aged 20-34 ($n = 160$, 69.0%), of male sex ($n = 140$,

**TABLE 1 |** Birth characteristics of cases of HLHS and hypoplastic left ventricle with additional CHD.

| Risk factors | All cases *n* (% of 343) | HLHS *n* (% of 244) | Hypoplastic left ventricle with additional CHD *n* (% of 99) |
|---|---|---|---|
| **Register** | | | |
| CARIS | 66 (19.2) | 57 (23.4) | 9 (9.1) |
| EMSYCAR | 162 (47.2) | 103 (42.2) | 59 (59.6) |
| NorCAS | 50 (14.6) | 41 (16.8) | 9 (9.1) |
| SWCAR | 65 (19) | 43 (17.6) | 22 (22.2) |
| **Year of birth** | | | |
| 1998–2005 | 137 (39.9) | 109 (44.7) | 28 (28.3) |
| 2006–2012 | 234 (68.2) | 157 (64.3) | 77 (77.8) |
| **Gestational age** | | | |
| Preterm (<37 weeks) | 50 (14.6) | 38 (15.6) | 12 (12.1) |
| Term/Post-term (≥37 weeks) | 280 (81.6) | 195 (79.9) | 85 (85.9) |
| Missing | 13 (3.8) | 11 (4.5) | 2 (2.0) |
| **Birth weight** | | | |
| Low (<2,500 g) | 63 (18.4) | 41 (16.8) | 22 (22.2) |
| Average (2,500–3,999 g) | 240 (70) | 171 (70.1) | 69 (69.7) |
| High (≥4000g) | 32 (9.3) | 26 (10.7) | 6 (6.1) |
| Missing | | | |
| **Maternal age** | | | |
| <20 | 44 (12.8) | 29 (11.9) | 15 (15.2) |
| 20–34 | 225 (65.6) | 160 (65.6) | 65 (65.7) |
| ≥35 | 61 (17.8) | 43 (17.6) | 18 (18.2) |
| Missing | 44 (12.8) | 29 (11.9) | 15 (15.2) |
| **Sex** | | | |
| Female | 139 (40.5) | 104 (42.6) | 35 (35.4) |
| Male | 204 (59.5) | 140 (57.4) | 64 (64.6) |
| **Socioeconomic deprivation (IMD)** | | | |
| Least deprived Q1 | 38 (11.1) | 27 (11.1) | 11 (11.1) |
| Q2 | 38 (11.1) | 25 (10.2) | 13 (13.1) |
| Q3 | 43 (12.5) | 21 (8.6) | 22 (22.2) |
| Q4 | 61 (17.8) | 46 (18.9) | 15 (15.2) |
| Most deprived Q5 | 92 (26.8) | 64 (26.2) | 28 (28.3) |
| Missing | 71 (20.7) | 61 (25.0) | 10 (10.1) |
| **Extra cardiac anomalies** | | | |
| Isolated CHD | 297 (86.6) | 220 (90.2) | 77 (77.8) |
| Structural ECA | 27 (7.9) | 12 (4.9) | 15 (15.2) |
| Chromosomal ECA | 19 (5.5) | 12 (4.9) | 7 (7.1) |

*HLHS, Hypoplastic Left Heart Syndrome; ECA, Extra Cardiac Anomaly, IMD, Index of Multiple Deprivation.*

57.3%) and to mothers residing in the most deprived areas (IMD quintile 5: $n = 35$, 30.2%) (**Table 1**).

Cases of hypoplastic left ventricle co-occurring with additional CHD, were more commonly: isolated CHD ($n = 77$, 77.8%), born at >37 weeks gestation ($n = 85$, 87.6%), of average birth weight ($n = 69$, 71.1%), to mothers aged 20–34 ($n = 65$, 66.3%), of male sex ($n = 64$, 64.6%) and to mothers residing in the most deprived areas (IMD quintile 5: $n = 28$, 31.4%) (**Table 1**). These cases most commonly occurred with ventricular septal defect ($n = 29$), AVSD ($n = 17$), DORV ($n = 13$), interrupted aortic arch ($n = 10$), and transposition of the great vessels ($n = 8$), with 41 cases having "other" or "unspecified" CHD.

**TABLE 2 |** Kaplan-Meier survival estimates for HLHS and hypoplastic left ventricle with additional CHD born between 1998 and 2012.

| Age | All cases Survival (95% CI) | HLHS Survival % (95% CI) | Hypoplastic left ventricle with additional CHD Survival % (95% CI) |
|---|---|---|---|
| 1 week | 86.3 (82.2, 89.5) | 84.4 (79.2, 88.4) | 90.9 (83.3, 95.2) |
| 1 month | 78.7 (74, 82.7) | 76.2 (70.4, 81.1) | 84.9 (76.1, 90.6) |
| 1 year | 66.5 (61.2, 71.2) | 63.5 (57.2, 69.2) | 73.7 (63.9, 81.3) |
| 5 years | 61.2 (55.9, 66.2) | 58.6 (52.2, 64.5) | 67.7 (57.5, 75.9) |
| 10 years | 56.2 (50.6, 61.4) | 54.6 (48.1, 60.7) | 59.2 (47.5, 69.2) |
| 15 years | 34.4 (25.8, 43.1) | 32.6 (23.5, 42.0) | 40.3 (19.6, 60.2) |

*HLHS, Hypoplastic Left Heart Syndrome.*

## Survival and Trends in Survival

Kaplan-Meier survival estimates for cases of HLHS born between 1998 and 2012 are shown in **Table 2**, with 84.4% of cases surviving the first week of life, 76.2% the first month, 63.5% to age 1 year, 58.6% to age 5 years, 54.6% to age 10 years, and 32.6% to age 15 years. Compared to cases born 1998–2005, survival of cases born 2006–2012 appeared to be slightly increased up to age 5 years (**Figure 2**), but similar thereafter. Due to violation of the proportional hazards assumption, survival in the two era's was not formally compared.

The Kaplan-Meier survival estimates for cases of hypoplastic left ventricle with additional CHD were slightly higher compared to cases of HLHS, with 90.9% of cases surviving the first week of life, 84.9% the first month, 73.7% to age 1 year, 67.7% to age 5 years, 59.2% to age 10 years, and 40.3% to age 15 years. However, the difference in survival between the two phenotypes was not statistically significant (log-rank test: $p = 0.14$). The association between year of birth and mortality among cases of hypoplastic left ventricle with additional CHD did not reach statistical significance (log-rank test: $p = 0.09$), survival appeared to be increased in those born 1998–2005 vs. 2006–2012 (**Figure 2**).

The survival rates at ages 1 month and 1 year for cases of HLHS and hypoplastic left heart variant were significantly lower than those of the general population of England and Wales (99.5 and 99.7%, respectively, test of proportions: $p < 0.001$).

## Predictors of Survival

In all cases combined, there were significant associations between mortality and gestational age at delivery ($p = 0.007$), birth weight ($p = 0.005$), and infant sex ($p = 0.01$) (**Table 3**). One year survival estimates increased with increasing gestational age at delivery (52.0, 67.5, and 91.7% for preterm, term and post-term births), although the association was only statistically significant in preterm vs. term births (HR = 1.77, 95% CI: 1.21, 2.57). One year

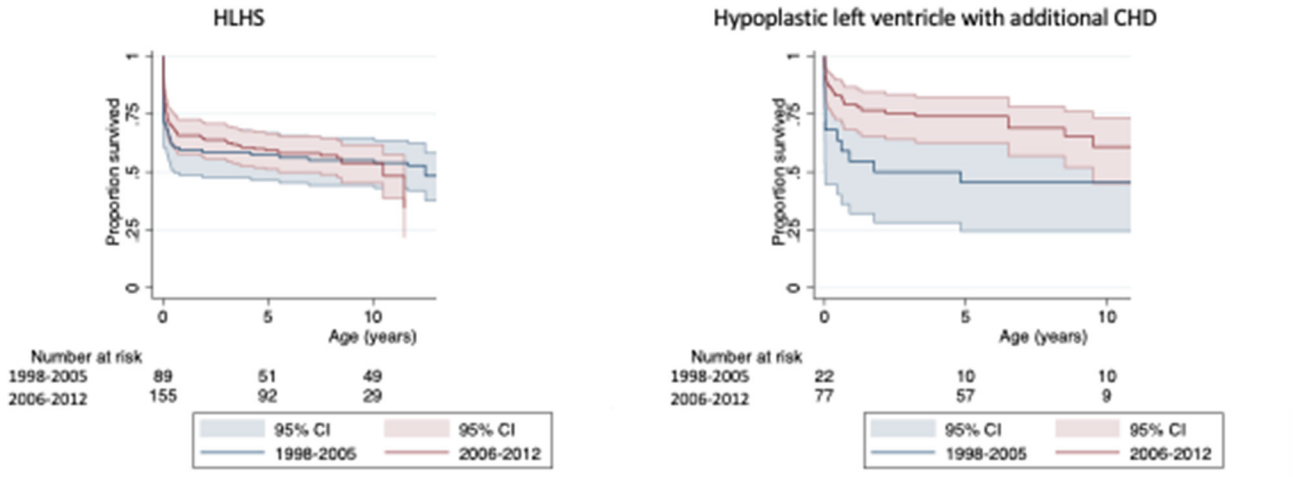


**FIGURE 2 |** Kaplan-Meier survival curves by year of birth.

survival estimates increased with increasing birth weight (57.1, 66.7, and 81.3% for low, average, and high birth weight), with the association being statistically significant in low vs. average birth weight cases (HR = 1.51, 95% CI: 1.09, 2.23) and high vs. average birth weight (HR = 0.50, 95% CI: 0.25, 0.98). Males had decreased risk of mortality compared to females (HR = 0.67, 95% CI: 0.50, 0.91). There was no evidence of an association between mortality and plurality ($p = 0.82$), maternal age ($p = 0.17$), socioeconomic deprivation ($p = 0.65$) or ECAs ($p = 0.86$).

# DISCUSSION

## Summary

Based on this population-based study of 343 traced cases of HLHS or hypoplastic left ventricle with additional CHD born in England and Wales, we have shown that survival to age 5 years in the twenty first century is as high as 59 and 68%, respectively. Survival was slightly greater amongst cases with hypoplastic left ventricle with major CHD compared to HLHS, although the difference was not statistically significant. This study shows that the greatest risk of mortality in those with HLHS or hypoplastic left ventricle and additional CHD occurs during the first week of life, although survival continued to decrease over the 15 years of follow-up. Survival estimates were increased in the later cohort (2006–2012 vs. 1998–2005) although for cases of HLHS they may only have been increased until age 5 years.

## Strengths

The main strength of this study is the use of high-quality data derived from four regional population-based congenital anomaly registers. The registers were notified of cases from multiple sources including prenatal ultrasound, cardiology databases, cytogenetics and post-mortems. As a result, there is likely very high ascertainment of cases. The ascertainment of cases at birth as opposed to at surgical intervention is a further strength as this has enabled pre-operative mortalities to be captured. However, this also means that we have included in our estimates those that opted for palliative care, which will have deflated the survival estimates. A recent survey of European surgeons reported that parental intention to treat was 95% in the UK, with the remaining 5% opting for comfort care (22). According to a US study of military personnel, the proportion of parents opting for comfort care is minimal (3%) since the 2000's and so these cases probably make up a very small proportion of our cohort (23).

A further strength of this study is that we attempted to separate cases of HLHS and cases of hypoplastic left ventricle with additional CHD subtypes such as DORV or AVSD. We have shown that survival estimates may vary between these two types of cases, with cases of hypoplastic left heart occurring with additional CHD having slightly improved prognosis when compared to classic cases of HLHS. Combining these cases may also over inflate the survival estimate associated with classic HLHS. Ideally, cases of HLHS would be coded as those that underwent a Norwood stage I procedure only, but unfortunately the data we used was not linked to information on surgical intervention. As a result we may have misclassified some cases of CHD with hypoplastic left ventricle as HLHS. Additionally, we may have misclassified cases that occurred with ventricular septal defects as we were not able to identify the specific phenotype. Where muscular VSD could occur as part of HLHS, the same cannot be said for conotruncal VSD. However, misclassification likely accounted for only minority of cases of VSD. However, our approach allows cases with HLHS that did not survive until Norwood stage I to be included in the analysis.

## Limitations

This study has some limitations. Firstly, "fuzzy matching" was used to link the congenital anomaly register data to death registrations. As a result, we were not able to trace the survival status of 28 cases. This could have caused bias in our survival estimates if the survival statuses of these cases were significantly different to the cases that were traced. These cases were slightly

**TABLE 3 |** Risk factors for mortality in cases of HLHS or hypoplastic left ventricle with additional CHD born 1998–2012.

| Risk factors | *N* (%) | Age 1 year Kaplan-Meier survival estimate | Hazard ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| **Gestational age** | | | | 0.007 |
| Preterm (<37 weeks) | 50 (14.5) | 52.0 (37.4, 64.7) | 1.77 (1.21, 2.57) | |
| Term (37–41 weeks) | 270 (78.3) | 67.5 (61.6 (72.8) | 1 (reference category) | |
| Post-term (≥42 weeks) | 12 (3.5) | 91.7 (53.9, 98.8) | 0.72 (0.29, 1.76) | |
| Missing | 13 (3.8) | | | |
| **Birth weight** | | | | 0.005 |
| Low (<2,500 g) | 63 (18.3) | 57.1 (44.0, 68.3) | 1.51 (1.09, 2.23) | |
| Average (2,500–3,999 g) | 240 (70.1) | 66.7 (60.3, 72.2) | 1 (reference category) | |
| High (≥4,000 g) | 32 (9.3) | 81.3 (63.0, 91.1) | 0.50 (0.25, 0.98) | |
| Missing | 8 (2.3) | | | |
| **Maternal age** | | | | 0.17 |
| <20 | 44 (13.0) | 54.6 (8.8, 67.8) | 1.42 (0.92, 2.19) | |
| 20–34 | 225 (65.5) | 68.9 (62.4, 74.5) | 1 (reference category) | |
| ≥35 | 61 (17.7) | 63.9 (50.6, 74.6) | 1.31 (0.88, 1.93) | |
| Missing | 13 (3.8) | | | |
| **Sex** | | | | |
| Female | 139 (40.3) | 59.7 (51.1, 67.3) | 1 (reference category) | 0.01 |
| Male | 204 (59.7) | 71.1 (64.3, 76.8) | 0.67 (0.50, 0.91) | |
| **Socioeconomic deprivation (IMD)** | | | | 0.65 |
| Least deprived Q1 | 38 (11.0) | 73.7 (56.6, 94.9) | 1 (reference category) | |
| Q2 | 38 (11.3) | 55.3 (38.3, 69.3) | 1.57 (82.9, 2.97) | |
| Q3 | 43 (12.5) | 69.8 (53.7, 81.2) | 1.06 (54.9, 2.04) | |
| Q4 | 61 (17.7) | 67.2 (53.9, 77.5) | 1.18 (0.65, 2.17) | |
| Most deprived Q5 | 93 (27.0) | 67.4 (56.8, 76.0) | 1.20 (0.68, 2.12) | |
| Missing | 71 (20.6) | | | |
| **Plurality** | | | | 0.82 |
| Singleton | 320 (93.3) | 66.3 (60.8, 71.1) | 1 (reference category) | |
| Multiple | 15 (4.3) | 66.7 (37.5, 84.6) | 1.09 (0.51, 2.33) | |
| Missing | 8 (2.0) | | | |
| **Extra-cardiac anomalies** | | | | 0.86 |
| Isolated | 297 (86.7) | 67.3 (61.7, 72.4) | 1 (reference category) | |
| Structural ECA | 27 (7.9) | 55.6 (35.2, 71.8) | 1.25 (0.72, 2.17) | |
| Chromosomal ECA | 19 (5.5) | 68.4 (42.8, 84.4) | 1.23 (0.67, 2.27) | |

*HLHS, Hypoplastic Left Heart Syndrome, ECA, Extra-Cardiac Anomaly, IMD, Index of Multiple Deprivation.*

more likely to be of low or high birth weight compared to the traced cohort and were more likely to have been born earlier in the study period, which suggests they might have had poorer survival than the traced cases. Given that 93% of the cohort were traced, the impact of any biases caused by excluding the untraced cases will be minimal. Future linkage studies will benefit by linking using National Health Service (NHS) number, which is now routinely recorded by the NCARDRS. An additional weakness was that our analysis of socioeconomic status (IMD) was restricted to cases born to mothers resident in England only, which reduced the power of this analysis. Moreover, due to the low prevalence of hypoplastic left ventricle with additional CHD, we may not have had adequate power to detect statistically significant differences in survival between the two time periods and when comparing to HLHS survival.

The BINOCARD did not routinely link to hospital episode statistics, so we were not able to present pre- or post-operative survival estimates relating to cardiac surgeries. Post-operative survival estimates for cases treated in the UK are available via NICOR, with 30 day post-operative survival for the Norwood, Glenn and Fontan procedures exceeding 90% (9). There is very limited information on modern pre-operative mortality rates, with the most recent UK study of cases born between 1992 and 1993 reporting that 32% of cases died with no intervention (12). However, it is not clear what proportion of these had an intention to treat. Moreover, we could not attribute mortalities to heart transplants which may have occurred at various points of the life course and likely represent post-neonatal deaths. While paediatric heart transplants are rare in the UK in general ($n$ = 23 in 2012, according to the UK National Transplant Registry),

the proportion of heart transplants for CHD doubled in the UK between 2002 and 2012 (24). The proportion of HLHS cases that result in transplant in the UK have not been recently reported, but according to the Children's Heart Federation are likely to account for a very small number of cases due to the availability of donor hearts within that age range (25). Linkage between the data recorded by the congenital anomaly registers and hospital episode statistics is now theoretically possible via the NCARDRS and is currently being explored by the authors. The estimates in this study could be used in conjunction with surgical survival rates now provided by the National CHD audit to build a more accurate picture of HLHS prognosis (9).

We found improved survival among cases of hypoplastic left ventricle with additional CHD compared to HLHS. However, this group represents a variety of different phenotypes which may have variable prognoses. Further research into the specific combinations of CHD subtypes is required to provide more specific survival estimates for these cases.

The BINOCARD recorded routinely collected demographic information for each case, but did not hold clinical information on treatments or interventions. Due to the small number of available demographic risk factors, the lack of clinical predictors and the relative rarity of some of the characteristics (e.g., post-term birth and high birthweight), we did not attempt to perform a multivariable analyses or examine causality. Mediation analyses could be performed to examine whether the negative impact of low birth weight is accounted for primarily by the effects of preterm birth. In future studies, a path analysis may be applied to examine the complexity of the association between socioeconomic deprivation and mortality, although we found little evidence of an association in the univariable setting.

## Comparison to Previous Studies

A previous UK study reported age 12 survival to be 21% for cases born during 1992–1993 (12), which is consistent with cases born in the early part of our study period. More recently, 5 year survival for cases of HLHS born in 2000–2015 was 56.3% in the UK, which is comparable to the 5 year survival reported in our study (58.6%) (13). According to the UK National CHD audit of surgeries performed between 2015 and 2016, 30 day post-operative survival was 92.8% following the Norwood procedure, 98.2% following Glenn procedure and 99.6% following Fontan (9). This suggests the greatest risk of mortality is associated with the stage 1 repair, which usually occurs in the first 1 or 2 weeks of life (8). This is reflected in our study which shows the highest decrease in survival during the first week and between the first week and first month of life. However, this high hazard rate may also represent cases that died pre-operatively or those that opted not to treat.

The survival estimates provided in this study relate to children born in England and Wales, who were entitled to free health care provided by the NHS. Our survival estimates may not be representative of other populations with different health care systems, for example in the USA where the health care system is not publicly funded, and CHD outcomes vary according to health insurance status (26). Perhaps for this reason, our survival estimates exceed those of 60 cases born in Atlanta (USA) between 1999 and 2005 (27), who had 10 year survival of 42.5% compared to our estimates of 54.7% (HLHS) and 48.0% hypoplastic left ventricle with additional CHD between 1998 and 2005. Differences in survival may also be due to differences in infant transplant provision, which is relatively uncommon in the UK but may remain as a parental choice in some centres in the USA (28). Conversely, in Denmark, which has a publicly funded health care system, 10 year survival for 143 children born with univentricular hearts between 2003 and 2015 exceeded our survival estimates at 72%, although cases were ascertained based on surgical procedures so some pre-operative mortalities may not have been ascertained (29). A different regional study in Denmark that ascertained 308 cases from a CHD register reported much lower 5 year survival estimate of 18.8% for cases of univentricular heart born between 2000 and 2009 (14). These differences in survival estimates may also be related to the definition of HLHS and the accuracy of coding, the decision to terminate following a prenatal diagnosis or to opt for comfort care, which vary by country and centre (22). In New Zealand, 5 year survival for cases of HLHS born between 2006 and 2010 was 62.1% although this estimate is based on 29 cases ascertained on surgical procedures (15). The applicability of our survival estimates to cases born in developing countries, where there is a paucity of population-based research into HLHS survival, is also not clear.

We found that preterm birth and low birth weight were associated with an increased risk of mortality, which is consistent with previous research (14, 27, 30). These factors may influence the age at which surgeries are performed, the stability at the time of operation, lung development and the technical aspects of surgical reconstruction, which can negatively impact on survival (31). Similar to previous studies, we also found no evidence that ECAs increase the risk of mortality (27). While this is not the case for most other CHD subtypes (32), HLHS is perhaps so clinically severe that additional congenital anomalies are overpowered in terms of mortality. In cohorts from Denmark and the USA, increased mortality rates among those with high vs. low levels of neighbourhood poverty has been reported (14, 27, 30). We did not find any statistically significant evidence of an association with IMD, which could perhaps be reflective of the differences in our health care systems. We found no association between mortality and plurality or maternal age with is consistent with several previous studies, although none of these are based on UK populations (14, 27, 30).

In our study, female sex was associated with an increased risk of mortality, possibly because these cases were more likely to be HLHS as opposed to hypoplastic left ventricle with additional CHD. Three population-based studies did not report a significant association between infant sex and mortality (14, 27, 30), although in two the mortality rate among females was greater (14, 27). In a USA based study, surgical mortality was significantly greater in females following the Norwood procedure, but not following the Glenn or Fontan procedures (33). Another US study also reported an increased risk of mortality in females following the Norwood procedure, although this was of borderline statistical significance (15.5% in males vs.

30.0% in females, $p = 0.07$) (34). Speculatively, females with HLHS could have increased likelihood of being born with a more severe HLHS phenotype, which would increase the gender disparity in mortality, but there is no research to corroborate this currently.

### Implications

We have shown survival up to age 15 for those born with HLHS has improved compared to previous studies based on earlier cohorts. This information is important for counselling parents when their child is diagnosed with HLHS. However, the survival estimates reported in this study need to be considered alongside data on quality of life and morbidity. There is some evidence that children with HLHS experience lower quality of life, increased risk of neurodevelopmental delay, behavioural problems and decreased functional status (35, 36). Given that survival was very rare until the 1990s and 2000s, there is also a paucity of information known on longer term survival and quality of life in adults living with palliated HLHS.

## DATA AVAILABILITY STATEMENT

Data was obtained from the National Congenital Anomaly and Rare Diseases Registration Service and linked to ONS death registrations via NHS Digital. For NCARDRS data requests please email phe.ncardrs@nhs.net. Linked data requests will require additional ethical approvals.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Newcastle & North Tyneside 2 Local Research Ethics Committee (13/NE/0188). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

KB was responsible for the statistical analysis of the data and drafting the initial manuscript. NM, ED, KL, and DT provided the data and critically reviewed and commented on the manuscript. JR, along with KB, conceived and designed the study and had input into the drafting and critical appraisal of the manuscript. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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# Why Does Child Mortality Decrease With Age? Modeling the Age-Associated Decrease in Mortality Rate Using WHO Metadata From 25 Countries

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**Background:** Our previous study analyzed the age trajectory of mortality (ATM) in 14 European countries, while this study aimed at investigating ATM in other continents and in countries with a higher level of mortality. Data from 11 Non-European countries were used.

**Methods:** The number of deaths was extracted from the WHO mortality database. The Halley method was used to calculate the mortality rates in all possible calendar years and all countries combined. This method enables us to combine more countries and more calendar years in one hypothetical population.

**Results:** The age trajectory of total mortality (ATTM) and also ATM due to specific groups of diseases were very similar in the 11 non-European countries and in the 14 European countries. The level of mortality did not affect the main results found in European countries. The inverse proportion was valid for ATTM in non-European countries with two exceptions.
Slower or no mortality decrease with age was detected in the first year of life, while the inverse proportion model was valid for the age range (1, 10) years in most of the main chapters of ICD10.

**Conclusions:** The decrease in child mortality with age may be explained as the result of the depletion of individuals with congenital impairment. The majority of deaths up to the age of 10 years were related to congenital impairments, and the decrease in child mortality rate with age was a demonstration of population heterogeneity. The congenital impairments were latent and may cause death even if no congenital impairment was detected.

**Keywords: mortality rate, age, childhood, congenital anomalies, WHO database**

## INTRODUCTION

Human mortality rate decreases with age after birth and increases with age in adults. This increase in adults is exponential and it is usually interpreted as a manifestation of aging and affects all individuals (1–23). Age is a deterministic variable in the relationship because coefficients of determination were higher than 0.99 (if age as a single independent variable is used to explain the changes of mortality rate, then more than 99% of variability is explained) (4, 19–22). All historical changes in healthcare such as reduction of mortality due to infectious diseases or due to cardiovascular diseases did not affect the shape of the relationship in the last two centuries and they changed only two parameters of the model (11, 12, 18, 23).

Faster changes in mortality rate with age occur during childhood (5, 8, 9, 20–28). The steep mortality decrease is accompanied by age-based changes in the causes of death (28). For example, congenital anomalies and impairments originating in the perinatal period reached more than 85% of all deaths during the first 4 weeks of life and only 10% in the age 5–10 years interval in 14 European countries [furthermore, "the age 5–10 interval" corresponded to the mathematical interval (5, 10)] years and to the age category 5–9 years in demography) (28). Simultaneously, the decrease of total mortality rate was smooth and was described by the model of the inverse proportion with a coefficient of determination higher than 0.99 (20–28).

The presented paper closely follows the previous study (28). Age trajectory of total mortality (ATTM) was more important than age trajectories of mortality due to specific diseases and the decrease of ATTM was described by the model of the inverse proportion up 10 years in the previous study (28).

With respect to the whole world, 14 European countries studied in the previous study came from a homogenous region (e.g., with the respect to the health system or economy level). This study aimed at investigating the previous results in other continents and in countries with a higher level of child mortality; data from 11 non-European countries were used.

## MATERIALS AND METHODS

The description of age trajectory of mortality (ATM) during the first year of life was essential to observed results, and the WHO mortality database was used. For these reasons, the selection of countries and calendar years in the database was based on two criteria:

a) The ICD10 classification was used.
b) Four age categories were used in the first year of life (it was the maximal number of age categories used in the database in the first year).

Unfortunately, the list of countries that comply to the second criterion was not big. For example, India, China, Russia, or African countries did not meet it. Finally, 11 countries were selected in the database.

Age was assumed as the main factor, and all other factors were assumed to be less significant (28). Furthermore, the existence of general mechanisms was assumed, as demonstrated in the ATM after birth. For these reasons, ATM was primarily constructed in as large a population as possible. Including more regions and calendar years within the analysis may eliminate all factors other than age, rendering the impact of age more visible. The same methods that were used in the previous study were used to calculate the mortality rate (28). The unit corresponding to the mortality rate was "person-years," which was the number of years lived by members of the population between age limits of age category.

### Aggregated Populations

Zero deaths in a given age group may make it impossible to construct ATM for some set of diseases. While zero deaths due to some disease may occur within a specific age category in a specific calendar year, at least one death may occur within the same age category in another calendar year. Consequently, the inclusion of additional calendar years and regions may remove this obstacle. The aggregation of more calendar years was first utilized by Edmond Halley in 1693 (29, 30). The method enables the calculation of the mortality rate within one age category based on the number of deaths and living persons in several years and more regions (28–31). Besides, it may be assumed that ATM may be smoother in an aggregated population according to the law of large numbers.

The WHO mortality database contains the number of deaths Di within specific age categories in different countries (32). The database uses the following four age categories for the first year of life: "0 days" [(0, 24) hours], "1–6 days" [(1, 7) days], "7–27 days" [(7, 28) days], and "28–365 days" [(28, 365) days]. One or two age categories are also used in the database in the first year and such data were not convenient. Cause of death was determined using the specific revision of the International Classification of Diseases in the database. Here, the calendar period used in each country corresponds to the period when the 10th revision (ICD10) and the four age categories were applied (33). The numbers of living people are obtained from the U.S. Census Bureau (Bureau of the Census 2019) (34).

The present study used data collected in the following 11 non-European countries: Argentina, Brazil, Peru, Venezuela, Chile, Colombia, Mexico, Japan, Australia, New Zealand, and the USA. The first six countries were aggregated to population P4 (South America) while the last five countries were aggregated to population P5. Besides, aggregated populations P1, P2, P3, and P14 were defined and used in the previous study (28). Finally, the data from 11 non-European countries and 14 European countries from the previous study were aggregated to the largest population P25 (28). The sum of the means of population sizes calculated in a specific country in a specific calendar period was 1,306,115,570 living persons per one calendar year (it may be interpreted as the average size of P25). Population P25 was about 3.3 times bigger than P14 and represented a significant portion of the world population. Calendar periods and means of population sizes of populations are shown in the second and in the third columns in **Table 1** (28). If no deaths were registered within a specific age category due to specific diseases in any population, ATM for

**TABLE 1 |** Population sizes and results of total mortality calculated during the first 10 years in all populations in the log-log scale.

| Population | Years | Size | Slope $\gamma$ | Lower | Upper | level $\mu_1$ | $\tilde{R}^2$ | $R_b^2$ | c/x |
|---|---|---|---|---|---|---|---|---|---|
| **Part A: Previous study** | | | | | | | | | |
| France | 2000–2014 | 61,466,098 | −0.984 | −1.03 | −0.93 | 58.0 | 0.9963 | 0.9965 | 0.47 |
| Germany | 1998–2015 | 80,892,654 | −0.987 | −1.04 | −0.94 | 54.5 | 0.9964 | 0.9967 | 0.54 |
| Italy | 2003–2014 | 59,026,383 | −1.007 | −1.07 | −0.94 | 49.3 | 0.9941 | 0.9948 | 0.81 |
| Spain | 1999–2015 | 44,137,863 | −0.960 | −1.02 | −0.90 | 58.3 | 0.9941 | 0.9931 | 0.17 |
| UK | 2001–2015 | 61,569,167 | −1.030 | −1.08 | −0.98 | 60.0 | 0.9963 | 0.9959 | 0.22 |
| **P1** | x | 307,092,165 | −0.997 | −1.05 | −0.95 | 56.6 | 0.9965 | 0.9970 | 0.87 |
| Czech Republic | 1994–2015 | 10,384,837 | −0.918 | −1.01 | −0.83 | 70.2 | 0.9873 | 0.9810 | 0.06 |
| Austria | 2002–2016 | 8,420,447 | −1.006 | −1.07 | −0.94 | 52.0 | 0.9944 | 0.9951 | 0.82 |
| Hungary | 1996–2015 | 10,055,552 | −1.008 | −1.08 | −0.94 | 90.8 | 0.9937 | 0.9945 | 0.79 |
| Poland | 1999–2015 | 38,570,112 | −1.023 | −1.09 | −0.96 | 79.9 | 0.9946 | 0.9948 | 0.41 |
| Slovakia | 1996–2014 | 5,407,663 | −0.951 | −1.03 | −0.87 | 77.6 | 0.9901 | 0.9887 | 0.19 |
| **P2** | x | 72,838,611 | −1.003 | −1.06 | −0.94 | 77.5 | 0.9948 | 0.9955 | 0.92 |
| Sweden | 1997–2015 | 9,215,809 | −0.968 | −1.02 | −0.91 | 46.5 | 0.9953 | 0.9948 | 0.22 |
| Norway | 1996–2015 | 4,708,433 | −0.966 | −1.01 | −0.92 | 54.6 | 0.9967 | 0.9959 | 0.13 |
| Denmark | 1994–2009 | 5,357,073 | −1.014 | −1.06 | −0.97 | 48.1 | 0.9970 | 0.9972 | 0.51 |
| Finland | 1996–2015 | 5,265,968 | −0.960 | −1.03 | −0.89 | 50.9 | 0.9919 | 0.9913 | 0.23 |
| **P3** | x | 24,547,283 | −0.975 | −1.03 | −0.92 | 49.8 | 0.9960 | 0.9959 | 0.30 |
| **P14** | x | 404,478,059 | −0.996 | −1.05 | −0.95 | 60.1 | 0.9962 | 0.9967 | 0.88 |
| **Part B: Present study** | | | | | | | | | |
| **P14** | x | 404,478,059 | −0.996 | −1.05 | −0.95 | 60.1 | 0.9962 | 0.9967 | 0.88 |
| Argentina | 2003–2014* | 40,916,085 | −1.020 | −1.09 | −0.95 | 156.2 | **0.9923** | 0.9928 | 0.55 |
| Brazil | 2006–2015 | 196,482,134 | −1.034 | −1.11 | −0.96 | 165.6 | 0.9933 | 0.9931 | 0.30 |
| Peru | 1999–2015* | 28,032,803 | −0.917 | **−0.98** | −0.85 | 187.4 | 0.9929 | 0.9857 | **0.02** |
| Venezuela | 1996–2013 | 26,147,140 | −0.992 | −1.08 | −0.90 | 224.1 | 0.9885 | 0.9899 | 0.85 |
| Chile | 1997–2015 | 16,109,643 | −1.012 | −1.07 | −0.95 | 111.4 | 0.9947 | 0.9952 | 0.66 |
| Colombia | 1997–2013* | 42,606,241 | −0.971 | −1.03 | −0.91 | 183.5 | 0.9945 | 0.9943 | 0.29 |
| **P4** | x | 350,294,046 | −1.007 | −1.08 | −0.94 | 174.6 | 0.9935 | 0.9943 | 0.80 |
| Mexico | 1998–2012* | 108,139,515 | −1.002 | −1.07 | −0.93 | 192.9 | 0.9930 | 0.9939 | 0.94 |
| Japan | 1995–2015 | 126,809,629 | −0.871 | **−0.92** | −0.82 | 57.1 | 0.9948 | 0.9735 | **0.00** |
| Australia | 1998–2015 | 20,616,599 | −1.003 | −1.07 | −0.94 | 61.4 | 0.9943 | 0.9950 | 0.92 |
| New Zealand | 2000–2013 | 4,094,611 | −0.985 | −1.06 | −0.91 | 79.3 | 0.9911 | 0.9920 | 0.66 |
| USA | 1999–2010* | 291,683,111 | −1.010 | −1.10 | −0.93 | 89.4 | 0.9903 | 0.9914 | 0.78 |
| **P5** | x | 551,343,465 | −0.994 | −1.05 | −0.94 | 102.2 | 0.9959 | 0.9964 | 0.81 |
| **P25** | x | 1,306,115,570 | −1.002 | −1.06 | −0.95 | 111.7 | 0.9961 | 0.9965 | 0.92 |

*Size population is mean of population sizes in specific calendar years. Data was not collected in the whole calendar interval labeled as "*" in the second column; exact calendar years were: in Argentina:2003, 2005–2014, in Peru:1999–2000, 2002–2008, 2011–2015, in Colombia:1997–1998, 2010–2013, in Mexico:1998, 2000, 2001, 2007, 2008, 2010, 2011, 2012 and in USA:1999–2005, 2009–2010. The column labeled as "slope $\gamma$" contains point estimation of the slope in the linear two parametric model in the log-log scale and the next two columns ("Lower" and "Upper") contain the limits of 95% confidence intervals of the parameter $\gamma$. The parameter $\mu_1$ is per 100,000 living per 1 year and was calculated in the two parametrical linear model. $\tilde{R}^2$ is the adjusted coefficient of determination calculated for one predictor and nine points in the linear two parametrical model. $R_b^2$ is the coefficient of determination calculated for the inverse proportion with a single parameter in the log-log scale (it was automatically also the adjusted coefficient of determination) (28). The last column labeled as "c/x" contains p-value of the standard Fisher's test, which determined that the linear model with two parameters does not provide a significantly better fit than the inverse proportion with a single parameter (which may slowly differ from the values of $\mu_1$ calculated in the two parametrical linear model).*

this population and set of diseases was not constructed. For example, no cases in the chapter "Pregnancy, childbirth, and the puerperium" (XV) were found within the age range (0, 10) years in all populations. Thus, this chapter was not relevant to the study.

Sums Di of death numbers from diseases of all main ICD10 chapters, within all populations and within all age categories, and sums Li of living individuals within specific age categories are in the file "25_Countries_Dolejs_Homolkova" Animations are in eight files in "mp4" format and other results are in the file "**Appendix**" The files may be found in the **Supplementary Material**.

The level of statistical significance was set to 0.05 for all tests. The arithmetic mean of the interval endpoints was used as a representative value for each age category. The time unit "1 year" was used in all age categories and in all calculations.

## Chapters of the ICD10

Cause of death may be considered less reliable information than the determination of age. On the other hand, ATM from specific diseases may show the composition of ATTM and may show some other important results (e.g., extraordinary different shape of ATM due to neoplasms) (26, 28).

Two chapters of ICD10 "Diseases of the eye and adnexa" (VII) and "Pregnancy, childbirth, and the puerperium" (XV) were not relevant to the study because only 10 cases were in chapter VII and zero cases were detected in chapter XV in the largest population P25 in the whole age interval (0, 10) years. ATM in all other chapters of the ICD10 and ATM due to congenital anomalies of the central nervous system (CACNS) were constructed. CACNS were the subset of the chapter "Congenital malformations, deformations, and chromosomal abnormalities" (XVII), and ATM due to CACNS was constructed because it showed a higher coefficient of determination up to higher ages in the previous studies (26–28).

Diseases from chapters for which the decrease in mortality was slower during the first year than after the first year were not related to congenital impairment were aggregated to the group labeled "Other diseases." This group contained chapters I–XV, without chapter II (Neoplasms) (28).

## Data Processing and Statistical Methods

Data processing, statistical methods, and model assessment were the same as in the previous studies (24, 27, 28). A simple software was developed using Visual Basic for Applications, and Microsoft (MS) Excel 2019 was used for the calculation of the sum of cases in each chapter and each age category. MS Excel was used at the first level of processing, along with more packages in R 4.0.2 for Windows for statistical calculations (linear and non-linear regression) and preparation of charts. The level of statistical significance was set to 0.05 for all tests. Overview of other possible models and more detailed description of model assessment may be found in previous studies (24, 27, 28).

# RESULTS

## Age Trajectory of Total Mortality

ATTM was more important and had a more general meaning than other ATMs that were constructed in specific groups of diseases. Besides, the determination of the cause of death may be burdened with some uncertainty (for example, it may be expected for cases registered in the 18th chapter of the ICD10: "Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified").

ATTM had reached the minimal value in all 32 studied populations (in 25 countries and 7 aggregated populations) in the age interval 5–10 years with three exceptions (the minimal value was in the next age interval 10–15 years in Slovakia, Peru, and Japan). All ATTMs were statistically evaluated in the age interval 5–10 years in all 32 populations, and the results calculated in the log–log scale are shown in **Table 1**. **Table 1** contains all populations from the previous study (14 countries and aggregated populations P1, P2, P3, and P14) and all populations from the presented study (11 countries and the aggregated populations P4, P5, and P25) (28).

## Inverse Proportion Assessment in the Log–Log Scale

The decrease of ATTM was visually linear in the log–log scale. It is shown in **Figure 1** for P25 and for all populations in the file "All_Populations_All_causes_Animation_1.mp4" presented in the **Supplementary Material**. The linearity in the log–log scale was tested in the quadratic model using the method of least squares (LS). The null hypothesis that the quadratic element was zero was not rejected ($p > 0.05$), while the linear element was significant ($p < 0.0001$) in all populations (the results are not shown in **Table 1**). Consequently, it was assumed that the decrease in total mortality with age was linear in the log–log scale. At the second step, a linear model with two parameters was developed in the log–log scale (27, 28). The parameters were $\ln[\mu_1]$, $\gamma$, where $\gamma$ is the slope of the straight line in the log–log scale. The two parameters, their standard deviations, and the adjusted coefficients of determination $R^2$ were calculated using the LS method for the age interval of 0–10 years. The hypothesis that the residuals were age-independent was not rejected ($p > 0.05$). The results of the linear regression are shown in **Table 1**.

The specific value $-1$ for the slope $\boldsymbol{\gamma}$ corresponded to the inverse proportion between mortality rate and age. Because the slopes $\boldsymbol{\gamma}$ were close to the value $-1$ in the fourth column in **Table 1**, the null hypothesis $H_o$: $\boldsymbol{\gamma} = -1$ was examined to test the inverse proportion at the last step (all formulas were described in detail in the previous study) (28). The inverse proportion with a single parameter was a nested model that includes the two-parameter linear model. The null hypothesis that the model with two parameters did not provide a significantly better fit than the inverse proportion was tested using a standard Fisher's test. The resulting $p$-values are shown in **Table 1** in the last column (the null hypothesis was rejected only in Peru and Japan). Because the slopes are more or less close to $-1$, the inverse proportion between total mortality and age may be the resulting model within the age range of 0–10 years.

## Chapters of the ICD10

Three main results described in the previous study were confirmed for non-European populations from three other continents and/or with higher mortality levels (all files mentioned below may be found in the **Supplementary Material**):

a) ATM due to CACNS decreased according to the inverse proportion up to higher ages. The model of inverse proportion was not rejected in all aggregated populations and in 12 countries in the age range (0, 15) years (it was the maximal age range where ATM due to CACNS was constructed). Besides, mortality minimum was reached in the age category (55, 60) years in the largest population P25 with $R_b^2 = 0.9953$. ATM due to CACNS in P25 is shown in **Figure 2**, and ATM due to CACNS in all 32 populations is shown in the file "All_Populations_CACNS_Animation_2.mp4." More

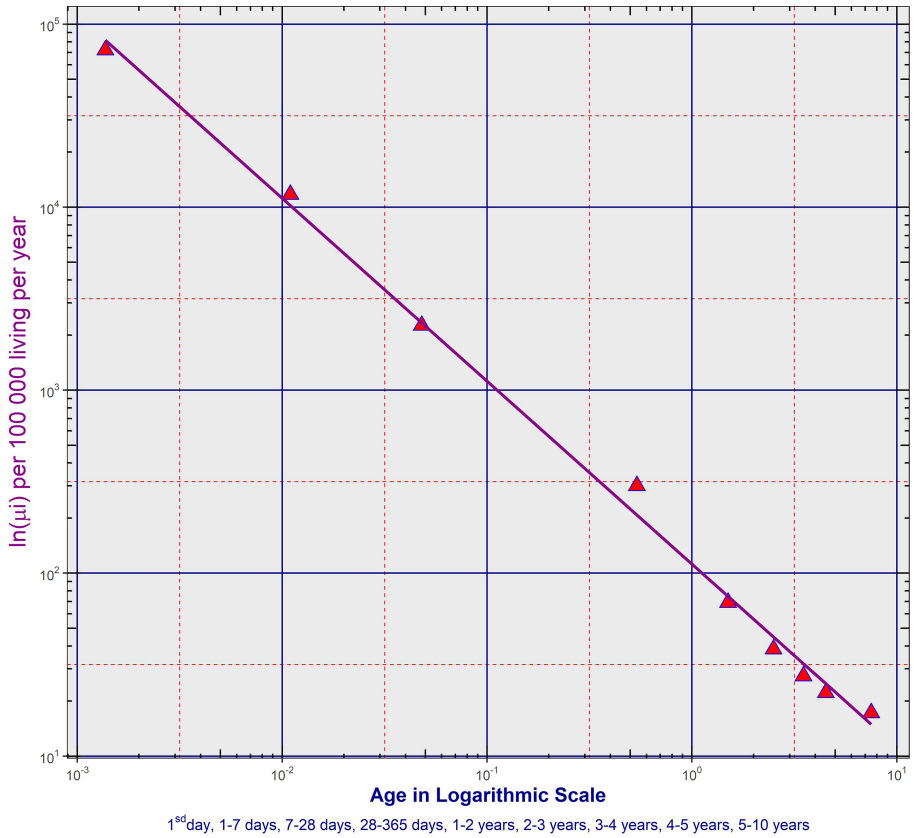


**FIGURE 1 |** Age trajectory of total mortality in P25. (All_Populations_All_causes_Animation_1.mp4).

detailed results calculated in ATM due to CACNS in all populations are in Table 2, and other notes are in the file "**Appendix**."

b) ATMs due to the majority of chapters of ICD10 were age-independent or slowly decreased during the first year, and simultaneously, these ATMs decreased according to the inverse proportion in the age range (1, 10) years. ATM due to the specific category "Other diseases" is shown in **Figure 3** in P25. ATMs due to "Other diseases" in all 32 populations are shown in the file "All_Populations_Other_diseases_Animation_3.mp4,"
ATMs due to the first chapter "Certain infectious and parasitic diseases" of ICD10 are shown in file "All_Populations_Chapter_1_Animation_4.mp4," and ATMs due to all chapters of ICD10 in P25 and P14 are shown in the file "All_Chapters_P25_and_P14_Animation_5.mp4."
TCIR explained child mortality decrease with age based on the sequential extinction of more severe impairments. It also explained the bending ATM using formula (2), which was derived in previous studies (25–28). If the maximal congenital individual risk of death $r_{max}$ in the born population is not big, then Equation (1) is valid:

$$\mu(x) = \frac{\mu_1}{x}.\left[1 - e^{(-r_{max}.x)}\right], \text{and for small } x: \cong \frac{\mu_1}{x}\left[1 - (1 - r_{max}.x)\right] = \mu_1.r_{max} \quad (1)$$

More detailed results calculated in ATM due to all chapters of ICD10 in population P25 are in Table 3 and other notes are in the file "**Appendix**."

c) The shapes of ATM due to neoplasms were very different. It was confirmed that ATMs due to neoplasms were age-independent in all populations after the first year of life (the independence of age was tested in the age range 1–10 years as in other chapters of ICD10 and also in the age range 1 month−15 years. Simultaneously, the ATM due to neoplasms decreased in the first month of life (the model of inverse proportion was rejected only in P1, Argentina and Venezuela where the decrease was slightly slower). ATM due to neoplasms in all populations is shown in the

file "All_Populations_Neoplasms_Animation_6.mp4." More detailed results calculated in ATM due to neoplasms in all populations are in Table 4, and other notes are in the file "**Appendix**."

## DISCUSSION

According to the theory of congenital individual risks of death (TCIR), the mortality decrease was explained as the result of depletion of individuals with more severe congenital impairments (25, 26, 28). The inverse proportion was explained in TCIR as the result of the specific distribution of congenital impairments in the born population. The spectrum of congenital anomalies was very wide because some individuals died during the first hours and others may survive to higher ages. The born population was considered as a whole and the changes of individual congenital risks of death were assumed as age-independent (possible individual changes of individual risk of death were assumed to be negligible with respect to differences in the whole population) (25, 26, 28). The evidence that empirical ATTM decreased according to the inverse proportion was interpreted by the following rule: "as more severe the impairment was than less frequent it was in born population" (25). The rule may be caused by selection in previous generations or by selection in the prenatal period (25, 26, 28).

The empirical decrease of ATMs was very fast in European countries after birth, and the absolute value of the decrease to 10 years was approximately the same as the absolute value of the increase with age in the age range 10–90 years (28). ATMs after birth were studied in the log–log scale where the assumptions of the regression model were satisfied. The model of inverse proportion is a formally linear model with the slope −1 in the log–log scale. The empirical coefficients of determination calculated in the inverse proportion in ATTM were very high (>0.99) (26–28). ATM due to congenital anomalies of the central nervous system (CACNS) decreased according to the model of inverse proportion over the age of 50 years and the coefficients of determination were higher than 0.99 (27, 28). The decrease of ATM with the slope −1 in the log–log scale was also observed in other groups of diseases in the age range 1–10 years while the decrease was slower during the first year of life (these ATMs were labeled "bending ATMs") (26, 28).

The inverse proportion was valid for ATTM in non-European countries with the two exceptions (Peru and Japan). Such high values of coefficient of determination $R_b^2$ in **Table 1** are uncommon, and the inverse proportion between total mortality and age was a deterministic relationship. It implies that, compared to the first day of life, the mortality rate was 10 times lower after 10 days, 100 times lower after 100 days, and 3,650 times lower after 10 years.

Because the adjusted coefficients of determination calculated for ATTM in the two parametric linear model for $R^2$ were high in Japan and Peru (0.9948 in Japan and 0.9929 in Peru), the lower value of $R_b^2$ was due to slopes that differ from the value −1. ATTMs in Peru and Japan were also exceptional with respect to the age category of the minimal mortality value (the minimal value of ATTM was reached in the age category 10–15 years in the two populations). The explanation of the results is difficult without any speculation. One possibility is that the slower decrease was related to the Japanese population, which is known for high life expectancy. The effect was less significant in Peru (with a steeper decrease than in Japan), and simultaneously, the population in Peru contains a subpopulation of Japanese (Japan Peruvians), which constitute ∼1.4% of the population of Peru. The speculation may show that the slower mortality decrease may be exclusive to the Japanese population. Another possibility is that the quality of data collection in Japan and Peru may be another reason for the non-conformance of the inverse proportion model. This may be true for one or both countries (35, 36). On the other hand, the determination of the age of death represents more reliable information. The used age categories were relatively wide and possible uncertainty may be found rather in the determination of the cause of death.

ATMs due to CACNS differed from other diseases because they reached the minimal value in higher ages, and the inverse proportion model was valid with high coefficients of determination (26–28). The age range 0–15 years was used here to compare ATM from CACNS as, in all populations studied, this was the lowest age range in which mortality decreased. All 32 ATMs due to CACNS were available in the age range 0–15 years. Curvature was not rejected in nine countries (Germany, Italy, Austria, Poland, Slovakia, Finland, Australia, New Zealand, and the USA), while it was rejected in all other countries and in all aggregated populations where the linear model was assumed. In the next step, the inverse proportion was tested in the remaining 16 countries and all 7 aggregated populations (in ATMs due to CACNS where the curvature was rejected). The inverse proportion was rejected only in France, Czech Republic, Hungary, and Brazil. Consequently, the inverse proportion was confirmed in 12 countries and in all aggregated populations in the age range (0, 15) years. The coefficients of determination calculated in the model of inverse proportion $R_b^2$ were very high, in general reaching the maximum value of 0.9942 in the largest population P25, and the value of 0.9937 in P14. All results are shown in Table 2 in **Appendix**. The coefficients of determination $R_b^2$ calculated for ATM from CACNS were almost as high as those calculated for total mortality (**Table 1** in the main text). For example, the coefficient of determination was 0.9965, for total mortality within the age range (0, 10) years in P25, while a value of 0.9938 for ATM from CACNS was found in P25 in the same age range. ATM from CACNS decreased up to 60 years in the largest population P25, and the coefficient of determination $R_b^2$ reached 0.9953 in the age range (0, 60) years in P25. The ATM is shown in **Figure 2** and all other ATMs due to CACNS are shown in the file "All_Populations_CACNS_Animation_2.mp4" in the **Supplementary Material**.

Results observed in ATM due to specific diseases also support TCIR. If TCIR is correct, a significant part of the deaths up to 10 years was, in fact, caused by congenital impairment, even in the cases registered in the category "Other diseases." Figure 4 in the file "**Appendix**" illustrates age changes of proportions of deaths from diseases categorized in specific chapters of ICD10. Because

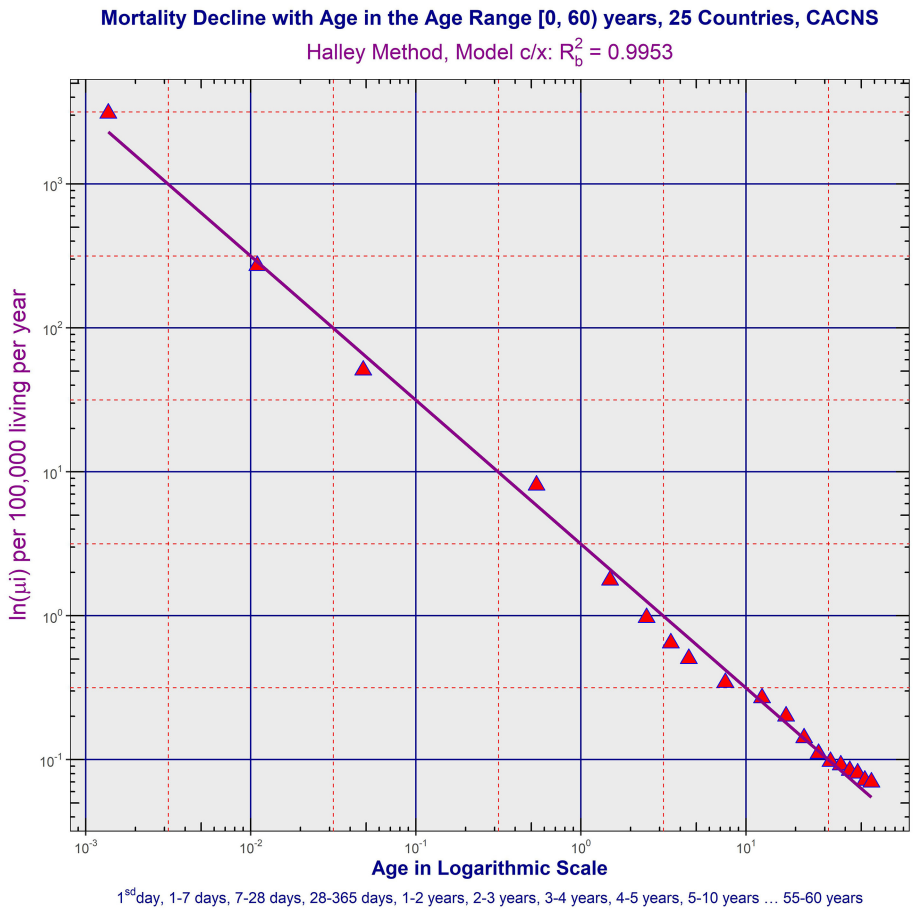


**FIGURE 2 |** Age trajectory of CACNS in P25. (All_Populations_CACNS_Animation_2.mp4).

ATM due to the majority of chapters decreased according to the inverse proportion after the first year, the deaths may have been caused by latent congenital impairment. For example, 23% of the deaths until the age of 10 years were due to conditions grouped in the category "Other diseases" in P25. Simultaneously, ATM due to "Other diseases" was bent according to Equation (2).

ATM due to neoplasms differed from other diseases. The evidence that ATMs due to neoplasms decreased in the first month of life and were age-independent within the age range (1 month, 15 years) may be important for the verification of any hypothesis that a specific type of neoplasm is only significant to a subpopulation with congenital predisposition, for example, a genetically susceptible subpopulation.

## Quality of WHO Data and Death Classification

The determination of cause of death according to ICD may be related to some dissimilarity in different countries (35–40). For example, mortality data from 27 countries of the WHO European region were analyzed for coronary heart disease. Comparisons of mortality rates from coronary heart disease as reported in national mortality registries between countries and over time were highly compromised (35). The studies also described the regional differences in death classification (35–40). It may be partially followed in the animations "All_Populations_Chapter_1_Animation_4.mp4," "All_Populations_Certain_conditions_in_perinatal_period_Animation_7.mp4," and "All_Populations_CA_Animation_8.mp4," which were presented in the **Supplementary Material**. Besides, bending ATMs were explained in TCIR by the fact that congenital defects were not observed in some cases, and these cases were not categorized as congenital anomalies. Two categories, CACNS and "Diseases of the central nervous system," and ATM calculated in the categories represented a typical illustration of the effect, and the two ATMs may be found in the animation "All_Chapters_P25_and_P14_Animation_5.mp4" constructed in two populations P14 and P25. The regional differences in death classification may also be followed in the aggregated group of diseases "Other diseases." It was created here for death cases that were not related to congenital impairment, malignant neoplasms, and accidents. The mortality level and shape of ATM due to "Other diseases" are shown in the animation "All_Populations_Other_diseases_Animation_3.mp4."

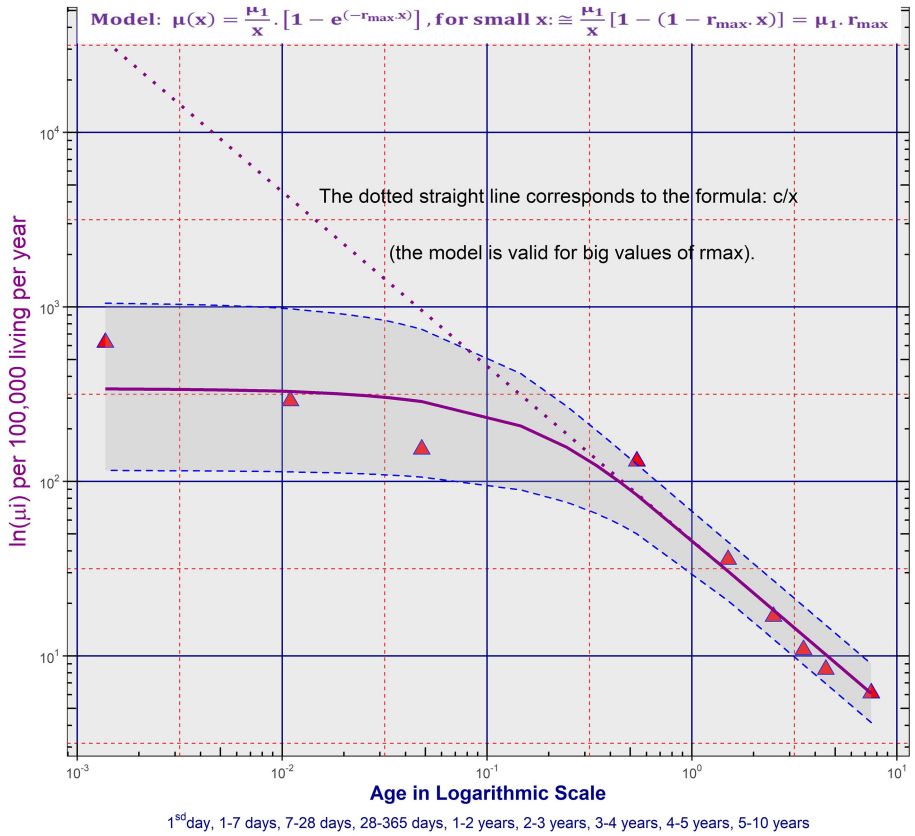


**FIGURE 3 |** Age trajectory of "Other diseases" in P25. (All_Populations_Other_diseases_Animation_3.mp4).

## Consequences in Clinical Practice

According to TCIR and ATM constructed in 25 studied populations, the majority of deaths up to the age of 10 years may be related to congenital impairment, and the decrease in child mortality rate with age was a demonstration of population heterogeneity. The explanation of the mortality decrease with age leads to the existence of latent congenital defects. Such latent congenital defects may cause death even if no congenital impairment was observed and the cases were aggregated here in the group "Other diseases." For example, death cases put in the first chapter of ICD10 "Certain infectious and parasitic diseases" may be associated with a congenital defect. Namely, if influenza was determined as a cause of death, then latent congenital impairment may be the dominant cause of death. Some other epidemiological studies also support the idea that a significantly higher proportion of individuals with an inherited predisposition are among these cases (41). These findings showed that there were two basic children's patients in clinical practice. The bigger group of patients was without congenital impairment and the smaller group was affected by congenital impairment. The heterogeneity in death cases was different (42, 43). The majority of death cases may be related to congenital impairment, which was latent. In other words, the group of patients with congenital impairment was relatively small in the whole population, but it may be dominant among dead up to the age of 10 years. More details of these findings may be found in the **Appendix**.

## CONCLUSION

ATTM and also ATM due to specific groups of diseases were very similar in 11 non-European countries and in 14 European countries. The level of mortality did not affect the main results found in 14 European countries.

Child mortality decrease with age may be explained as the result of the depletion of individuals with congenital impairment. The majority of deaths up to the age of 10 years were related to congenital impairments and the decrease in child mortality rate with age was a demonstration of population heterogeneity. The congenital impairments were

latent and may cause death even if no congenital impairment was detected.

All results are based on published data, and the data are presented as a supplement in the file **Table 1**. It contains the numbers of living people in each age category, and each population is shown in **Table 1**. Besides, it contains the numbers of deaths in each age category, in each ICD10 chapter, and in each population aggregated in a calendar period. Consequently, more epidemiological findings may be done using simple calculations and the data.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

JD realized statistical computations and composed the main text. HH checked all parts related to pediatrics and clinical interpretations. Both authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

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# A Prevalence Estimation of Exstrophy and Epispadias in Germany From Public Health Insurance Data

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**Introduction:** The prevalence of rare diseases is very important for health care research. According to the European Surveillance of Congenital Anomalies (EUROCAT) registers, the live prevalence for exstrophy and/or epispadias (grades 1–3) is reported with 1:23,255 (95% CI: 1:26,316; 1:20,000). A Europe-wide prevalence evaluation based on reports from excellence centers estimates a prevalence for exstrophies of 1:32,200 and for isolated epispadias of 1:96,800 in 2010. However, the frequency of exstrophy [International Statistical Classification of Diseases and Related Health Problems revision 10 (ICD-10): Q64.1] and epispadias (ICD-10: Q64.0) treated in different age groups in Germany remains unclear.

**Material and Method:** Public health insurance data from 71 million people (approximately 87% of the population) were provided by the German Institute for Medical Documentation and Information (DIMDI) in accordance to the German Social Insurance Code for this research purpose. DIMDI analyzed the data source for the ICD diagnoses exstrophy and epispadias between 2009 and 2011. As provided data were robust over the years, averaged data are mentioned. Detailed subgroup analysis of small numbers was forbidden due to privacy protection.

**Results:** Annually, 126 persons of all ages with epispadias and 244 with exstrophy are treated as inpatients. In the observed population, 34 infants (<1 year of age) with epispadias and 19 with exstrophy (58% male) are treated as outpatients each year. This corresponds to an estimated live prevalence of 1:11,000 (95% CI: 1:14,700; 1:8,400) for EEC (exstrophy–epispadias complex), more specifically a prevalence of 1:17,142 for epispadias and of 1:30,675 for exstrophy. The male-to-female ratio for exstrophy is 1.4:1 for infants and 1.6:1 for all minors. In children and adolescents, 349 epispadias and 393 exstrophies (up to the age of 17) are treated annually, whereas adults with exstrophy and even more with epispadias make comparatively less use of medical care.

**Conclusion:** With the help of DIMDI data, the live prevalence of bladder exstrophy and epispadias in Germany could be estimated. The prevalence of epispadias was higher than in previous reports, in which milder epispadias phenotypes (grade 1 or 2) may not have been included. These analyses might enlighten knowledge about nationwide incidence and treatment numbers of rare diseases such as the EEC.

**Keywords: prevalence, epispadias, bladder exstrophy epispadias complex (EEC), health insurance, public health, bladder exstrophy**

## INTRODUCTION

The bladder exstrophy–epispadias complex (EEC) is one of the most serious congenital midline defects in humans. Today, the EEC is understood as a spectrum from an isolated genito-urethral defect affecting males and females (epispadias), to classical bladder exstrophy with an open bladder and pelvis in addition to the genital defect culminating in a multiorgan anomaly—the cloacal exstrophy. As it is shown in various recent long-term outcome studies, comparatively little substantial improvements in functional outcomes were achieved (1, 2). Although a rare disease, EEC treatment in Germany is decentralized with possible profound long-term sequelae such as unfavorable outcome related to small hospital treatment numbers (3, 4). A central aspect here remains that robust data regarding the incidence and live prevalence of EEC are still lacking. Although in 2009 a network for congenital uro-rectal malformations (CURE-Net) was established in Germany and a nationwide and centralized data collection about newborns with EEC was formed, obtained data remain incomplete as reporting was voluntary. The only EEC prevalence study in Germany estimated live prevalence from EEC reported cases through excellence centers (5). Still, prenatal terminated pregnancy rates of EEC affected individuals are not easily detectable. There exist some estimates from European Surveillance of Congenital Anomalies (EUROCAT) registries (6, 7). The incidence of individual malformation phenotypes may vary to an unknown extent across the spectrum of EEC (8). Accordingly, the number of children born in Germany per year or living individuals with EEC remains unclear.

As a consequence, scientific conclusions of clinical research remain limited in respect to their validity. Furthermore, the unmet need for more intensified and specific professional care for individuals with EEC of all ages cannot be structured, and patient-centered health system planning cannot be established. Incidence and prevalence data might put officials in the position, like in other rare diseases and other European countries, to bundle both professional and economic resources and thus might substantially improve the health care situation of people with EEC of all ages in Germany.

As an initial approach, free accessible codes of the operation and procedure classification system (OPS) from German general hospital sources were analyzed. However, in contrast to anorectal malformation (9), EEC relevant procedure codes were not unique. Recently, a legally guaranteed access to public insurance data in Germany was made available for scientific purposes. This covers the vast majority of the population; only high incomes have private insurances. Due to the great lack of reliable knowledge of the scientific community about the true numbers of EEC cases in Germany, the CURE-Net scientific consortium established this request for an anonymized analysis of unique data of EEC patients with public health insurance for the first three available years 2009, 2010, and 2011. The German Institute for Medical Documentation and Information (DIMDI) was assigned to a data source investigation to analyze the individual EEC patients according to the exact ICD-10 (International Statistical Classification of Diseases and Related Health Problems revision 10) diagnosis Q64.1 (bladder exstrophy) and Q64.0 (epispadias) from the years 2009 to 2011.

The purpose of this study is to evaluate the live prevalence of the EEC in Germany, to assess the male-to-female ratio, and to consider the treatment incidence of various age groups with the help of the German insurance documentation, including a representative nationwide population. Furthermore, the results of this methodology will be compared with further prevalence estimations.

## MATERIALS AND METHODS

In the German private and public health insurance system, each patient served is categorized according to ICD-10 diagnoses, such as exstrophy (Q64.1), epispadias (Q64.0), cloaca (Q43.7), and possibly including cloaca exstrophy. The diagnoses serve for financial settlement for ambulatory and inpatients bills with a plausibility check. Although it is not specified within the ICD code clinically, epispadias can be differentiated according to the meatus location in glandular (grade 1), penile (grade 2), or penopubic (grade 3) (10). Similarly, as there is no specific ICD code for cloaca exstrophy, ICD code Q43.7 includes cloacal malformations as well; however, data for cloaca codes were also requested in spite of limited tolerable conclusions.

These health care data are provided by the DIMDI institute as part of the Federal Ministry of Health (BMG) and operates the "Information System Health Care Data" on its behalf in the course of the Data Transparency Ordinance. The request of data evaluation via a direct access is strongly limited to authorized institutions, such as universities, for research purposes and was allowed from 2009 onwards. Data of the public health insurances cover approximately 87% of the German population which is rated here to median 71,014,351 people (11). The request was written with a pre-specified Structured Query Language (SQL) script to be analyzed and prepared after application against

payment by the "Data Preparation Office" at DIMDI. DIMDI provides an analysis of the so-called "DaTraV data" according to §303d of the Social security code five (SGB) V. As only anonymized data such as numbers of diagnoses of ambulatory and stationary patients a year were retrospectively made available from the DIMDI institute to the authors, no ethical approval is needed according to German law.

The data sets for three years (2009–2011) were provided and divided into three age strata for outpatient data and without age strata for inpatients. Additionally, specific case numbers of three requested ICD-10 codes related to EEC such as Q64.0, Q64.1, and Q43.7 were allocated. There is a statement from DIMDI that in their reporting each case is only counted once, meaning that no double cases are included. Due to data protection reasons, some detailed analyses were not allowed in subgroups of diagnosis, sex, and age group because of extremely small case numbers (<5). Due to the expected patient numbers in the case of rare diseases, the standard limit of 30 cases per group had already been reduced. Nevertheless, we could not obtain all favored sex and age strata. We received limited strata for three age levels: below 1 year of age, 1–17 years, and adults 18 years onwards. The data of approximately 71 million German insurance cases ($n = 71{,}014{,}351$) each year were analyzed over three consecutive years, corresponding to approximately 87% of the complete German population. This rate is calculated from the reported denominator data below 1 year and the published birth rate of the corresponding years: 582,832/668,586 of average births 2009–2011. The denominator in the estimation of live prevalence are nationwide public available newborns at the Federal Statistical Office (11). The first number is the average reported number of insurance cases below 1 year of age for the years 2009, 2010, and 2011. These numerators are used as the denominator for EEC rates as shown in all tables.

## RESULTS

The inpatient data showed that each year generally 370 inpatients with public insurance were treated with bladder exstrophy or any epispadias in Germany from 2009 to 2011 (**Table 1**). According to their phenotype an average of 126 persons with public insurance had epispadias (range 101–144), and 244 had bladder exstrophy (range 216–260) each year. Since 87% of the population has statuary insurance, we estimate that approximately a total of 425 patients were treated each year. Due to very low case numbers and therefore data security reasons, differentiation between gender and age groups was not possible. The very rare cloaca malformation (Q43.7), which may include cases of cloacal exstrophy, was reported on average with 64 individuals per year (range 55–80) in an astonishingly high number in comparison to the bladder exstrophy cases.

Outpatient data of adults (individuals 18 years onwards) indicated that 362 adults (range 351–371) with epispadias and 743 adults (range 736–747) with exstrophy were treated in the ambulatory setting. The male-to-female ratio was available only for exstrophy, showing a quite stable distribution with a male predominance of 1.322 (range 1.247–1.394) (**Table 2**) in adult treatment.

In the group of children and adolescents from 1 to 17 years, 315 (range 313–318) epispadias and 374 (range 367–379) exstrophies are treated annually in outpatient care, i.e., an average of 18.5 epispadias and 22 exstrophies per year (together 1:16,097). The male-to-female ratio for exstrophy is 1.597 for all minors with a range between the years from 1.561 to 1.681 (**Table 3**).

In the group below 1 year of age, each year on average 34 infants with epispadias (i.e., 39 Germany-wide; 1:17,142) and 19 with exstrophy (11 of them male; 58%; i.e., 22 Germany-wide; 1:30,675) are treated at least once as outpatients (**Table 4**). These data likely represent the most valid prevalence estimation considering the need for surgery during this period. Among the infant group, exstrophy was noted in one out of every 27,166 males and one out of every 35,501 females. This corresponds to an estimated live prevalence for the complete EEC of 1:10,997 (95% CI: 1:14,700; 1:8,400).

Comparing the patient numbers in the three age groups with the Cochrane-Armitage-trend-test, it becomes apparent that the attending incidence in adolescence and even more in adulthood is significantly lower for epispadias ($p < 0.001$) and exstrophy ($p < 0.001$).

## DISCUSSION

There is no doubt that epidemiological and clinical research in rare diseases need basic incidence and prevalence data. Due to the lack of a mandatory reporting of congenital anomalies to a nationwide birth registry in many European countries including Germany, only estimations from EUROCAT or German birth registries, the National American Insurance database, or published European epidemiological studies are available for research purposes (5, 10, 12–15). The German EUROCAT-Registry in Saxony-Anhalt which covers approximately 2% of the German population reports for the last decade an incidence for epispadias 1:34,500 (95% CI: 1:15,900; 1:100,000) and for exstrophy 1:29,400 (95% CI: 1:14,500;1:76,900) (6). These results can only be considered as an incomplete and insufficient random sample. The prevalence for EEC reported in Germany was estimated with 6.7 per 100,000 live births (1:14,900). The population-based registries ($n = 76$ members) in EUROCAT are active and regularly contacting birth clinics throughout Europe to transmit data on congenital anomaly cases in their region in a standardized way. EUROCAT indicated the prevalence of bladder exstrophy and epispadias with 4.3 per 100,000 (1:23,300) live births in their last published report in 2017 (7). In all European registries live incidences were between 1:33,300 in 2015 and 1:16,700 in 2016 (cf. **Figure 1**). The international clearinghouse for birth defects monitoring systems provided an average prevalence rate of 3.3 per 100,000 (1:30,300) for bladder exstrophy and 2.4 per 100,000 (1:41,700) for isolated epispadias (12). A National American Insurance database study (15) showed a significant increase for epispadias from 8.0/100,000 to 11.6/100,000 and a decrease in the birth prevalence of bladder exstrophy from 2.4/100,000 to 1.6/100,000 during the years

**TABLE 1 |** Inpatients with EEC, and due to small cell numbers no age and sex stratification.

| Type of malformation (ICD-10 code) | Basic population (0–99 years) of insurance sample (denominator) | Epispadias (Q64.0)[a] | Bladder Exstrophy (Q64.1)[a] | Cloaca (Q43.7) (which may include cloaca exstrophy)[a] |
|---|---|---|---|---|
| Year 2009 | 71,059,552 | 133 | 260 | 55 |
| Year 2010 | 70,958,660 | 144 | 257 | 57 |
| Year 2011 | 71,024,842 | 101 | 216 | 80 |
| Average in years 2009–2011 | 71,014,351 | 126 each year | 244 each year | 64 each year |

[a]*Due to N < 5 in stratified cells, no sex or age data are given.*

**TABLE 2 |** Outpatient data of adult (18 years onwards) EEC patients.

| Type of malformation (ICD-10 code) | Year 2009 | Year 2010 | Year 2011 | Average in years 2009–2011 |
|---|---|---|---|---|
| Basic adult (>17 years) of insurance sample (denominator) | T 59,258,500 = M 27,465,227 + F 31,793,273 Ratio: 0.864[a] | T 59,296,294 = M 27,508,664 + F 31,787,630 Ratio: 0.865[a] | T 59,467,858 = M 27,633,780 + F 31,834,078 Ratio: 0.868[a] | T 59,340,884 = M 27,535,890 + F 31,804,994 Ratio: 0.866[a] |
| Epispadias[b] (Q64.0) | T 351 P 1:168,828 | T 363 P 1:163,351 | T 371 P 1:160,291 | T 362 P 1:163,925 |
| Exstrophy (Q64.1) | T 747 = M 435 + F 312 Ratio: 1.394 P 1:79,329[a] | T 746 = M 414 + F 332 Ratio: 1.247 P 1:79,486[a] | T 736 = M 420 + F 316 Ratio: 1.329 P 1:80,799[a] | T 743 = M 423 + F 320 Ratio: 1.322 P 1:79,867[a] |
| Cloaca (Q43.7)[c] | T 108 P 1:657,959 | T 102 P 1:695,673 | T 126 P 1:563,689 | T 112 P 1:634,057 |

*T, total, i.e., sum of female and male cases; F, female; M, male; Ratio, male-to-female ratio; P, live prevalence.*
[a]*Probably biased due to death rate, coding, or new detection.*
[b]*No sex strata.*
[c]*Cells below 5, hence no presentation for any age strata or sex.*

from 1997 till 2009 (15). This decrease may have occurred as a result of an increase in prenatal pregnancy termination, which was noted in at least 25% after prenatally diagnosed bladder exstrophy cases in the EUROCAT registry (7, 16). However, antenatal diagnosis was made in 3% of male epispadias, 16.4% in bladder exstrophy, and 28% of cloacal exstrophy according to malformation severity (5). From German CURE-Net data we know that only 8.8% of all available participants with classical exstrophies were prenatally diagnosed.

With another method, a prospective survey from the European Society of Pediatric Urology calculated EEC incidence by national reporting through expert centers (5). EEC incidence was calculated for the whole EEC with 1 in 30,000, for male epispadias 1:101,000 live births, 1:1,300,000 for female epispadias, and for isolated bladder exstrophy 1:46,000 (5). The incidence in Germany alone for the year 2010 was rated to be each 1:32,200 for exstrophy ($n = 21$), 1:96,800 for male epispadias ($n = 7$), and 1:677,900 for female epispadias ($n = 1$) (5).

The most valid live prevalence data are listed in **Table 4**. These data are most valid since all EEC newborns were promptly examined and generally received immediate surgical treatment. According to the presented DIMDI data (2009–2011) the live prevalence for exstrophy was 1:30,675 (in males 1:27,166 and in females 1:35,501). This would mean that 22 exstrophy individuals are born each year in Germany. In 2010 this would have been nearly concordant with the 21 reported exstrophy cases from the Cervellione et al. study (5). Although prenatal exstrophy diagnosis seems to be more often reported than previously, exstrophy prevalence from DIMDI data remained quite stable over the reported years, indirectly suggesting against a significant increase in the rate of prenatal termination for this diagnosis. However, there is only indirect evidence for this statement.

For epispadias, DIMDI prevalence was calculated to be 1:17,142, much higher than previously reported (5). This number corresponds to 39 epispadias of all types each year for the complete German newborn population. The German EUROCAT registry (6) reported an incidence of 1:34,500, with a large confidence interval. In the independent patient registry CURE-Net only a minority of participants had epispadias (14% in both newborns and in the older age cases) compared to exstrophy (8). The majority had the most severe epispadias grade 3 (80% of the newborn and 78% of older age epispadias cases), whereas only a minority of patients had epispadias grade 1 (10% of newborn/13% of older epispadias patients) and epispadias grade 2 (10% of newborn/9% of older age epispadias patients) (8). This report is at odds with the dominant observation that the less severe phenotypes occur most frequently. This is most likely a result of many cases of isolated epispadias, especially grades 1 and 2, being treated in smaller hospitals and so not being reported or included in the previously reported data from excellence centers (5). Additionally, while severe epispadias is associated with risk for incontinence, in up to 75% (10), lower grade epispadias

**TABLE 3 |** Outpatient data of children and adolescents 1–17 years of age with EEC.

| Type of malformation (ICD-10 code) | Year 2009 | Year 2010 | Year 2011 | Average in years 2009–2011 |
|---|---|---|---|---|
| Basic population (1–17 years) of insurance sample (denominator) | T 11,226,731 = M 5,757,645 + F 5,469,086<br>Ratio: 1.053 | T 11,070,155 = M 5,678,681 + F 5,391,474<br>Ratio: 1.053 | T 10,975,021 = M 5,630,061 + F 5,344,960<br>Ratio: 1.053 | T 11,090,636 = M 5,688,796 + F 5,401,840<br>Ratio: 1.053 |
| Epispadias[a] (Q64.0) | T 313<br>P 1:35,868 | T 314<br>P 1:35,255 | T 318<br>P 1:34,513 | T 315<br>P 1:35,208 |
| Exstrophy (Q64.1) | T 378 = M 237 + F 141<br>Ratio: 1.681<br>P 1:29,700 | T 379 = M 231 + F 148<br>Ratio: 1.561<br>P 1:29,209 | T 365 = M 223 + F 142<br>Ratio: 1.570<br>P 1:30,069 | T 374 = M 230 + F 144<br>Ratio: 1.597<br>P 1:29,654 |
| Cloaca (Q43.7)[b] | T 108<br>P 1:657,959 | T 102<br>P 1:695,673 | T 126<br>P 1:563,689 | T 112<br>P 1:634,057 |

*T, total, i.e., sum of female and male cases; F, female; M, male; Ratio, male-to-female ratio; P, live prevalence.*
[a]*No sex strata.*
[b]*Cells below 5, hence no presentation for any age strata or sex.*

**TABLE 4 |** Outpatients below 1 year of age with EEC malformation for live prevalence estimation.

| Type of malformation (ICD-10 code) | Year 2009 | Year 2010 | Year 2011 | Average in years 2009–2011 |
|---|---|---|---|---|
| Total birth rate in Germany | T 665,126 = M 341,249 + F 323,877<br>Ratio: 1,054 | T 677,947 = M 347,237 + F 330,710<br>Ratio: 1,050 | T 662,685 = M 339,899 + F 322,786<br>Ratio: 1,053 | T 668,586 = M 342,795 + F 325,791<br>Ratio: 1,052 |
| Birth rate (<1 year) of insurance sample (denominator) i.e., ∼87.2% | T 574,321 = M 294,727 + F 279,594<br>Ratio: 1.054 | T 592,211 = M 303,306 + F 288,905<br>Ratio: 1.050 | T 581,963 = M 298,430 + F 283,533<br>Ratio: 1.053 | T 582,832 = M 298,821 + F 284,011<br>Ratio: 1.052 |
| Epispadias[a] (Q64.0) | T 35<br>P 1:16,409 | T 37<br>P 1:16,006 | T 30<br>P 1:19,399 | T 34<br>P 1:17,142 |
| Exstrophy (Q64.1) | T 22 = M 11 + F 11<br>Ratio: 1.0<br>P 1:26,106 | T 19 = M 11 + F 8<br>Ratio: 1.4<br>P 1:31,169 | T 15 = M 10 + F 5<br>Ratio: 2.0<br>P 1:38,798 | T 19 = M 11 + F 8<br>Ratio: 1.4<br>P 1:30,675 |
| Cloaca (Q43.7)[b] | T 108<br>P 1:657,959 | T 102<br>P 1:695,673 | T 126<br>P 1:563,689 | T 112<br>P 1:634,057 |

*T, total, i.e., sum of female and male cases; F, female; M, male; Ratio, male-to-female ratio; P, live prevalence.*
[a]*No sex strata.*
[b]*Cells below 5, hence no presentation for any age strata or sex.*

patients are much more likely to be continent. Because of this they do not seek medical care as often and so may not be included and reported in the data from major centers (5) or even specific registries.

The lower male-to-female ratio in adults is likely caused by male-to-female ratio changes in the general population due to higher overall survival of females. Variable male-to-female ratios were previously reported (5, 10, 12–15). Epidemiological studies reported a male-to-female ratio of 2.2:1 for epispadias and a 1.8:1 male predominance for classical bladder exstrophy (13) as well as of 1.4:1 for epispadias and 2.8:1 for exstrophy (14). In CURE-Net, the majority of participants were male (68%), with a male-to-female ratio of 2.2:1 and 2.1:1 in the newborn and older age patient groups, respectively, (8). Taken together, a male predominance in EEC is consistently reported, even in the DIMDI data over several age groups. The incidence of female epispadias, in contrast, is probably underestimated because the condition is seldom diagnosed at birth.

Compared to infants (5.8 per 100,000), older epispadias patients seem to make significantly less use of medical care during childhood. Therefore, their prevalence in the insurance data appears to be decreasing. In the age group from 1 to 17 years, approximately only 18.5 epispadias patients (2.8 per 100,000) were treated per year, whereas about 22 exstrophy patients (3.4 per 100,000) were visited per year (**Tables 3**, **4**). For exstrophy, the prevalence and use of medical care does not significantly change between infants and adolescents. However, in adulthood, even less frequent outpatient treatment per year for epispadias (0.6 per 100,000) and for exstrophy (1.3 per 100,000) is apparent. This is probably due to less medical care in this age group, but it could also generate an impression of a lower live prevalence in older age or lower incidence in former years, as it was already reported for epispadias (15). However, the true reasons for this finding remain unclear.

For cloaca (Q43.7) only a global live prevalence (1:634,057) could be calculated, which is likely biased due to lack of coding in older ages. This could be one reason for the

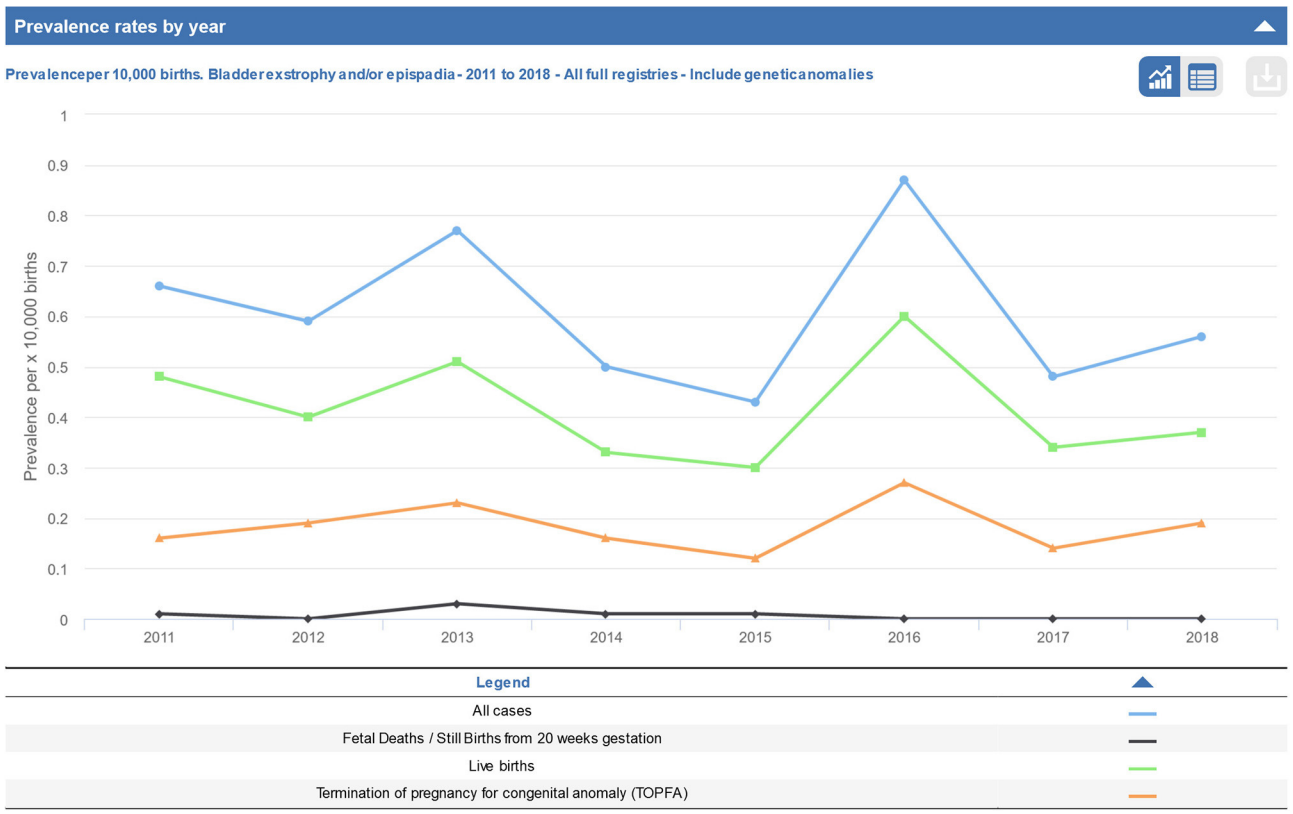


**FIGURE 1 |** Prevalence rate per year across all registries of bladder exstrophy and/or epispadias from 2011 to 2018 (taken from EUROCAT report).

low frequency ($n < 5$), which do not permit further age stratification. Another reason may be low numbers in the smallest age group of infant babies below 1 year of age. If the case number for this smallest group is reduced, the prevalence would be not more than 1:116,566, both far below the otherwise reported prevalence of 1:50,000 (17). This further supports the need for representative reporting among infants.

As already stated, insurance data only provide evidence about live prevalence of the anomaly. Furthermore, limitations of any data collection may have an unpredictable encryption error. Even assuming that all male exstrophy patients below 1 year of age with both diagnosis (Q64.0 and Q64.1) were simultaneously reported, the live incidence for epispadias would still be over the previously reported level: (34 – 11 = 23; hence, at least 1:25,341 or 3.9 per 100,000, if not 5.8 epispadias per 100,000). The reported ICD code Q43.7 includes cloacal exstrophies and other cloacal anomalies to an unknown extent with no possibility to discriminate these entities exactly by this insurance data analysis. From systematic scientific point of view complete insurance data were reported here. Furthermore, DIMDI ensured that each case was encoded once. However, as data seem to be quite consistent over the years, such an error can most probably be excluded. In addition, especially for inpatient treatment, quality assurance by the medical service of the health insurance companies is usually monitored with plausibility check. Possible incorrect or indecisive data are inquired and sent back to the hospitals for further discussion or correction if the accounting could not be proven and was incorrect. Whether this includes internal medical treatment or operations cannot be said. Furthermore, no information is given from DIMDI analysis whether complications with these diagnoses or the diagnosis itself warranted the inpatient hospital stay. Further medical analysis about associated diagnoses or procedures, however, would definitively allow further conclusions. One major benefit of these insurance data is (a) that they have been validated by the insurances to protect from incorrect billing and (b) that they clearly refer to specific individuals even for several visits during 1 year, and hence double counting is prevented. Further, (c) they are quite complete and thus representative for about 71 million people with public health insurances representing about 87% of the German population. Although the data from private insurances were not accessible, no systemic error can be expected in this limited selection, due to the nature of the anomaly. However, as this is a specific German analysis, we believe that this data retrieval methodology may be applied for further research purposes in Germany. Additionally, it could be adjusted to other countries with comparable nationwide and valid insurance data.

Taken together, analyzing DIMDI data sources allows a more precise prevalence estimation of rare diseases such as those within the spectrum of EEC. Furthermore, it provides valuable information about both inpatient and outpatient treatment needs among German patients of all age groups. Detailed additional data including operation procedures, complications, or comorbidities would improve knowledge about the medical needs of individuals with complex and rare anomalies.

## CONCLUSION

Although any data collection has limitations, these DIMDI insurance data for Germany seem to be quite reliable and complete over the consecutive years 2009–2011. While comparable live prevalence of exstrophy has been previously reported by a different methodological approach, our calculations yielded a higher lifetime prevalence of epispadias at 1:17,142. It is likely that milder epispadias (grade 1 or 2) are insufficiently recorded in the currently available data sources such as European survey, CURE-Net and EUROCAT Registry Malformation Monitoring Saxony-Anhalt, or the personal experience of self-help groups.

For the first time, national coverage data for different age groups were analyzed. Adult or adolescent epispadias and exstrophy patients are less likely to seek medical care. In conclusion, this report offers a first impression of the potential analyses possible using the legally guaranteed access to insurance data in Germany.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

EJ and A-KE had the idea and wrote the manuscript. EJ performed all calculations. NZ and A-KE wrote the proposal to the DIMDI. EJ and HR initiated Network for Systematic Investigation of the Molecular Causes, Clinical Implications and Psychosocial Outcome of Congenital Uro-Rectal Malformations (CURE-Net). All authors read and approved the final manuscript.

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# The Voice of Parents of Children With a Congenital Anomaly – A EUROlinkCAT Study

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EUROlinkCAT aims to investigate the health and educational outcomes of children with congenital anomalies for the first 10 years of their lives. We also aim to facilitate the development of a more reciprocal relationship between families with children with congenital anomalies, health and social care professionals, and researchers by conducting focus groups. The aim of the focus groups and parent interviews was to investigate parental experiences of having a child with a heart defect requiring surgery, cleft lip, spina bifida or Down Syndrome and to identify their research priorities. In total, seven interviews with 12 parents and eight focus groups with 58 parents and two caregivers were conducted in four European countries. We found that parents request more positive information with a focus on quality of life and what the children can achieve rather than solely on the negative aspects and limitations of the congenital anomaly. Some parents also highlighted discrepancies between the family's need for support and the lack of support received from the local authority. Finally, it was challenging for the parents to address specific research priorities. Future research should therefore focus on the potential of a child with a congenital anomaly.

**Keywords: caregiver, congenital anomalies, child, family, communication**

## INTRODUCTION

Congenital anomalies are a major contributor to infant and childhood morbidity and mortality (1–3). Major congenital anomalies affect one in 50 live births (4) with congenital heart defects being the most frequent (4). Survival of children with congenital anomalies has improved (5), so up-to-date knowledge about childhood morbidity is required in order to counsel parents both after a prenatal or postnatal diagnosis. With increasing survival, more families are caring for infants and children with congenital anomalies and face many everyday challenges (6–8). EUROlinkCAT is a 5 year research project (9) with the aim to establish a linked European cohort of children with congenital anomalies partnering with EUROCAT (European Surveillance of Congenital Anomalies in Europe) registries (10). EUROlinkCAT aims to expand the knowledge on the survival, health and education of children in Europe born with major congenital anomalies and to investigate health inequalities. Previous studies of parental involvement in health research have identified issues that

clinical researchers may not be aware of (11) and EUROlinkCAT acknowledges that parents have extensive knowledge on their child's symptoms, the health care system and challenges occurring in everyday life.

To incorporate parental needs and expectations into the healthcare system and define research priorities their involvement is imperative. Little is known about parental involvement in health research, despite the increasing interest of doing so (11). The part of the EUROlinkCAT project reported here aims to investigate parent's experiences, needs and wishes both for improvement of the collaboration between parents and the healthcare system and for future research. Thus, we want to take parent involvement to a new level by inviting them to participate in a large research project across EU countries.

The EUROlinkCAT project aims to close the gap between research and clinical treatment, and to ensure evidence-based practice where parent's knowledge and perspectives are included. This study provides new knowledge to be used to leverage changes in clinical practice incorporating parents' needs and help researchers follow parental priorities for conducting future research studies.

The aim of the study was to investigate parental experiences of having a child with one of four congenital anomalies: Down syndrome, spina bifida, cleft lip or severe congenital heart defects requiring surgery. We asked parents questions in two key areas: "What is important for you having a child born with a congenital anomaly? And what should the researcher focus on in upcoming projects?"

# METHODS

## Study Design

Due to the aim of investigating parental experiences of having a child with a congenital anomaly we conducted a qualitative approach. The qualitative approach gave us the opportunity to hold an holistic position and obtain in-depth knowledge about the parent's experiences and their research priorities (12). To obtain the needed information from the parents we conducted interviews and focus groups. The interviews were conducted first in order to identify what parents experience when living with a child with one of the four congenital anomalies and to understand their research priorities. Secondly, the interviews were carried out to test and qualify the interview guide for focus groups. The interviews identified challenges when talking with the parents about their research priorities.

## Sample

As the total group of congenital anomalies included in the EUROlinkCAT study is very heterogeneous we decided that parents of children with four pre-defined congenital anomaly groups with different health problems covering intellectual disability, physical disability, visible defects and non-visible defects with higher mortality, would be included. The four anomaly groups selected were congenital heart defects requiring surgery (non-visible defect with higher mortality), cleft lip (visible defect), spina bifida (physical disability) and Down syndrome (intellectual disability and often associated with congenital heart defect). Besides the four criteria for health problems the anomaly groups were chosen with consideration of not being too rare, resulting in having challenges with inclusion.

## Recruitment

Parents were recruited *via* five EUROCAT registries in Denmark, Spain, Poland, and Italy: Funen County Registry in Denmark, Valencian Region Registry (Registro poblacional de Anomalías Congénitas de la Comunitat Valenciana) in Spain, Wielkopolska Registries in Poland, and Emilia- Romagna Registry (Indagine Malformazioni congenite in Emilia Romagna-IMER) in Italy, Tuscany Registry (Registro Toscano Difetti Congeniti-RTDC) in Italy.

Participants were consecutive recruited through an invitation either from the collaborating hospital physicians or through patient associations for the included anomalies. Inclusion criteria was being biological parent or grandparent of a child aged between one and 10 years old with one of the selected congenital anomalies. Participants had to be able to speak the local language of their country or English. All parents participating in the interviews and focus groups had been regularly in contact with a hospital due to their child's diagnosis. The rationale for choosing Denmark, Spain, Poland and Italy was to have as broad a perspective as possible in relation to culture, organization of health system and social circumstances as possible.

## Data Collection

Semi-structured interviews were conducted in the Region of Southern Denmark by PhD KGH and Professor JC. The Spanish focus group with parents of children with Cleft Palate and Cleft lip was conducted in a meeting room in Barcelona. The focus group was conducted in Barcelona because of a close collaboration between clinicians in Barcelona and the researchers in the Valencian registry. One focus group in Poland with parents of children with Down syndrome was conducted in at the Department of Medical Genetics, Poznan University of Medical Sciences. The other Polish focus groups with parents of children with Spina Bifida and Cleft lip took place in SWPS University of Social Sciences in Katowice and The Specialist Children's Hospital in Olsztyn, respectively. In total, four focus groups were conducted in Italy; for parents of children with Down syndrome a focus group took place in the Trisomy 21 Onlus Association in Florence. The focus group for parents of children with cleft lip was held at St Anna Hospital in Cona Ferrara, and for parents of children with spina bifida the focus group was held at Hotel Parma & Congressi in Parma. Finally, a focus group with parents of children with congenital heart defect took place in the Heart Hospital "Gaetano Pasquinucci" in Massa.

Prior to each interview and focus group, the parents were introduced to the participating researchers and the aim of the study. The focus groups were carried out by researchers from the local EUROCAT registry; BCs AN and BCs AP in Italy and MSc AJ and Professor ALB in Poland. In Spain the focus group was carried out by researchers from the local EUROCAT registry and sociologist Lucía Páramo. EUROlinkCAT produced an online focus group manual to allow researchers to prepare and train for the focus groups. This would also support rigor during

the study. There was no relationship between the researchers and the participants prior to the interviews and focus groups. One researcher interviewed and the second researcher took field notes during the focus group. Initially, an interview guide was developed for the interviews in Denmark based on the literature and according to the study aim. As it was challenging for the parents to answer questions about research priorities we added follow-up questions to the interview guide for the interviewers to use during the focus groups to support talking and discussion. The themes in the interview guide included: parents' experience of the pregnancy, birth, daily life and, priorities for future research. To support the questions raised by the interviewer during the focus groups, cards with the themes were visual for the parents to ensure focus on each theme.

The interviews in Denmark were conducted between March and July 2016 and the focus groups in Spain, Poland and Italy were conducted between February 2018 and November 2019. All interviews and focus groups were audio-recorded and transcribed by the researchers from each registry and translated into English. The objective field notes from the focus groups were used during transcripts of the interviews, to help subsidize parental statements (i.e., father crying). The parents participating in interviews in Denmark were provided with the interview transcript after the interviews and were encouraged to read it and provide feedback if the written material did not coincide with their views. None of the parents provided feedback to the transcripts. Of ethical reason, parents participating in focus groups were not provided with interview transcripts while they contained data from other people than themselves.

## Analysis

Data were analyzed using systematic text condensation (STC), which is inspired by Giorgi and adjusted by Malterud (13). STC is a method suitable for descriptive analysis of phenomenon for the development of new descriptions and concepts. A STC analysis consists of decontextualisation and recontextualisation of the data. STC is a procedure of four steps: (I) read transcripts repeatedly to identify themes, (II) identify and code units of meaning, (III) identify sub-groups of codes from step II and develop condensates from them and, (IV) describe experiences based on the condensates (14). To have multiple perspectives during the first process of the analysis, KGH and JC individually extracted themes from data in interview and focus group transcripts. Hereafter themes were triangulated in a face-to-face process where each researcher presented identified themes and from where they derived in data (interview transcripts). JC and KGH reached consensus of themes and presented the themes for the entire research group which agreed. KGH and JC completed steps II-IV, presented the full analysis for the research group to have their comments and finally, all researchers approved the analysis and the presentation of findings.

## Ethics

In Denmark the study was approved by the Danish Data Protection Agency (2008-58-0035) and by the hospital management.

**TABLE 1 |** Participants in interviews and focus groups.

| | Mother, *n* | Father, *n* | Caregiver, *n* |
|---|---|---|---|
| **Individual interviews** | | | |
| Down syndrome, Denmark | 1 | | |
| Spina bifida, Denmark | 2 | 2 | |
| Cleft lip and palate, Denmark | 2 | 2 | |
| Congenital heart defects, Denmark | 2 | 1 | |
| **Total** | **7** | **5** | |
| **Focus groups** | | | |
| Cleft lip and palate, Poland | 7 | 1 | |
| Spina Bifida, Poland | 7 | 4 | 1 |
| Down syndrome, Poland | 4 | | |
| Down syndrome, Italy | 5 | 3 | |
| Spina bifida, Italy | 3 | 2 | |
| Cleft lip and palate, Italy | 7 | 3 | 1 |
| Congenital heart defects, Italy | 4 | 2 | |
| Cleft palate, Spain | 6 | | |
| **Total** | **43** | **15** | **2** |

In Italy, after presenting the study to the Regional Health Authority, hospital board, and university it was confirmed that this work was considered a normal part of the registries activities and that specific approval was not required. However, the IMER Registry and the RTDC Registry operate under law decree, which does not allow parents of children in the registry to be contacted directly, so therefore recruitment was conducted through clinicians and parent/patient organizations of children with the condition.

In Poland there was no obligation to have specific ethical approval for this work, as organizing focus groups is one of the tasks of EUROlinkCAT overall which had already received ethics approval.

In Spain there was no obligation to have specific ethical approval for this work, as organizing focus groups is one of the tasks of EUROlinkCAT overall which had already received ethics approval.

According to the Helsinki declaration (15) the parents in the four regions received written and oral information about the study. The parents gave written informed consent to participate prior to the interviews and focus groups. The parents could withdraw from the study at any time with no consequences for the future treatment of their child or themselves.

# RESULTS

A total of 12 parents participated in seven interviews and 58 parents and two caregivers participated in eight focus groups (**Table 1**). The interviews lasted between 55 and 104 min. The focus groups lasted between 180 and 200 min. Overall, the analysis identified no notable differences in parent's experiences and expectations identified in the interviews and focus groups across the four countries.

**TABLE 2 |** Systematic text condensation of transcripts.

| Themes | From themes to codes | | Sub-categories | Overall categories |
|---|---|---|---|---|
| | Quotes | Codes | | |
| | | | | **The challenges of tailored information** |
| Presentation of diagnosis | *She (the Doctor) said all the worst things about spina bifida* (mother, spina bifida, Poland) | The parents expressed that they felt clinicians had a tendency to only present the worst case scenario of the anomaly. | Discrepancy | |
| Support | *I could have used spending time with other mothers with disabled children* (mother, down syndrome, Italy) | Parents feel that it is important to have contact with other parents experienced in parenting a child with an identical anomaly to hear the good stories. | Searching for peers | |
| Empathy | *It is very important with human relationship* (father, cleft lip, Italy) | Some parents perceived a lack of empathy from the clinicians. | Compassion | |
| | | | | **Parenting in between compassion and bureaucracy** |
| The child | *We live in the countryside and love the forest and we wanted him to be able to join our walks in the forest. When he was two, he managed the ATV all by himself. He should not be limited* (father, spina bifida, Denmark) | The parents expressed that they wanted to support their child in their development. | Parental love | |
| Parental experiences | *So much money had been spent on saving his life, but after his discharge the municipality would not help us* (mother, congenital heart disease, Denmark) | One of the major challenges the parents experienced in daily life with their child was the bureaucracy when applying for support or help supplies. | Fighting battles | |
| Surroundings | *So it's something that I believe you see in people's faces. And I imagine the lip, which is more of a cosmetic thing, people are not very tactful and I've heard them say terrible things* (mother, cleft palate, Spain) | Parent and children with visible anomalies experienced being starred at. Parents perceive that people often don't know what to say or do leading to hurtful reactions or comments. | Being exposed | |

Analysis of the interviews and focus groups identified two overall themes (**Table 2**); *The challenges of tailored information* and *Parenting in between compassion and bureaucracy*.

## The Challenges of Tailored Information

A recurrent observation from the analysis was that parents that received a prenatal diagnosis of the congenital anomaly were happy they had received it before the child was born so they could prepare themselves. However, the parents that did not know about their child's diagnosis before birth were happy that they did not know prior to the birth of their child. A father stated that he could not bear the thought of having chosen an abortion if they had known about the anomaly before birth.

Around the time of diagnosis, regardless if it was prenatal or at birth, the parents were very reliant on open and trustworthy communication with clinicians. When clinicians engage in an open dialog and signal sincere empathy, with both spoken- and body language, the parents perceived the information about the diagnosis was given with a sense of trust. Further, parents feel acknowledged when communication occurs parallel to receiving compassion and care from the nurses. However, some parents in this study did not experience such compassion from their clinicians.

> *"He [the clinician] explained the analysis and said that I was affected by a fetus with….. as if it was cancer to be removed"* (mother, Down syndrome, Poland).

Some of the parents felt that information provided by the clinicians was not neutral but colored by the clinicians own beliefs. Parents found this unprofessional. Most parents felt dependent on clinicians, creating difficulties where clinicians have opinions that the parents did not share. This was identified by the discrepancy between the information the clinicians provided and the information the parents requested. Many of the parents of children with Down syndrome, spina bifida and cleft lip perceived that the clinicians in the hospital focus too much on the physical malformation/anomaly instead of all the other aspects that define a human being. Clinicians provided information concerning what the child would not be capable of due to his/her congenital anomaly instead of focusing on the competences and the potential for development that the child could have. A few parents stated they wanted more positive information about what their child would be capable of instead of only being told what their child would never be capable of.

> *"….the doctor immediately describes what the worst scenario is. He doesn't tell their potential"* (mother, spina bifida, Italy)

The parents with children with severe congenital heart defect requiring surgery experienced the information around the diagnosis as balanced. When the parents felt sufficiently informed about the diagnosis they did not search for further information. However, parents who did not feel sufficiently informed searched

for more information turning to social media and Google. Thus, today's parents do search the internet for information, but there are two dimensions of internet searching after having a child with a diagnosis. It can be a source of lots of information, but also *an ugly beast* as one mother described it. When doing internet searches, there was no filter helping the parents interpret the information. To obtain more information and have a broader perspective on their child's diagnosis, the parents reached out to other parents, either through Facebook groups or patient associations.

> *"The best contact is with a parent, not a psychologist"* (mother, Down syndrome, Italy).

Some parents withdrew their membership from diagnosis specific Facebook groups because there was too much focus on the negative aspects of the diagnosis. The parents differed according to when and why they needed to get in contact with peers, but all needed the contact at some point. Two parents described that they did not have the strength to engage with other parents until a year after the diagnosis, even though they felt insufficiently informed and lacked trust in health care professionals. Their home health nurse then helped them engage with peers. On the other hand, some parents asked for peers right after birth. One mother described that she gave birth to her child and later discovered that another woman had given birth to a child with Down syndrome at the same ward at the same time, but no one thought about connecting them: "*It would have been a great opportunity to talk and support each other*" (mother, Down syndrome, Italy). Some parents had not yet met with other parents.

The parents participating in the congenital heart defect focus group expressed the need for support from a psychologist. It was challenging for these parents not knowing the outcomes of their child's surgery as the doctors were unable to fully guarantee a successful outcome. These parents felt unsure whether they would see their child alive again, which was emotionally exhausting. The parents also requested psychological support for siblings.

## Parenting in Between Compassion and Bureaucracy

Everyday life for the parents is filled with infinite joy for their children. The parents participating in interviews and focus groups concentrated on quality of life for their children and possessed the belief that physical or cognitive impairment is not equivalent with low quality of life. After the new-born period, and when family life with new routines has settled, the parents work hard to support the development of their child. As the child gets older, challenges for the child with physical and/or cognitive impairment increase and it becomes more visible to the parents that their child is different from children without a congenital anomaly. Despite the child's challenges, the parents said they think about opportunities rather than limitations. Parents with children with Down syndrome or spina bifida know that training is essential for development of competences for their child. However, the many of the parents perceived lack of support from their clinician.

> *"I was told from a doctor that these children are very difficult and I shouldn't expect anything from him. That was awful, how could he say that?"* (mother, Down syndrome, Italy)

Parents of children with cleft palate described challenges with feeding their child and lacked information and practical guidance. As one mother describes the information, she gained from a doctor:

> *"Feed the baby in an upright position and in case she chokes, perform basic resuscitation. And after a year I'll operate on her and she'll be as good as new"* (mother, cleft palate, Spain)

In this case, the mother felt insecure and was overwhelmed with the fact that surgery was not within a year. Many parents perceived to be left alone with the feeding challenges resulting in emotions of guilt and anger.

Experiences of guilt and blame appeared in more ways. The mothers blamed themselves for their child's anomaly and kept reflecting on their behavior, medication and nutrition in the weeks where the anomaly typically arose. Further, parents described situations where they were questioned why they continued the pregnancy. Finally, some parents of children with cleft lip had experienced people asking them to stay indoor with their child not to frighten the public with the appearance of the child.

Fighting for support was a common theme among the parents. Some parents expressed that the worse part of having a child with a congenital anomaly, and thereby special needs, is the constant battle with the local authorities. Parents of children with congenital heart defects who often suffer infections found it difficult to manage their jobs. Some parents had to work less hours and others had to quit their jobs entirely resulting in financial pressure for the family.

> *"So much money had been spent on saving his life, but after his discharge the local authority would not help us. I had to quit my job, because he was always sick. . . . and ended up as divorced."* (mother, congenital heart defect, Denmark).

In general, the majority of parents lack support from the local authority to cope with their family's situation even though they may have a legal right (for example for special schooling or reduced working hours). Parents find fighting battles with the local authority for support is very resource demanding and emotionally draining. When parents' expectations for sufficient help for training their child exceeded the offers provided from hospital or local authority, they wanted to train their child themselves. However, it was not always possible for the parents due to abilities, resources and economic obligations.

> *"I would love to work part-time so I could increase intellectual training with her, because studies show that these children can learn. But I'm not supported in that perception."* (mother, Down syndrome, Denmark).

Battles with the local authorities are also demanding and requires a lot of competences, stubbornness, and time. Time that the children could have benefitted from. All parents expressed that they repeatedly applied for extra hours of work or other forms of help, but their requests often resulted in rejection after rejection. Parents felt at times that they had to act as a lawyer protecting their child's rights.

*"To be Honest, We Take Care Privately Because My Patience With the Whole System Is Running out"* (Father, Spina Bifida, Italy)

The parents felt that these interactions with the local authority were the worse part about having child with a congenital anomaly. The lack of support that parents received led to parents believing that their child is not acknowledged as a human being with potential.

When questions concerning the parent's wishes and priorities for future research were raised, it was challenging for the parents to give specific answers. However, we identified that the research priorities for the parents were around quality of life (for all four anomaly groups), cognitive development (Down syndrome, spina bifida, and congenital heart defects), and how much training results in improved cognitive and physical outcome (for all four anomaly groups).

# DISCUSSION

The overall aim of this qualitative study was to investigate parental experiences of having a child with one of four congenital anomalies: Down syndrome, spina bifida, cleft lip or severe congenital heart defects requiring surgery. Despite cultural differences and different hospital settings, the parents had the same worries, identical expectations and experiences with information from clinicians.

## Holistic and Empathic Communication

The research questions identified that informing parents of their child's condition, both pre- and postnatal, requires careful presentation of the diagnosis and its impact on the child's life. Studies concerning parental perspectives about being informed of an anomaly in their child have been widely studied (16–20) and recommendations for information strategies have been presented when a congenital anomaly is diagnosed prenatally (21) and postnatal (22). We identified that parents requested more holistic information about the diagnosis and the prognosis taking the child's abilities and future possibilities in consideration. This is identical with the findings from Davies et al. (23), who identified that when health care providers focus on the positive when interacting with parents of children with complex and chronic conditions it resulted in positive outcomes for both parents and health care providers. However, physicians are legally obliged to inform about the risks of the condition and potential short and long-term complications of planned procedures and surgeries (24) and this does not always correlate with what the parents want to hear.

We also identified that some parents in this study perceived that they lacked empathy from physicians providing information about their child's anomaly and they therefore searched for more information from associations, peers, and the Internet. Empathy has been identified as one of the main elements when interacting with parents with ill children (23). Hilton-Kamm identified that parent's perception of the physician's compassion and empathy when providing information about a congenital heart defect diagnosis is inversely related of the parents seeking second opinions (25) and that they request more information during the perinatal and neonatal period than physicians provide (26).

## Parental Concerns

We also identified that having a child with a congenital anomaly leads to numerous concerns. It was clear from the interviews and focus group discussions that the parents fully accept the disabilities of their child and fight for the rights of their child. Another study of parents expecting a child with a congenital anomaly has identified that the parents already during pregnancy focus on characteristics and strengths rather than disabilities or obstacles (27). The parents in our study were generally more concerned about long-term prognosis and quality of life than of the risk of surgery in infancy. This correlates with the objective of EUROlinkCAT with examine morbidity and education to 10 years of age. The findings from this study indicates that parents experiences challenges and they appear both in hospital- and community settings. Many clinicians and sectors are involved when a child is born with a congenital anomaly and this continues throughout childhood. To increase parent support cross-sectoral collaboration must be prioritized to ensure their needs.

## Research Priorities

What the parents requested was research on how parents can increase the quality of life for their children and what the parents can do to optimize the long-term outcome for their child. A case study has shown that specific training of children with Down syndrome increased motor and cognitive development (28), but to our knowledge the impact on the amount of parental training has not been studied in relation to long-term motor and cognitive outcome as well as the impact on quality of life. Carlsson et al. have involved couples who experienced a prenatal diagnosis of a congenital heart defect to assess their perspectives on research priorities (29). They found that their participants requested more research on written material, similar to the parents in our study. For counseling parents after a diagnosis of a congenital anomaly, holistic up-to-date information on short and long-term outcomes are needed. Finally, the parent's research priorities focused on long-term outcomes and quality of life as important themes.

## Implications for Clinical Practice

Our findings indicate that encouragement and compassion can be the keys to give the family a better start with their new baby. Thus, we suggest that clinicians working with these families meet them with kindness and compassion focusing on the child's possibilities and abilities. Further, the clinicians must see the family's needs in a holistic perspective involving support persons from primary care and local authorities in general. Finally, we suggest more research focusing on the family's needs and wishes.

## Methodological Considerations

A limitation to consider is that data were collected in five regions in four European countries. It was planned that focus groups should be carried out with parents of all four anomaly groups in the participating countries and further, more focus groups were planned in other European countries (Malta, Portugal and England). However, the COVID-19 situation in Europe haltered the continuation for the focus groups. This study expresses the opinions of parents with live children whose views may be very different from parents who decide termination of pregnancy due to fetal anomaly. Our initial expectation was that there would be differences across the countries in parental experience and perceptions due to cultural and social circumstances, and organization of health systems. Further, another limit concerns the multi-dimensional heterogeneities that exist in congenital anomalies. This might be particularly present in the CHD group with different heart defects requiring different clinical management and prognosis of the children. It was a requirement for inclusion that surgery was performed to avoid inclusion of less severe heart defects. However, we mainly identified similarities.

Parents of children up to 10 years of age were included in the study why recall must be considered. Further, clinical practice and the approach to parents of children with congenital anomalies have developed during the last decade.

During the interviews and focus groups we identified that it may be challenging for people who are not familiar with research to identify research possibilities and limitations. It requires help during the interviews to encourage the parents to express their priorities for future research.

Parents in the study lead challenging everyday lives so we expected last minute dropouts and included a minimum of ten parents for each focus group. For two focus groups we experienced last minute drop-outs from parents and therefore two focus groups were carried out with less than the recommended number of participants (13). However, we did not experience that it negatively affected the dynamic and discussion among the participants. No parents participating in interview or focus groups withdraw from the study. All parents participating expressed their satisfaction with participation in a focus group and the opportunity to release their emotions, say their opinion, and share their experiences with other parents. Parents communicate with each other *via* social media, but face to face contacts in small groups are rare. The energy and insights generated by the focus groups will be used for further involvement of parents and health professionals. All parents were offered to contact the researchers after interviews and focus groups if in need for a more personal talk. The focus groups were facilitated by the strong working relationships between EUROCAT Registry leaders, clinicians and patient associations with which they have long-standing relationships. The research group is interdisciplinary consisting of physicians, nurses, and epidemiologists from different countries. This provided the group with different perspectives and pre-understanding. All researchers took active part in collecting data. A common platform for training of the researches for conducting focus groups, an interview guide and a template of how to transcribe data where developed to minimize the effect of individual beliefs and assumptions.

# CONCLUSION

The parents request more positive information instead of solely focusing on the negative aspects of living with the anomaly regardless if the child is diagnosed during pregnancy or at birth. It was challenging for the parents to directly address specific research priorities, but we identified that the parents had great concerns about their children's quality of life and cognitive and physical achievements and were caught between seeking the best possibilities for their child and the limited resources that the public system can provide.

# DATA AVAILABILITY STATEMENT

Data supporting the conclusions of this article can be made available by the authors upon request.

# ETHICS STATEMENT

The studies involving human participants were reviewed and approved by EUROlinkCAT under the Danish Data Protection Agency (2008-58-0035). Written informed consent was not required for the study on human participants, in accordance with the local legislation and institutional requirements.

# AUTHOR CONTRIBUTIONS

KH: methodology, investigation, formal analysis, writing original draft, and writing review and editing. AN: investigation, resources, visualization, supervision, and writing original draft. AP and AL: investigation and resources. AJ-D: investigation. EG: supervision, project administration, and funding acquisition. CC-C: investigation and writing review and editing. JC: conceptualization, methodology, formal analysis, investigation, writing original draft, and writing review and editing. All authors read and approved the final version of the manuscript.

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