Neural tube defects and co-occurring anomalies in Europe, 1980-2015

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Abstract

Study questions: Does the proportion of neural tube defects (NTDs) in which there are co-occurring anomalies of other systems vary by geographical area and time in Europe between 1980 and 2015? Which group of factors, e.g., sex, age, family history, condition at discovery, and prenatal screening during pregnancy that potentially affect ascertainment of isolated NTD cases and co-occurring anomalies, are associated with the presence of these anomalies?

Methods: Data on fetuses or births with NTDs recorded in 28 European Surveillance of Congenital Anomalies (EUROCAT) registries operating throughout or during a part of the period 1980-2015, were obtained and analysed. Information on the corresponding denominators of total births for each registry were compiled, comprising approximately 15.7 million births. We developed algorithms to classify NTDs that occurred as part of certain recognized genetic and unknown etiology syndromes, isolated NTD cases (either with no other anomaly reported or only an anomaly considered to be secondary to the NTD), and NTDs not occurring as part of recognized syndromes but with one or more co-occurring anomalies. We assessed factors that could impact ascertainment of isolated NTD cases and NTD cases with co-occurring anomalies. We also conducted a sensitivity analysis to evaluate the effect of family history and consanguinity on NTD outcomes. Lastly, we reported on the prevalence of NTDs across Eastern, Western, Northern, and Southern Europe as defined by the World Bank.

Results: A total of 14,703 fetuses or births with NTDs were recorded in the EUROCAT registries. The total number of births (live and stillbirths) that occurred during the periods when the EUROCAT registries were operating was 15.7 million. The overall prevalence at birth of NTDs in Eastern Europe was 11.6 per 10,000 births, in Western Europe 11.0 per 10,000 births, in Northern Europe 10.5 cases per 10,000 births and in Southern Europe 6.7 per 10,000 births. The most prevalent genetic syndromes were chromosomal anomalies, particularly trisomy 18. The most common secondary anomaly was Arnold-Chiari syndrome which occurred mostly with spina bifida. The most prevalent associated anomalies were limb defects, followed by congenital heart defects, abdominal wall, and urinary system defects. We found anencephaly to be most prevalent in isolated NTD cases without secondary anomalies and spina bifida in secondary anomalies and most of the co-occurring anomalies. For factors that could affect ascertainment, we found that birth type, birth weight, family history, maternal and paternal age (≥30 years) were risk factors for isolated NTD cases and for co-occurring anomalies. Lastly, approximately 48% of NTDs occurred in Northern Europe, followed 29% in Western Europe, 16% in Southern Europe, and 7.6% in Eastern Europe.

Conclusion: In the absence of folate fortification, isolated NTD cases showed higher prevalence than NTDs with co-occurring anomalies over the past 35 years across Europe. This research suggests that Europe should develop and implement policies and guidelines for folate fortification to help prevent simpler NTD cases. Further research and data are needed for newer EUROCAT registries to predict trends and prevalence of NTDs.

Contribution and involvement: This project was done in collaboration with the experts from the School of Epidemiology and Public Health (SEPH) and dysmorphologists that provided support with regards to NTD classification. AB spearheaded the development of the research protocol and thesis manuscript with funding and support from JL and TR. Evaluation of the output of cases from the algorithm was provided to AB by JL, TR, JE, FB, AN, EB, ML, and the EUROCAT Joint Research Centre.

Chapter 1: Introduction

1.1 Background

Neural tube defects (NTDs) are defects of the central nervous system (CNS) that occur when the neural tube fails to completely close during embryonic development (Greene and Copp, 2014). These defects occur in more than 300,000 newborns worldwide annually, resulting in annual loss of approximately 6.2 million daily adjusted life years (DALYs) (Botto et al., 1999; Greene and Copp, 2014). The most common NTDs are anencephaly, spina bifida, and encephalocele. Anencephaly is the most severe, with virtual absence of the forebrain and the skull vault (Elwood et al., 1992). The failure of the brain to form is due to improper sealing of the rostral neural tube (Berry et al., 1999a). Most infants with anencephaly are stillborn, although as the brain stem including the respiratory centres may be intact, liveborn infants may survive for a short period of time, of the order of a few days (Elwood et al., 1992). Spina bifida is a general term encompassing many varieties of lesions due to midline separation of the vertebrae (Elwood et al., 1992a). In open spina bifida, there is exposed neural tissue, an open spinal canal, and incomplete skin cover. If there is skin cover, the lesion often becomes cystic, and is classified as spina bifida cystica. In vital statistics records in most countries, and in clinical and epidemiological studies, cases of spina bifida with anencephaly are typically classified in the anencephaly group (Elwood and Little, 1992). Encephalocele is a sac-like protrusion of the brain and the membranes that cover it through an opening in the skull (Avagliano et al., 2019; CDC, 2020b). NTDs may be detected prenatally by ultrasound. In addition, open defects allow cerebrospinal fluid to leak into the amniotic fluid, enabling prenatal detection from measurements of alpha-fetoprotein and other substances in amniotic fluid and maternal serum (Elwood et al., 1992a). In a systematic review of studies published in the period 1990-2012, the overall frequency of induced abortion following prenatal diagnosis was 83% for anencephaly (range, 59-100%) and 63% for spina bifida (range, 31-97%) (Johnson et al., 2012).

Between the 1930s/1940s and the mid-1970s, there was a decline in the prevalence at birth of NTDs, or mortality due to these defects, in the British Isles, continental Europe, the USA and Canada; data from other countries are sparse (Elwood and Little, 1992a). Since then, time trends have been greatly affected by antenatal diagnosis and selective induced abortion. Without adjustment for the effects of selective induced abortion, the prevalence at birth of NTDs in the 1980s was around 10-20 per 10,000 births in Great Britain (England, Scotland, and Wales), continental Europe, North America, and Australia. More recently, in a systematic review of reports published in the period 1990-2014, more than half of the World Health Organization (WHO) member states did not have any data relevant on NTD surveillance; in those that did have such data, there was considerable variation in prevalence at birth (defined to include live births, fetal deaths, and terminations of pregnancy for fetal anomaly in the numerator, and live births and fetal deaths in the denominator) between and within countries (Zaganjor et al., 2016). The median prevalence per 10,000 births (range) was 6.9 (0.3–199.4) in the Western Pacific Region, 9.0 ((1.3–35.9) in Europe, 11.5 (3.3–27.9) in the Americas, 11.7 (5.2–75.4) in Africa, 15.8 (1.9–66.2) in South-East Asia, and 21.9 (2.1–124.1) in the Eastern Mediterranean Region.

1.2 Risk factors

Risk factors for NTDs include genetic predisposition, sex, age, lower socio-economic status, maternal diabetes, maternal obesity, maternal hyperthermia, maternal use of valproic acid, low folate status, chronic illness, and possibly other aspects of maternal nutrition, air pollution, problematic substance use and occupational chemical exposures (Little, 1992; Little and Elwood, 1992a; Elwood et al., 1992b, c; Gilbert et al., 2013; Tanoshima et al., 2015; Donnan et al., 2016; Avagliano et al., 2019; Jia et al., 2019; Liu et al., 2019; Spinder et al., 2019). Regarding genetic predisposition, recurrence risks of about 5% have been reported in the British Isles, and 2.5-3% in other areas, and about 10% when there is a history of two or more affected fetuses or infants (Little, 1992; Avagliano et al., 2019). Concordance rates are higher in monozygotic than in dizygotic twins (Little and Elwood 1992b, Avagliano et al., 2019). Chromosomal anomalies are reported to be involved in less than 10% of cases (Avagliano et al., 2019). Some other specific genetic syndromes include NTDs, including Meckel-Gruber syndrome, but these account for a very small proportion of the prevalence at birth of NTDs (Little and Nevin, 1992; Lynch 2005). Overall NTDs, including anencephaly and encephalocele are more prevalent in females and have sex-specific disproportionate effects to folic acid supplementation (Liu et al., 2018). The risk of NTDs is associated with low SES, measured by parental occupation, education, or household income (Brender & Suarez, 1990; Canfield, et al., 1996; Farley et al., 2002; Meyer & Siega-Riz, 2002).

1.3 Prenatal screening

Screening amongst women varies worldwide which is influenced by availability, cost, access to, and quality of screening (Boyd et al., 2008). Screening policies in Europe vary between regions where countries that had screening policies reported higher prevalence structural malformations and chromosomal anomalies, compared to countries with no policies (Boyd et al., 2008). There was variation in the scheduling of screening during the three trimesters between regions. Women would be more likely to opt in for prenatal screening if a traumatic event occurred during pregnancy or perhaps if anomalies were prevalent in their family history (Boyd et al., 2008). It is also seen that detection rate between countries that offered first, and second-trimester screening differed from those using a mixed first or second-trimester screening policy. Countries within Europe using similar screening policies also varied in detection rates because of other factors such as maternal age, availability of patient decision aids, peer approval, cost, time concerns, and overall awareness of the benefits of prenatal screening (Boyd et al., 2008; Lépine et al., 2016). It is important to consider these differences at the population level when addressing strategizing and implementation of preventative policies to tackle NTDs. Since the late 1970s, NTD screening has been offered based on measuring serum-alpha-fetoprotein (MSAFP) levels, between 15 and 22 weeks of gestation to maximize accuracy and minimizing false positive rates (Garne et al., 2010; NICE, 2019; Nizard, 2010; Wang et al., 2009).

In many jurisdictions, prenatal screening for NTDs occurs simultaneously with screening for Down syndrome (trisomy 21), and other fetal aneuploidies with the screening packages being diverse in terms of the number of tests included and their frequency (Wilson et al., 2014; Nizard, 2010). However, since 2008, the UK National Institute for Clinical Excellence has recommended that ultrasound screening does not have to be paired with alpha-fetoprotein testing (NICE, 2019). In most regions, routine second trimester ultrasound enables visualization of anatomical structures and supplements the screening tests that are specific to NTDs and aneuploidies, along with potentially detecting other types of fetal anomalies. There appears to be limited information on how differences between prenatal screening systems have impacted the distribution of NTDs by presence or absence of additional anomalies: However, the consensus is that prevalence rates are higher in countries that have relied upon screening policies that focus on first trimester screening

(Wilson et al., 2014; Lépine et al., 2016). A study from South Carolina found that there was little difference in the proportion of NTDs with anomalies of other systems detected by ultrasonography, MSAFP screening, amniotic fluid alpha-fetoprotein (AFP) screen or at delivery (Stevenson et al., 2004). They also found that in most cases, the co-occurring anomalies that occurred alongside NTDs were either components of a multiple malformation syndrome or secondary to the NTD, highlighting the multifaceted impact of NTDs (Stevenson et al., 2004). Apart from chromosomal anomalies, prenatal screening of NTDs also revealed fetuses with structural malformations, ventriculomegaly and midline anomalies (Stevenson et al., 2004). Since chromosomal abnormalities were also present and were typically detected after 20 weeks, posing legal issues, it is important that chromosomal analysis and fetal autopsy is incorporated into genetic counseling for pregnant mothers to make an informed decision (Siddesh et al., 2017).

There are many outcomes that can result because of congenital anomalies (CA). The most common chromosomal CA is Down syndrome, affecting 1 in 800 babies (Lobo & Zhaurova, 2008). A study conducted by Dolk and Colleagues in Europe discovered that the most common non chromosomal CA were Congenital heart defects (CHD), followed by limb defects, anomalies of the urinary system, and nervous system (Dolk et al., 2010). The risk of having a child with these conditions increases with maternal age, family genetics, and environment exposure to teratogens, such as radiation, alcohol, and medications during pregnancy. Chromosomal abnormalities can generally be detected prenatally via karyotyping and non-invasive prenatal testing (NIPT) from 8 weeks onward (Yang et al., 2020). Due to variation in prenatal screening practices across Europe, there are large differences in screening practices and policies along with organizational and cultural variation which directly impact detection rates of chromosomal abnormalities and NTDs (Boyd et al., 2008). Another variation that is seen across Europe is in abortion laws between countries with most European countries permitting abortion and a few allowing for it under strict conditions (i.e., endangering maternal health) (Berer, 2017; Economist, 2020). Similarly, many European countries have set acceptable fetal age cut-off for abortions and terminations of pregnancy for fetal anomaly (TOPFA) (Boyd et al., 2008). Since CAs have a large spectrum of consequences that vary in severity associated with it, many families opt to abort following TOPFA. “According to WHO, 17%-42% of infant mortality was attributed to congenital anomaly” (Boyle et al., 2018). The average infant mortality due to CAs in 11 EUROCAT countries was 1.1 per 1000 births, with higher rates seen in countries where TOPFA was illegal (Malta 3.0, Ireland 2.1) (Boyle et al., 2018). Between these two countries, TOPFA is still illegal in Malta whereas Ireland has recently approved it with restrictions (Broido, 1995). The average TOPFA prevalence was 4.6 per 1000, which is nearly three times more prevalent than the rate of stillbirths (0.6 per 1000) and infant deaths combined (Boyle et al., 2018. Since the decision of TOPFA is made after prenatal detection, this also impacted the overall prevalence of post-neonatal survivors that were diagnosed with non-lethal CAs (Broido, 1995).

Moreover, it is interesting to note that termination of pregnancy was legalized in 2019 in Northern Ireland under the abortion act which has applied to the rest of the UK since 1967. The abortion act of 1967 did not extend to Northern Ireland up until October 2019, and prior to this, it was likely for individuals to crossover into England, Scotland or Wales to have a termination of pregnancy following prenatal diagnosis., This was followed up with financial burdens, arising from having to pay out of pocket for this procedure until the UK government was legally bound to introduce access to abortion in Northern Ireland by March 2020. This would allow for abortion of cases of rape, incest, severe fetal abnormality and if there is a physical or mental threat to the woman's health. Lastly, with the advancement in prenatal screening and improvement in the technologies used, it would be optimal to obtain more data on the issue as this can potentially explain the variation in the proportion of NTD cases across various European regions.

1.4 Multiple pregnancies

In higher income countries, the rates of multiple births have been increasing steadily since the 1970s, partly due to increasing maternal age and the increase in the use of assisted reproductive technologies (Blondel & Kaminski, 2002; Collins, 2007; Fell & Joseph, 2012; Pison et al., 2015). There is evidence suggesting a higher proportion of births from multiple pregnancies compared with singleton pregnancies have fetal anomalies: however, evidence about the risk of NTDs in twins is inconsistent (Ben-Ami et al., 2005; Boyle et al., 2013; Little & Nevin, 1989; Little & Bryan, 1986; Mastroiacovo et al., 1999; Pison et al., 2015; Ramos-Arroyo, 1991; Sargiotto et al., 2015; Windham et al., 1982). In a multicentre study in Europe, the rate of prenatal diagnosis was similar between multiple and singleton pregnancies, but termination of pregnancy after prenatal diagnosis was less common in multiple pregnancies (B. Boyle et al., 2013). There does not appear to be any ongoing investigations of anomalies associated with NTDs in fetuses/births from multiple pregnancies, due to statistical power limitations.

EUROCAT is a European Network of population-based registries for the epidemiological surveillance of congenital anomalies collecting information on 1.7 million births each year (30% of births in Europe) from 51 registries. Of these 51 registries, 23 registries contribute from anonymous individual full members or associate members specific data (Tucker et al., 2018). These registries help to assess the impact of developments in prenatal screening and policies, and act to provide a framework that is geared around discovering causes of congenital anomalies, with the goal of developing effective preventative strategies (Tucker et al., 2018).

1.5 International Classification of Diseases (ICD9 and ICD10) with British Pediatric Association Extension

The international classification of diseases (ICD) codes are internationally recognized diagnostic codes that are assigned to identify illnesses. It is the global standard for health data, clinical documentation and statistical aggregation that allows for sharing of health records for a patient between different systems (WHO, 2021). The versions that were evident in the dataset were ICD9 and ICD10. For births prior to 2002, EUROCAT used ICD9 coding and ICD10 for births after (EUROCAT, 2020). Both ICD9 and ICD10 codes were divided into subgroups based on the types of anomalies. In ICD9, congenital anomalies existed between 7400 to 7599 while for ICD10, congenital anomalies existed in the Q chapter. ICD9 was completely numeric whereas ICD10 implemented characters. The major difference between ICD9 and ICD10 was the number of characters involved. ICD9 had up to five characters and ICD10 can have up to seven. ICD10 offers higher specificity than ICD9 as it contains approximately 4x times the number of codes (13,000 for ICD9 versus 68,000 for ICD10) (Holman et al., 2018). Since ICD10 codes are more detailed and abundant, they can help to improve clinical, financial, and administrative performance measurements, care and disease process management, and reporting on the efficacy on new medical technology (Holman et al., 2018).
Since specificity varied, extensions were created that were added on to the existing diagnostic coding framework. The British Pediatric Association (BPA) extension was the addition of a single

digit at the end of the diagnostic that would further specify the location of the anomaly and sub-anomalies that fell under those groups. For example, Q05 is the ICD10 code for spina bifida; however, Q051 is classified as thoracic spina bifida with hydrocephalus (EUROCAT, 2008). Due to the consistency and recognizability of the diagnostic codes, they were easily transferrable between different healthcare systems since the codes mean the same, irrespective of geography. This allowed researchers and healthcare professionals to conduct surveillance-based analysis and were able to categorize illnesses as secondary to NTD or co-occurring with NTDs.

1.6 NTDs and co-occurring anomalies

Congenital anomaly registries have been established for well over 40 years (Modell et al., 2018). These registries collect detailed information about individual birthweight, sex, type of anomaly, family history, maternal and paternal demographics, and exposure during pregnancy (EUROCAT, 2018). The objectives of these registries are to provide epidemiological information on CA that can aid in monitoring the impact of early teratogen exposure to a fetus, and evaluating the efficacy of interventions (EUROCAT, 2018).

Congenital anomalies are multi-faceted and can present a spectrum of limitations, ranging from chromosomal to physical (Persson et al., 2017). They can result in minor and major anomalies which can severely diminish the quality of life (QoL) and lifespan of an individual (CDC, 2019). Population surveillance programs indicate that classifying CAs based on etiology results in further categorizing cases, which is a step closer to developing a strategic approach to mitigate the negative effects and intervene early on (Calzolari et al., 2014). Since CAs can occur as isolated or with additional co-occurring anomalies, detailed surveillance of co-occurring cases are essential as they are more sensitive to teratogenic exposure compared to isolated cases (Calzolari et al., 2014). However, the challenge here is that the underlying cause of many CAs remain unknown and is assumed to be environmentally related (Opitz, 1994). Although the literature suggests that the four main causes of CAs are genetics, chemical exposure during pregnancy, chromosomal, and infections, almost 50% of all CA cases cannot be linked to a specific underlying cause (WHO, 2021).

Since NTDs are defects that occur during embryonic development, they can impact individuals in a variety of ways ranging from motor or cognitive impairment to death. Some NTDs occur in conjunction with chromosomal disorders, and genetic syndromes such as Meckel-Gruber syndrome: however, the proportion is small (Barisic et al., 2015). When genetic syndromes and chromosomal anomalies are excluded, the proportion of cases that have additional malformations varies between studies, ranging from 9.1% to 33.3% in the population-based or multi-hospital studies included (Table 1) (Barisic et al., 2015). In Table 1, studies that focused on single hospitals were excluded to minimize referral bias arising, for example, from the fact that proportions of cases with associated anomalies in spina bifida clinics would be expected to be lower than in population-based studies (Stevenson et al., 2004). In Canada, among the cases of NTDs identified over a ten-year period, 17% of cases had associated malformations, half of whom displayed components of a recognizable syndrome (Lowry, 2008). In Europe, based on 13 EUROCAT registries in the period 1980-1987, the proportion of cases with additional malformations was 15.2%, with levels being higher in Continental Europe (17.9%) than in Ireland and the UK (14.6%) (Dolk et al., 1991). Two studies conducted in France reported proportions of 12.1% and 20.5% while an Irish and Swedish study reported 22.4% and 16.6%, respectively (Källén et al., 1998; McDonnell et al., 1999; Stoll et al., 2011). In an Iranian cohort study, co-occurring anomalies were reported in 66% of NTD cases, with higher prevalence in fetuses with spina bifida (Moradi et al., 2017). They also reported that CNS anomalies were the most common category of co-occurring anomalies, and that Chiari II was found in all cases of open spina bifida, followed by extremities anomalies and spine deformities (Moradi et al., 2017).

The variability in the proportion of cases with additional anomalies may in part be due to differences between studies in the methods used to identify NTDs associated with recognized syndromes and sequences. Not all studies utilize karyotyping and autopsy to identify cases (Dolk et al., 1991; Källén et al., 1998; Stevenson et al., 2004). Variation may also be due to differences in inclusion of fetuses from terminated or spontaneously aborted pregnancies and if they were included, there is major discrepancy in the timing of termination or spontaneous abortion (Källén et al., 1998; Stevenson et al., 2000). It has been suggested that in areas of low prevalence at birth, the proportion of cases associated with anomalies of other systems are higher as compared to other areas: However, this is not strongly supported in a 1980-1987 EUROCAT data analysis (H. Dolk, de Wals, et al., 1991; Stevenson et al., 2000). Another possible reason for variability in these cases could be explained by the variation in frequencies of susceptibility genotypes, such as those for MTHFR C677T variant (Wilcken et al., 2003). This can potentially explain the higher prevalence at birth of NTDs in the UK, Ireland, Canada, and China (Elwood et al., 1992; Moore et al., 1997). By increasing folate levels through fortification in Canada along with diet and supplementation in China, it may have decreased geographical variation attributable to genetic susceptibility and provided a protective effect since increased requirement for folate in more susceptible women has been met (Berry et al., 1999b; De Wals et al., 2007; Melnick & Marazita, 1998). The geographical differences in case presentation may also be due to variation in dietary and cooking practices, exposure to teratogens, maternal diabetes and obesity, and maternal age (Government of Canada, 2017).

1.7 Anomalies secondary to NTDs

There are known anomalies that occur in addition to NTDs that have been deemed as secondary. Although there are discrepancies within the literature, there is consensus on some anomalies. Isolated NTD cases are fetuses that either only have an NTD or they have an NTD with 1 or more secondary anomalies. Anomalies that are secondary to the presence of an NTD are anomalies that are a result of the primary NTD that is present within the fetus at birth. Although an individual can have multiple anomalies, if the anomalies that occurred are classified as secondary, then the fetus/baby would be deemed as having an isolated NTD. There are many secondary anomalies that can occur with an NTD that would be classified as an isolated NTD; for example, an NTD with Arnold-Chari malformation, NTD with anomalies of the corpus callosum, and anencephaly with adrenal hypoplasia (EUROCAT, 2018). Some musculoskeletal anomalies, such as clubfoot or hip dysplasia that occur with an NTD can be classified as anomalies secondary to NTDs.

From this isolated NTD group, genetic syndromes such as chromosomal anomalies, amniotic bands, Meckel-Gruber, and OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects) and minor anomalies are excluded. This is because when genetic syndromes are present, other anomalies that are prevalent in the fetus are primarily because of that genetic predisposition. Fetuses with chromosomal anomalies also have additional co-occurring anomalies, making it challenging to pinpoint the underlying reason for the onset of the additional anomalies.

1.8 Folic acid fortification

The findings on supplement use of folic acid and fortification multivitamin have been supported by the Atlanta Birth Defects Case-Control Study, suggesting a reduction in overall occurrence of birth defects, in addition to neural tube defects (Botto et al., 2004). There is also a difference in impact of folic acid fortification on sex where females showed a greater reduction in prevalence of NTDs, particularly anencephaly and cervico-thoracic spina bifida (Poletta et al., 2018).

In addition, another important factor that is seen to have a tremendous impact on overall prevalence and incidence of CAs is the lack of folate fortification policies across Europe. Folate fortification is the addition of a B vitamin called folic acid to enriched grain products such as bread, pasta, rice, and cereal (CDC, 2020). In 1996, the U.S. Food and Drug Administration authorized fortification and implemented it fully by 1998 (CDC, 2020). Since fortification implementation and use of folic acid supplements in the U.S., it has resulted in an average of 1,300 babies being born each year without NTDs who might otherwise be affected (CDC, 2020). This equates to approximately a 35% reduction in NTD cases (CDC, 2020). Even though fortification has been shown to have protective effects against simple NTDs, fortification policies are virtually nonexistent across Europe (CDC, 2020). A 2013 study in Ireland has shown an overall decrease in the number of foods fortified with folic acid over 10 years. Ireland is the only European country that initiated voluntary fortification. Around 4,500 pregnancies in the European Union are annually affected by a defect of the brain and spine, with an estimated 72% of pregnancies being terminated. Europe did not see a decrease in the overall prevalence of birth defects despite their attempts at increasing consumption of folic acid via supplementation (Pachón et al., 2013). In contrast, 35,000 cases of the most common NTDs (spina bifida and anencephaly) were prevented in 58 countries where fortification was mandatory (Ruxton, 2019). For these reasons, detailed surveillance registries across Europe are needed that collect complete case details of individuals, their families, and potential teratogenic exposures that can be linked to their anomalies, like EUROCAT.

1.9 Study questions

For the present study, I sought to answer the following research questions:

1. Does the proportion of NTDs in which there are co-occurring anomalies of other systems (e.g., genetic, musculoskeletal, nervous system, digestive, respiratory, and reproductive) vary by geographical area and over time in Europe between 1980 and 2015?
2. Which group of factors, e.g., sex, birth type, birth weight, prenatal test, condition at discovery, karyotype, post-mortem exam that affected ascertainment of co-occurring anomalies are associated with the presence of these anomalies?

Specific research objectives

1. To determine the proportion of cases with co-occurring anomalies from 1980-2015 by analyzing case series.
2. To assess the prevalence of NTD cases with co-occurring anomalies and how they vary between regions and over time.
3. To compare factors that could influence ascertainment of anomalies, and maternal risk factors for presence of overall co-occurring anomalies. We will also compare the overall characteristics of the fetus or infant between NTD cases with co-occurring anomalies and isolated NTD cases.

2.0 Methods

2.1 Study Design

This population-based cohort study used the EUROCAT database to assess all pregnant women, 15 to 49 years of age, from 1980-2015. We compared cases of NTDs with co-occurring anomalies with isolated NTD cases across Europe. The outcome of interest is assessing the proportion of isolated NTD cases and NTD cases with co-occurring anomalies between and within regions of Europe and analyzing the maternal risk factors that could have influenced the onset of cases.

2.2 Inclusion Criteria

The inclusion criteria will include all pregnant women aged 15 to 49 in Europe from 1980-2015. We will include live births (LB), fetal death (FD) from 20 weeks of gestation (by stillbirth, miscarriage, preterm labor, infection, and placental problems such as inadequate blood flow). We will also include fetuses that were terminated for fetal anomaly following prenatal diagnosis (TOPFA).

Registers to be included

The EUROCAT registry included All full-member registries (Austria: Styria; Belgium: Antwerp; Croatia: Zagreb; France: Auvergne, Isle de Reunion, French West Indies, Brittany, Paris; Germany: Saxony-Anhalt; Ireland: Cork & Kerry, Dublin; Italy: Emilia Romagna, Tuscany; Malta; Netherlands: North; Norway; Poland: Wielkopolska; Portugal: South; Spain: Basque Country, Valencia Region; Switzerland: Vaud; Ukraine: OMNI-NET; UK: South West England, Northern England, Thames Valley, East Midlands & South Yorkshire, Wales, Wessex).

Data were available from 28 EUROCAT registries operating in all or part of the period 1980-2015. The data included specific malformations (ICD9 or ICD10 diagnostic codes and descriptive text, EUROCAT Groups) identified in each fetus or baby. Information was available on pregnancy outcome via civil registration (liveborn baby, stillborn baby, termination of pregnancy), condition of the fetus/baby at the time of discovery of the anomaly (prenatal screen, condition at birth, age of discovery of congenital anomaly, malformation in siblings), examinations done on the fetus/baby (autopsy, karyotype, first positive prenatal test), other characteristics of the fetus/baby (sex, weight, birth year, country of discovery, surgical procedure(s) for anomaly, survival beyond one week of age), maternal and paternal risk factors (age, consanguinity, types of drugs used during pregnancy, illness before and during pregnancy, gestational length, mother and father's family with anomalies). The number of unique individuals in the dataset was 14,703. We excluded fetuses that had missing data about anomalies present in the fetus (n=11). The dataset contained 176 variables, most of them binomial, some categorical, and others contained text.

We examined the data descriptively and found that specific ICD codes did not always appear to correspond to EUROCAT subgroups. Therefore, we developed two algorithms utilizing ICD10-BPA and ICD9-BPA codes to identify isolated neural tube defects (NTDs), NTDs associated with specific genetic syndromes, and co-occurring anomalies of NTDs that did not appear to be part of recognized genetic syndromes. The algorithms were developed using Python 3 OpenPyxl and Pandas (scripts are provided in the Appendices to this document).

Isolated NTDs have been defined as anomalies that exist as NTDs on their own (spina bifida, anencephaly, encephalocele, and iniencephaly) or NTDs with anomalies that are secondary and are not a result of underlying genetic syndromes. Cases of NTDs with the following anomalies were categorized as isolated cases: CNS anomalies such as Arnold Chiari malformation, agenesis, or other anomalies of the corpus callosum, hydrocephaly, aqueductal stenosis, microcephaly, diastematomyelia, and similar spinal cord anomalies, hip dysplasia, clubfoot, and other joint deformation or contractures, and spine anomalies that relate to the site of the lesion, hydronephrosis or reflux, and adrenal gland hypoplasia or other cranial NTD. NTDs that occurred as part of a recognized genetic syndrome were separated into their subgroup. These cases were NTDs with chromosomal anomalies, Meckel-Gruber, OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects), single-gene disorder, and amniotic bands. Anomalies that did not fall into the groups described above were classified as co-occurring. For fetuses to be diagnosed with a co-occurring anomaly, they must present an NTD with at least one co-occurring anomaly. Lastly, to diagnose multiple co-occurring anomalies, they had to present an NTD with more than one anomaly from different subgroups. These anomalies can be defects of the abdominal wall, heart, urinary system, respiratory system, genital system, eye, ear face and neck, digestive, limb, orofacial, and others. The literature and EUROCAT guide were used to make these divisions. The following information and subgroup divisions are presented in the appendix.

Since every patient within the dataset had an NTD, this would allow us to map the rates of individual co-occurring anomalies by year and European region, as specified by the adapted version of the United Nations Statistics Division (2020, using M49 codes classification) (North, South, East, and West). We defined the following European regions as such: Eastern (Poland and Ukraine); Northern (adapted, with Ireland and UK being one group, as in Dolk et al. (1991), and the other group comprising Denmark and Norway); Southern (Croatia, Italy, Malta, Portugal, and Spain); and Western (Austria, Belgium, France, Germany, Netherlands, and Switzerland).

2.3 Algorithm 1 development

The first algorithm was based on section 3.3 (page 92) of the EUROCAT subgroups of congenital anomalies table in the EUROCAT Guide 1.4 and Reference Documents (EUROCAT, 2018). This table was used to code all cases of congenital anomalies and uses ICD10-BPA codes and maps the ICD9-BPA codes with the minor anomalies. The algorithm scanned the eight malformation columns that contained the code, and once it matched a EUROCAT subgroup, it would output a value of 1 for that EUROCAT subgroup. For example: if a patient row contained a Q792 code, the algorithm would output this as "1" for the abdominal wall defect subgroup. In the occurrence where a patient had multiple ICD10-BPA/ICD9-BPA codes, it would output a value of 1 to multiple EUROCAT subgroups. Specific variations of the subgroup codes were entered (see appendix), which controlled for minor anomalies.

Each unique four-, five-, six- or seven-character ICD10-BPA or ICD9-BPA code (i.e., Q00 vs. Q0001 being the same) was manually entered into the transcript yielding approximately 2500 unique ICD10-BPA and ICD9-BPA codes. We did NOT truncate codes as we observed that doing so resulted in incomplete data capture on our previous attempt. This method allowed for controlling which codes were read by the algorithm while omitting the minor anomalies pre-and post-2005.

From this algorithm, the following subgroups were generated: Nervous System (types of NTDs), Eye (Cataracts, Glaucoma, Anophthalmus/microphthalmos), Ear face and neck (Anotia), Congenital Heart Defects (21 conditions), Respiratory (Choanal atresia, Cystic adenomatous malformation of lung), Oro-facial clefts (Cleft lip with or without cleft palate, Cleft palate), Digestive system (8 conditions), Abdominal wall defects (Gastroschisis, Omphalocele), Urinary (5 conditions), Genital (Hypospadias, Indeterminate sex), Limb (5 conditions), Other anomalies (14 conditions), and Chromosomal (5 conditions). The anomalies with their codes that fell within each subgroup are presented in the appendix.

2.4 Algorithm 2 development

To identify isolated neural tube defect (NTD) cases, the steps in section 3.4, "Multiple Congenital Anomaly Algorithm" on page 97 of the EUROCAT 1.4 Guide were followed. We began by isolating all participants with only NTD codes specified in one or more columns of variables malfo1-malfo8. Then, we outputted cases that had Oro-facial clefts ICD10-BPA and ICD9-BPA codes associated with NTD codes. Next, chromosomal rearrangements (Q95 or 7584) were extracted that were present in addition to an NTD code. Although NTDs that were part of specific genetic syndromes are excluded, balanced chromosomal rearrangements were included due to the instructions outlined by EUROCAT in their algorithm.

We extracted all known sequences or combinations of anomalies without another anomaly code. This step extracted cases such as spina bifida with hydrocephalus and spina bifida with talipes equinovarus. The next few steps of the algorithm focused on extracting combinations of isolated NTD with secondary anomalies. The following cases were extracted; anencephaly with adrenal hypoplasia, NTD (encephalocele or spina bifida) with Arnold Chiari, anencephaly with adrenal gland hypoplasia or other cranial NTD, and NTD (anencephaly, encephalocele or spina bifida) with either of the following: agenesis or other anomalies of the corpus callosum, microcephaly, diastematomyelia, and similar spinal cord anomalies, musculoskeletal anomalies such as hip dysplasia and other joint deformation or contractures, and spine anomalies that relate to the site of the lesion, urinary tract dysfunction leading to hydronephrosis or reflux and exstrophy. Lastly, all the individual extracted groups were summated and classified as isolated NTD cases. To verify the algorithm's output, a comparison analysis was done with the malformation diagnostic codes and text variables to ensure concordance.

We identified genetic syndromes cases and syndromes with unknown etiology associated with NTDs, to exclude these cases in examining NTDs with co-occurring anomalies. Genetic syndromes included chromosomal anomalies, Meckel-Gruber, and single-gene disorders. We also identified syndromes with unknown etiology such as amniotic bands, OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects) (Keppler-Noreuil, 2001; NORD, 2018). The OEIS complex was handled by looking for a combination of ICD9-BPA and ICD10-BPA codes in the malfo1-malfo8 columns. The code Q6410 for cloacal exstrophy was scanned for OEIS complex diagnosis. In the case where this was not found, we looked for a combination of diagnostic codes for omphalocele, exstrophy, imperforate anus, and spinal defect (i.e., spina bifida). We verified this approach by cross-referencing this with the text columns that contained written diagnoses for each patient. The respective ICD10-BPA and ICD9-BPA codes for the exclusions of genetic syndrome cases are Q90-Q92, Q93, Q96-Q99, 758.0-758.3, 758.5-758.9, Q79.80, 762.80, Q64.10, 759.890, Q61.90, 759.89, Q44.71, Q74.84, Q75.1, Q75.4, Q75.81, Q87, Q93.6, D82.1, 755.81, 756.01, 756.04, 759.8, and 279.10. We reported the number, type, and location of genetic and unknown etiology syndromes that were excluded. For genetic syndromes, fetuses were classified as having a genetic syndrome associated with NTDs if any of the codes mentioned were present in the malfo1-malfo8 column. Since most genetic syndrome cases occur in conjunction with other anomalies, fetuses with multiple anomalies with at least one diagnostic code for a genetic syndrome were categorized under that genetic syndrome category (Chen, 2007; Liu et al., 2019).

Lastly, anomalies that did not fall in the isolated or genetic syndromes groups were classified as co-occurring. As mentioned in algorithm one description, there are co-occurring anomalies of many systems such as cardiac, renal, respiratory, limb, genital, abdominal wall defects, etc. We reported on the rates, type, and location of the centre of co-occurring anomalies that were observed.

Using the combination from the two algorithms and running them sequentially, we produced output with the following variables:

Isolated NTD

NTD (nervous system) +:

Abdominal Wall Defect

- Congenital Heart Defect

- Urinary system anomaly

- Respiratory system anomaly

- Genital system anomaly

- Eye defects

- Ear, Face, Neck anomaly

- Digestive system anomaly

- Limb defects

- Orofacial clefts

- Other anomalies

- >1 (multiple) co-occurring anomalies that are not secondary and not due to an underlying genetic syndrome.

Having formed these groups, we manually removed minor anomalies from each group using the EUROCAT guide 1.4 and reference document that specifies minor anomalies pre-and post-2005 (changing from ICD9-BPA to ICD10-BPA). Further data cleaning was conducted to remove blanks and missing values for analytical purposes. Table 1 below contains all the demographic variables within the dataset scanned and compared with the coding scheme in the EUROCAT 1.4 guide and reference document. Erroneous data entries were minimal (additional category entry for a variable not specified in the EUROCAT guide (e.g., FIRSTPRE (first positive prenatal test) had multiple entries of 0 even though there is no category 0 defined by EUROCAT) and were treated as missing. It is suspected that the entry of "0" for FIRSTPRE was meant to be "10," which indicates no prenatal test was performed. The frequencies for these variables are presented in Table 1. Any data entry errors, or inconsistencies seen in the dataset were documented.

2.5 Statistical analysis

Statistical analysis was conducted using Excel and Stata SE 16. We included live births (LB), fetal death (FD) from 20 weeks of gestation (by stillbirth, miscarriage, preterm labor, infection, and placental problems such as inadequate blood flow). We also included fetuses that were terminated for fetal anomaly following prenatal diagnosis (TOPFA). All analysis components relate to LB, FD, or TOPFA with neural tube defects, with or without co-occurring anomalies of any type. The prevalence rates of NTDs, and the three individual NTD subtypes in all births, were calculated for the four European regions. We calculated proportions stratified by ten-year period (with and without restriction to registries that have data available across periods, because registries have variable periods of observations within the proposed overall study period 1980-2015) and sex. Sensitivity analyses was done, including the exclusion of data from registries with an upper age limit for recording congenital anomalies of one month or less (Zagreb, Paris, Mainz, Emilia-Romagna, Barcelona, South Portugal) and exclusion of data from registries with an upper age limit of more than one year (Odense, Strasbourg, Cork & Kerry, Dublin, SE Ireland, North Netherlands, Wielkopolska, Vaud, SW England, N England, E Midlands, and S Yorkshire, and Wessex). For the analysis of the relationship between the proportion of cases with anomalies of other systems and population prevalence, population prevalence will be defined as the number of affected cases in live births, stillbirths, spontaneous abortion, and fetuses from terminated pregnancy divided by the total number of births (live and still) per 10,000. We used Poisson regression to model the relationship between the proportion of NTDs with co-occurring anomalies (all types together in primary analysis; secondary analysis by most common types, anticipated to be limb defects, cardiac defects, and abdominal wall defects) and (a) prevalence rate of isolated NTD; (b) prevalence rate of co-occurring anomalies (excluding those occurring with NTDs). We calculated proportions of co-occurring anomalies, genetic syndromes, and secondary anomalies by NTD subgroups (anencephalus, encephalocele, and spina bifida). A sensitivity analysis assessed the proportion of isolated and co-occurring NTD cases controlling for family history with or without reported consanguinity. Lastly, with the exclusions of genetic syndromes and secondary anomalies to classify isolated NTDs, the output of complex cases was assessed via blind duplicate dysmorphological review.

2.6 Ethical consideration

The University of Ottawa's Office of Research Ethics and Integrity approved the study protocol and manuscript. Special access for the data was requested from EUROCAT Joint Research Centre. The data files were password protected and were not shared externally. The participants in the dataset were anonymized, and sensitive personal identification information was not available. The researchers involved do not have a conflict of interest.

3.0 Results

3.1 Characteristics of case series

The dataset from this study included 14,703 participants from Europe between 1980 to 2015. The data were collected in 28 EUROCAT centres. Of the 14,703 fetuses, 9,277 were from pregnancies terminated due to fetal anomaly (TOPFA) (Table 1). Most of the anomalies in the fetus were discovered prenatally. There were more female babies with congenital anomalies than male babies. Approximately 67% of fetuses were alive at the time of discovery of their condition. Many of the babies/fetuses delivered were from singleton pregnancies and from the data available, it was the mother's first pregnancy. Most women did not undergo assisted conception or and few of the affected fetuses or babies underwent a surgical procedure for the malformation. From those that provided information on medication intake during the first trimester, most women reported not taking any medications. Lastly, of the fetuses/babies that were delivered, a substantial proportion did not survive beyond one week of age.

Table 1. Demographic characteristics associated with 14,703 births/fetuses with NTDs in the EUROCAT dataset.

Demographic Characteristic	Frequency (Mean)
Birth Type	
Livebirth	**4,384**
Stillbirth	**1,026**
Termination of Pregnancy due to Fetal Anomaly (TOPFA)	**9,277**
Missing	**16**
Note: for these 16 missing fetuses/babies, 13 were fetuses with isolated NTDs and 3 had multiple co-occurring anomalies	
When the baby was first suspected of having a congenital anomaly (When discovered)	
Birth	**2,014**
Less than 1 week	**139**
1-4 weeks	**12**
1-12 months	**35**
Over 12 months	**10**
Prenatal diagnosis in live fetus	**11,413**
At Spontaneous Abortion	**10**

Postnatal diagnosis, age not known	**15**
Missing	**1,055**
Note: of the 1,055 missing, 591 had live birth, 235 had stillbirth, and 223 had TOPFA.	
First Positive Prenatal Test	
Ultrasound at GA<14 weeks	**1,589**
Ultrasound at GA 14-21 weeks	**2,098**
Ultrasound at GA ≥ 22 weeks	**595**
Ultrasound GA not known	**236**
Serum/ combined screening	**93**
CVS or amniocentesis	**31**
Other test positive	**25**
Test(s) performed, result negative	**199**
No test performed	**19**
Missing	**9,818**
Number of Babies/Fetuses delivered	
Singleton	**14,033**
Twins	**593**
Triplets	**21**
Quadruplets	**3**
Missing	**44**
Number of Malformed in Multiple sets	
1	**2,043**
2	**86**
3	**2**
Missing	**12,572**
Condition at Discovery	
Alive	**9,814**
Dead	**354**
Missing	**4,535**
Mean Gestational age at discovery of anomaly (week)	**(18.4)**
Missing	*4,837*
Mean Gestational Length at birth (week)	**(23.6)**
Missing	**666**
Mean Birth Weight (g)	**(1534.8)**
Missing	**5,109**
Sex	
Male	**5,441**
Female	**5,849**
Indeterminate	**143**
Missing	**3,270**
Karyotype	
Performed, results known	**2,694**
Performed, results not known	**243**

Not performed	**3,706**
Probe test performed	**65**
Failed	**63**
Missing	**7,932**
Post-mortem Examination	
Performed, results known	**3,823**
Macerated fetus	**37**
Performed, fetus not recorded to be macerated, but results not known	**176**
Not performed	**3,533**
Missing	**7,134**
Mean Age of Mother (years)	**(27.1)**
15-25	**4,353**
26-35	**8,001**
36-45	**1,957**
46-49	**20**
Missing	**356**
Note: 8 total were excluded: 7 fetuses were excluded since the mother's age was less than 15 and 1 had mom greater than 49 years of age.	
Mean Age of Father (years)	**(31.5)**
15-25	**814**
26-35	**2,251**
36-45	**951**
46+	**129**
Missing	**10,558**
Note: The oldest age of the father was 70 and oldest mother was 53. The youngest father was 15 and the youngest mother was 13.	
Total number of Previous Pregnancies	
None	**4,001**
One	**3,059**
Two	**1,692**
Three	**806**
Four and more	**866**
Missing	**4,279**
Note: approximately 22 women had more 10 or more pregnancies in their lifetime. Highest number of previous pregnancies was 19	
Mother's Family with Anomalies	
Same	**96**
Other	**259**
Same and Other	**22**
No	**4,196**
Missing	**10,130**

Father's Family with Anomalies	
Same	**55**
Other	**134**
Same and Other	**16**
No	**4,132**
Missing	**10,366**
Siblings with Anomalies	
Same	**194**
Other	**346**
Same and Other	**57**
No	**5,233**
Missing	**8,873**
Consanguinity	
Not related or relationship more distant than second cousin	**5,179**
Relationship of second cousin or closer	**57**
Missing	**9,467**
Assisted Conception	
No	**5,804**
Induced Ovulation only	**88**
Artificial Insemination	**28**
IVF: In vitro fertilization	**105**
Gamete intra fallopian transfer (GIFT)	**3**
ICSI: Intracytoplasmic sperm injection	**23**
Egg Donation	**5**
Other	**28**
Missing	**8,619**
First Trimester Medication	
Yes, medication taken in first trimester	**261**
No medication taken in first trimester	**674**
Undermined	**104**
Medication taken, but timing unknown	**34**
Missing	**13,630**
First Surgical Procedure for Malformation	
Performed (or expected) in the first year of life	**548**
Performed (or expected) after the first year of life	**21**
Prenatal surgery	**12**
No surgery required	**885**
Anomaly too severe for surgery	**287**
Died before surgery	**9**
Missing	**12,941**

Note: Complete for all livebirths and fetal deaths, only if there was prenatal surgery.	
Survival beyond One Week of Age	
Yes	**2,716**
No	**10,484**
Alive at discharge <1week	**15**
Missing	**1,488**
Note: If a child does not survive beyond one week of age, this includes stillbirths and abortions.	

3.2 Associated anomalies

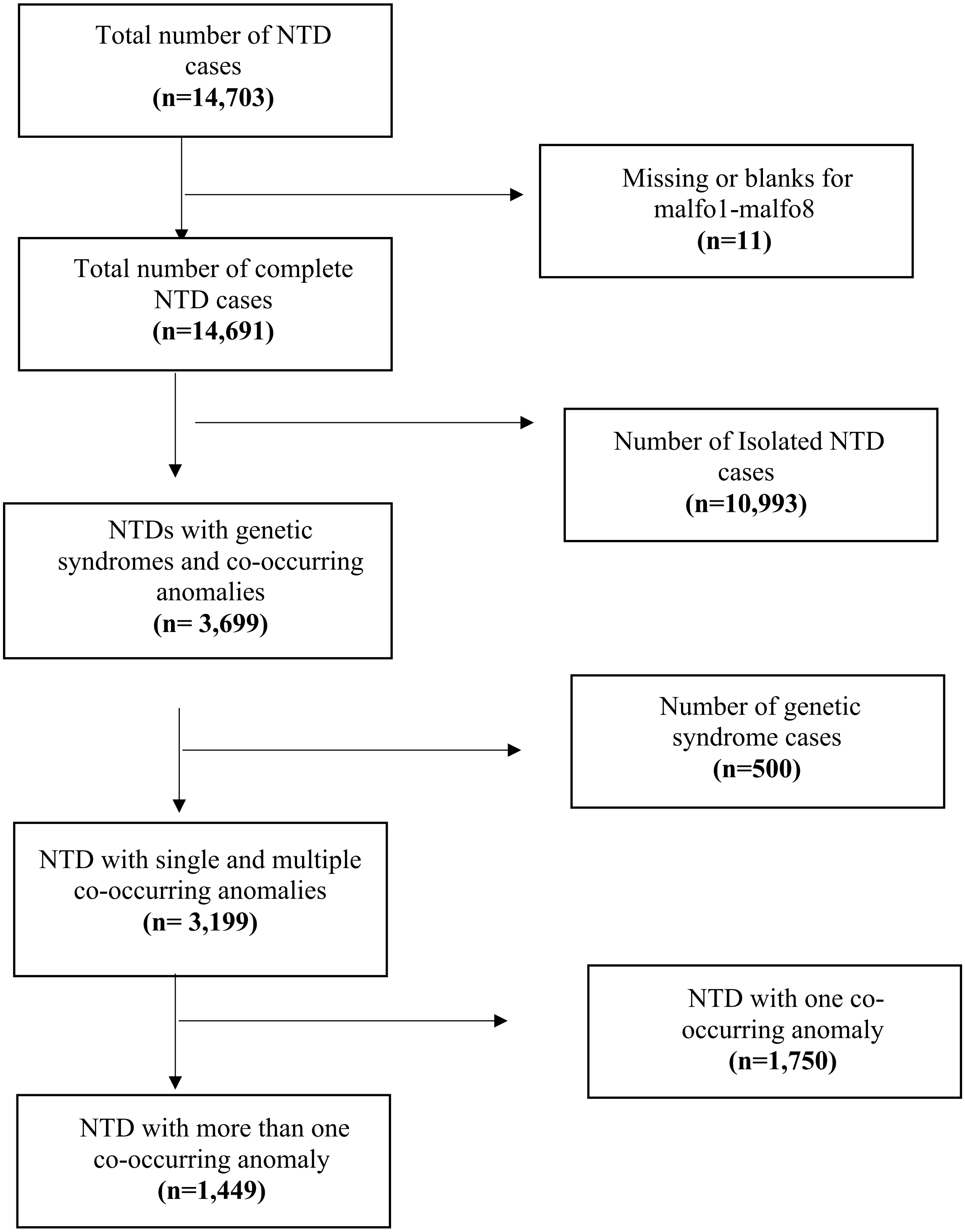

Figure 1. Flowchart of number and types of cases observed in the EUROCAT dataset, 1980-2015.

3.3 Syndromic NTDs

A total of 500 fetuses/babies affected by NTD had recognized genetic syndromes or syndromes of unknown etiology (Figure 1). Table 2 shows the conditions reported on based on NTD subtype. Of the recognized syndromes, chromosomal anomalies were the most prevalent, with majority of them occurring with spina bifida. Meckel-Gruber syndrome was most prevalent with encephalocele. For the unknown etiology syndromes, amniotic bands were most prevalent with anencephaly while OEIS complex only occurred with spina bifida. These rates are for fetuses/babies that had these syndromes occur in isolation with the NTD subgroups. The overall prevalence of Meckel-Gruber in this study is 0.62 per 100,000 births. The most common chromosomal anomaly observed was Trisomy 18 (Edward's syndrome).

Table 2. The number of Neural Tube Defects (NTDs) cases with recognized genetic syndromes or syndromes of unknown etiology.

Category	NTD of any type (n=500)	Spina Bifida	Anencephaly	Encephalocele
NTD occurring as part of recognized genetic syndromes				
Meckel-Gruber	96	7	9	80
Chromosomal	218	138	54	26
Single-gene disorder	72	31	16	25
NTD occurring as part of syndromes of unknown etiology				
Amniotic bands	92	9	52	31
OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects)	22	22		

These anomalies occur with one NTD code. For these fetuses or babies, they would have a total of two malformation codes (one for NTD, one for genetic syndrome or unknown etiology syndrome).

3.4 NTDs classified as isolated

In the dataset, more than 74% of affected fetuses/births had isolated NTDs. Of 10,993 fetuses/births classified as having isolated NTDs, 8,675 fetuses/births did not have any anomaly coded as secondary to the presence of the NTD. Of the remaining 2,318 fetuses/births, 1,074 had Arnold Chiari syndrome, followed by 500 hydrocephaly cases (Table 3). These anomalies were secondary to the presence of an NTD and were not due to a recognized genetic syndrome or syndrome of unknown etiology. The most prevalent secondary anomalies were Arnold Chiari syndrome, hydrocephaly, and clubfoot (talipes equinovarus). Anencephaly and spina bifida were the most common isolated NTDs that occurred without secondary anomalies. NTD cases with secondary anomalies were more prevalent with spina bifida, except for adrenal gland hypoplasia that was most prevalent with anencephaly.

Table 3. The number of NTD cases classified as isolated.

Isolated NTD with or without secondary anomalies	Frequency (n=10993)	Spina Bifida	Anencephaly	Encephalocele
NTDs coded as having no secondary anomalies	8,675	3,604	4,285	786
NTDs coded as having anomalies that are secondary to the NTD				
Arnold Chiari Syndrome	1,074	1,060	14	
Agenesis of the corpus callosum	60	37	1	21
Hydrocephaly	500	403	5	92
Microcephaly	86	41	5	40
Diastematomyelia	39	36	1	2
Hip dysplasia	140	133		7
Talipes Equinovarus (clubfoot)	339	310	21	8
Hydronephrosis	53	32	12	9
Adrenal gland hypoplasia	27		25	2

3.5 NTDs with co-occurring anomalies

Anomalies were defined as co-occurring if they were not secondary to the presence of an NTD and were not due to an underlying syndrome. The most prevalent co-occurring anomalies based on subgroup were limb defects, congenital heart defects, abdominal wall defects, and urinary system defects (Table 4). For anencephaly, the most prevalent were defects of the abdominal wall, respiratory system, and eye, and orofacial clefts. For spina bifida, the most prevalent were congenital heart defects, anomalies of the urinary system, genital system, ear face and neck, digestive system, limbs and of more than one system. Thus, the first two subgroup defects and urinary system defects were most prevalent with spina bifida while abdominal wall defects were most prevalent with anencephaly. Encephalocele was only prevalent with other defects which included anomalies such as congenital skin disorders, vater/vacterl, laterality anomalies, teratogenic syndromes, maternal infections, valproate syndrome, fetal alcohol syndrome, situs invertus, and conjoined twins. Multiple co-occurring anomalies were defined as the presence of one or more co-occurring anomalies within the same fetus/babies that were not secondary and were not due to an underlying known syndrome. Multiple co-occurring anomalies were most prevalent with spina bifida, followed by encephalocele, and anencephaly. Of the 3,199 fetuses that had a total of 4,675 associated anomalies, approximately 46% were multiple co-occurring anomalies.

Table 4. The number NTD cases with co-occurring anomalies not explained by an underlying syndrome or sequence, by type of NTD and type of co-occurring anomaly.

NTDs + co-occurring anomalies	Number of babies/fetuses with co-occurring anomaly of this type (n=3,199)	Spina bifida	Anencephaly	Encephalocele
Abdominal wall defects	190	50	116	24
Congenital Heart Defect	211	100	71	40
Urinary system	190	121	35	34
Respiratory system	58	21	28	9
Genital system	42	21	14	7
Eye defects	42	3	23	16
Ear, Face, Neck	13	8	2	3
Digestive system	162	74	58	30
Limb defects	653	537	56	60
Orofacial clefts	110	24	49	37
Other	79	24	20	35
>1 (multiple) co-occurring anomalies that are not secondary and/or not due to an underlying genetic syndrome	1,449	733	313	403

Note: row counts are not mutually exclusive in the third column.
This table reports fetuses that had an NTD and one associated anomaly. Individuals that had more than 1 anomaly from different subgroups were categorized as having multiple associated anomalies.

3.5.1 Factors that affected ascertainment of co-occurring anomalies

Most fetuses/babies that underwent TOPFA had an isolated NTD (Table 5). Majority of the fetuses that were born were premature, weighing at an average of 1.5 kg or 1,534.8g. In most instances, karyotyping was recorded as not having been done. It is interesting to note that isolated NTD was more prevalent in macerated fetuses. Most of the fetuses/babies were alive with an isolated NTDs when their anomaly was discovered.

Table 5. Factors that affected ascertainment of co-occurring anomalies by birth type, birth weight (<500g, 500-999g, 1,000g or more), prenatal test, condition at discovery, karyotype, post-mortem exam with column percentages.

Factors that affect ascertainment of co-occurring anomalies	**Isolated NTD (N=10,993)**	**NTD with co-occurring anomalies (N=3,199)**
Birth type		
Livebirths	2,860 (26.0)	1,001 (31.3)
Stillbirths	661 (6.0)	194 (6.1)
TOPFA	6,495 (59.0)	2,000 (62.5)
Missing	977 (9.0)	4 (0.1)
Birth weight (g)		
<500	2,444 (22.2)	1,368 (42.8)
500-999	605 (5.6)	346 (10.8)
1,000 or more	3,102 (28.2)	1,443 (45.1)
Missing	4,842 (44.0)	42 (1.3)
Karyotype		
Performed, results known	1,388 (12.6)	1,222 (38.2)
Performed, results not known	142 (1.3)	100 (3.1)
Not performed	2,761 (25.1)	944 (29.5)
Probe test performed	29 (0.26)	32 (1.0)
Failed	38 (0.34)	25 (0.8)
Missing	6,635 (60.4)	876 (27.4)
Post-mortem Examination		
Performed, results known	865 (7.9)	568 (17.8)
Macerated fetus	28 (0.25)	9 (0.3)
Performed, fetus not recorded to be macerated, but results not known	41 (0.37)	15 (0.5)
Not performed	2,598 (23.6)	892 (27.9)
Missing	7,461 (67.9)	1,715 (53.5)
Condition at Discovery		
Alive	6,686 (60.8)	2,979 (93.1)
Dead	228 (2.1)	125 (3.9)

Missing	4,079 (37.1)	95 (3.0)

3.5.2 Risk factors associated with co-occurring anomalies

The average maternal and paternal ages were 27 years and 31.5 years of age. High maternal age (age≥ 30) was deemed to be higher risk for fetuses with isolated anomalies since out of 10,993 isolated NTD cases, 5,433 occurred between 26-35 age range.

Majority of the fetuses/babies resulted from a singleton pregnancy. The average gestational age and length at the time of congenital anomaly discovery was 18.4 weeks and 23.6 weeks. Maternal and paternal age correlated with the highest proportion between 26 to 35 years of age. Both isolated NTD cases and NTDs with co-occurring anomalies had a high proportion of fetuses/babies that were not born from assisted conception. Although both categories had mothers that used medication during the first trimester, the ratio of those that used medication in the co-occurring anomalies group was higher compared to the isolated NTD group. However, a higher proportion of mothers in both categories did not use medications during their first trimester

Table 6. Risk factors such as number of babies/fetuses delivered, number of malformed in multiple sets, maternal age, paternal age, sex, maternal family history, paternal family history, affected siblings, assisted conception, medication in first trimester with column percentages.

Risk factors	**Isolated NTD (N=10,993)**	**NTD with co-occurring anomalies (N=3,199)**
Number of babies/fetuses delivered		
Singleton	9,594 (87.3)	2,970 (92.8)
Twins	379 (3.4)	208 (6.5)
Triplets	17 (0.2)	4 (0.1)
Missing	968 (9.1)	17 (0.6)
Number of Malformed in Multiple sets		
1	1,383 (12.6)	649 (20.3)
2	44 (0.4)	42 (1.3)
3	2 (0.02)	0
Missing	9,564 (86.98)	2,508 (78.4))
Mother's age (years)		
15-25	3,032 (27.6)	1,093 (34.2)

26-35	5,433 (49.5)	1,466 (45.8)
36-45	1,256 (11.4)	605 (18.9)
46-49	15 (0.1)	5 (0.2)
Missing	1,257 (11.4)	30 (0.9)
Father's age (years)		
15-25	590 (5.4)	223 (7.0)
26-35	1,535 (14.0)	710 (22.1)
36-45	624 (5.7)	319 (10.0)
46+	80 (0.7)	50 (1.6)
Missing	8,164 (74.2)	1,897 (59.3)
Sex		
Male	3,470 (31.6)	1,383 (43.2)
Female	3,796 (34.5)	1,667 (52.1)
Indeterminate	90 (0.8)	53 (1.7)
Missing	3,637 (33.1)	96 (3.0)
Mother's family with anomalies		
Same	53 (0.5)	39 (1.2)
Other	146 (1.3)	112 (3.5)
Same and other	15 (0.1)	7 (0.2)
No	2,760 (25.1)	1,402 (43.8)
Missing	8,019 (73.0)	1,639 (51.3)
Father's family with anomalies		
Same	34 (0.2)	21 (0.7)
Other	84 (0.8)	49 (1.5)
Same and other	11 (0.1)	5 (0.2)
No	2,700 (24.6)	1,397 (43.7)
Missing	8,164 (74.3)	1,727 (53.9)
Siblings with anomalies		
Same	121 (1.1)	73 (2.3)
Other	204 (1.9)	139 (4.3)
Same and other	38 (0.3)	18 (0.6)
No	3,412 (31.0)	1,784 (55.8)
Missing	7,218 (65.7)	1,185 (37.0)
Assisted Conception		
No	3,855 (35.1)	1,909 (59.7)
Induced Ovulation only	53 (0.5)	33 (1.0)
Artificial Insemination	14 (0.1)	13 (0.4)
IVF: In vitro fertilization	65 (0.6)	40 (1.3)
Gamete intra fallopian transfer (GIFT)	2 (0.02)	1 (0.03)
ICSI: Intracytoplasmic sperm injection	14 (0.1)	8 (0.25)
Egg Donation	2 (0.02)	3 (0.09)

Other	18 (0.2)	10 (0.3)
Missing	6,970 (63.36)	1,182 (36.93)
First Trimester Medication		
Yes, medication taken in first trimester	168 (1.5)	160 (5.0)
No medication taken in first trimester	454 (4.1)	213 (6.7)
Undermined	64 (0.6)	40 (1.3)
Medication taken, but timing unknown	16 (0.1)	16 (0.5)
Missing	10,291 (93.7)	2,770 (86.5)

A sensitivity analysis was done to compare the impact of consanguinity and family history on NTD case presentation (Table 7). Proportion of isolated NTDs and NTDs with co-occurring anomalies were higher without consanguinity, indicating consanguinity having a protective effect with family history on NTDs.

Table 7. Sensitivity analysis of family history with or without reported consanguinity and its impact on isolated NTD vs NTD with co-occurring anomalies.

Family history and consanguinity	Isolated NTD	NTD with co-occurring anomalies
Maternal family history		
With consanguinity	16	14
Without consanguinity	2,358	1,225
Paternal family history		
With consanguinity	15	14
Without consanguinity	2,302	1,199

As expected, isolated NTD frequency was higher across the 4 European regions. The approximate ratio of isolated NTD cases to NTD cases with co-occurring anomalies was 3:1 (Table 8). Isolated NTD cases were most prevalent in Northern Europe. Southern Europe reported a higher ratio of isolated NTD cases to NTD cases with co-occurring anomalies compared to Western Europe. Eastern Europe did not report any genetic syndromes or syndromes with unknown etiology. From 2000 to 2009, the ratio of isolated NTD cases to NTD cases with co-occurring anomalies was greater than 4:1. Lastly, Northern Europe reported the highest prevalence of NTD cases with genetic syndromes and/or unknown etiology syndromes.

Table 8. Isolated NTD cases versus NTDs with co-occurring anomalies by European Region and 10-year period of delivery.

European Region	Isolated NTD Frequency	NTDs with co-occurring anomalies	Total Frequency (not including genetic syndromes)
North	**80-89:** 515 **90-99:** 548 **00-09:** 2,464 **10-15:** 1431	**80-89:** 170 **90-99:** 306 **00-09:** 854 **10-15:** 583	**80-89: 685** **90-99: 854** **00-09: 3,318** **10-15: 2,014** **N= 6,871** **N= 7,033 (47.8%)** total including genetic syndromes
South	**80-89:** 180 **90-99:** 396 **00-09:** 630 **10-15:** 467	**80-89:** 68 **90-99:** 204 **00-09:** 224 **10-15:** 133	**80-89: 248** **90-99: 600** **00-09: 854** **10-15: 600** **N= 2,302** **N= 2,310 (15.7%)** total including genetic syndromes
East	**80-89:** 0 **90-99:** 31 **00-09:** 503 **10-15:** 346	**80-89:** 0 **90-99:** 9 **00-09:** 121 **10-15**: 101	**80-89: 0** **90-99: 40** **00-09: 624** **10-15: 447** **N= 1,111 (7.6%)** (No genetic syndromes occurred)
West	**80-89:** 412 **90-99:** 596 **00-09:** 833 **10-15:** 676	**80-89:** 299 **90-99:** 440 **00-09:** 527 **10-15:** 418	**80-89: 711** **90-99: 1,036** **00-09: 1,360** **10-15: 1,094** **N= 4,201** **N=4,249 (28.9%)** total including genetic syndromes

***Eastern Europe:** Ukraine, Poland, no Hungary – [62, 67]
***Western Europe**: Austria, Belgium, France, Germany, Netherlands, and Switzerland – [5,13,20,29,33,39,60,66,81,88]
***Northern Europe**: Ireland and UK being one group and Norway (No Denmark) – [10,49,57,59,68,70,72,73,84]
***Southern Europe**: Croatia, Italy, Malta, Portugal, and Spain– [8,18,21,23,28,30,86]

4.0 Discussion

This population-based case series used the EUROCAT database to assess all pregnant women, 15 to 49 years of age, from 1980-2015. We compared cases of isolated NTDs with NTDs with co-occurring anomalies of other systems across Europe. The outcome of interest was assessing the proportion of NTD cases between and within regions of Europe, along with analyzing the maternal risk factors that could have influenced the onset of cases. We also presented trends in the prevalence of isolated NTDs and NTDs with co-occurring anomalies of other systems by region, risk, and temporality in Europe, from 1980-2015 to understand the patterns of NTD cases. The total number of births (livebirths and stillbirths) in Europe from 1980-2015 was approximately 15.7 million. The overall prevalence of NTDs (including syndromes, co-occurring anomalies, secondary anomalies) across Europe from the dataset was 9.4 per 10,000 births. We found that majority of the cases that occurred across Europe have isolated NTD cases without secondary anomalies. The NTD most prevalent for these cases was anencephaly followed by spina bifida and encephalocele. Of the isolated NTD cases with secondary anomalies, Arnold-Chiari syndrome, hydrocephaly, and clubfoot were the most prevalent, which occurred primarily with spina bifida. This is because initial development errors arise early, and the resulting malformations may involve any germ layers (Pantanowitz, 2003). Due to the complexity and severity of anencephaly, multiple genes interact with environmental agents to cause the malformation. Studies have reported anencephaly to be 100% lethal within the first year of life (Dickman et al., 2016). Most babies/fetuses with anencephaly die before birth, with the pregnancy ending in a miscarriage (Cleveland Clinic, 2020). Those that are born die within hours, days, or weeks (Cleveland Clinic, 2020). Since anencephaly is a disorder of the brain, whereas spina bifida is a spine disorder, many anomalies are associated with spina bifida (CDC, 2020; NHS, 2020). This could also be due to having a semi-functioning brain in spina bifida instead of anencephaly, which can permit a fetus/baby to live a longer life (CDC, 2020).

The prevalence of isolated NTDs in Europe is 7.0 per 10,000 births with variations in geographical region. The prevalence of NTDs with co-occurring anomalies in Europe is 2.0 per 10,000 births. We see from the data that most of the isolated NTDs were anencephaly which occurred without secondary anomalies. Of the remaining NTDs with secondary anomalies, the majority occurred with spina bifida, consistent with the literature (Pantanowitz, 2003; NICHD, 2016). Arnold-Chiari was most prevalent with spina bifida and was less prevalent with anencephaly (Chen, 2007).

As seen in table 2, chromosomal anomalies were most prevalent. Fetuses with NTDs carry a risk of chromosomal abnormalities, which vary with maternal age, family history, and other structural abnormalities (Chen, 2007). Of the genetic and unknown etiology syndromes presented, chromosomal anomalies have the most significant number of variables. The most common chromosomal anomaly observed was Trisomy 18 (Edward's syndrome), like the findings in the literature (Chen, 2007; Copp et al., 2017). The mean prevalence of Meckel-Gruber in Europe is 2.6 per 100,000 births with regional differences (Barisic et al., 2014) whereas, the study's overall prevalence of Meckel-Gruber in this study is 0.62 per 100,000 births. Like the genetic syndromes, the isolated NTD cases were slightly underreported because fetuses were limited to having two malformations. If a fetus had more than two anomalies, including anomalies that would categorize it as having an isolated NTD, they were grouped under the multiple anomalies group. This could result in overreporting of the multiple anomalies group. However, the EUROCAT and literature guidelines were followed for categorizing isolated NTD cases and are specified in the study protocol (EUROCAT, 2018a).

To classify NTDs with an underlying syndrome, the fetuses/babies were restricted to two anomalies. This could result in an underreporting of these syndromes and the overall count of the anomalies. If a fetus/baby had multiple anomalies of the same subgroup, the anomalies would not be summed but instead given an overall value of 1. Although regional differences in genetic syndromes exist, the overall rate was perhaps underreported due to limitations in the algorithm developed for NTD case ascertainment. Discrepancies within the literature exist in terms of what classifies a syndrome to be genetic. Hence, guidelines provided by EUROCAT were followed. It is possible that EUROCAT's approach to case ascertainment of genetic and unknown etiological syndromes may be incomplete and requires further development as more European centres begin to operate and provide data. The prevalence of Meckel-Gruber was 0.62 per 10,000 births; this result is lower than previous reports due to incomplete ascertainment of the diagnostic codes by the algorithm, resulting in underreporting the prevalence. It is likely that with improvements to the algorithm, more fetuses would present a higher count of chromosomal anomalies than Meckel-Gruber, as suggested in the literature (Jayakar, 2019; Wellesley et al., 2012).

For NTDs with co-occurring anomalies, we see that limb defects, followed by congenital heart defects, abdominal wall, and urinary system defects, were most prevalent. We also find that majority of the fetuses/babies had multiple co-occurring anomalies with a spina bifida NTD due to the variability of the impact of spina bifida on the body and its association with other systems (CDC, 2020). We notice that, on average, a fetus/baby had two or more co-occurring anomalies, with most of them also occurring with spina bifida.

For the trends of isolated NTDs and NTDs with co-occurring anomalies across Europe, from 1980-2015, isolated NTD frequency was higher across the 4 European regions. The population prevalence of Eastern Europe is 11.6 cases per 10,000 births. The population prevalence of Western Europe was 11.0 cases per 10,000 births. The population prevalence of Northern Europe was 10.5 cases per 10,000 births. The population prevalence of Southern Europe was 6.7 cases per 10,000 births. A possible link for the high prevalence of Eastern Europe may be due to the radioactivity from the Chernobyl nuclear disaster in 1986 (Akar, 2015; Mocan et al., 1990). A study assessing the effects of the Chernobyl radiation on the prevalence of NTDs in Turkey found a statistically significant increase in NTDs and anencephaly after 1986 from 2.12 and 1.29 per 1000 births to 4.39 and 2.46 per 1000 births, respectively (Mocan et al., 1990). The approximate ratio of isolated NTD cases to NTD cases with co-occurring anomalies was 3:1. Isolated NTD cases were most prevalent in Northern Europe. Southern Europe reported a higher ratio of isolated NTD cases to NTD cases with co-occurring anomalies than Western Europe. Eastern Europe did not report any genetic syndromes or syndromes with unknown etiology. From 2000 to 2009, the ratio of isolated NTD cases to NTD cases with co-occurring anomalies was greater than 4:1. Northern Europe reported the highest prevalence of NTD cases with genetic syndromes and unknown etiology syndromes. This could be explained by many factors affecting ascertainment and underlying risk factors that could impact disease etiology.

Many factors affect ascertainment of co-occurring anomalies such as birth type, birthweight, karyotype, post-mortem examination, and condition at discovery. We found that most of the

fetuses/babies were terminated due to fetal anomaly (TOPFA). Of the remaining, there were significantly more livebirths than stillbirths for both isolated NTD cases and co-occurring anomalies. We also found that from a total of 5,426 livebirths and stillbirths, 2,990 of those fetuses/babies were not underweight and deemed to be premature (born before 37 weeks of age and less than 2,500g) (Stanford Children's Health, 2021). For karyotyping, a significant proportion of individuals opted out of getting a karyotype. This could be due to the financial burden likely to be borne by provider that can result from requesting a karyotype that can cost around £1,000 or more (NHS, 2021). Due to the variations in health policies across Europe, the test may not have been available in some lesser European developed countries. If it were available, it would cost substantially more than developed European countries.
Similarly, a significant portion of families did not perform a post-mortem examination due to the underlying financial burden (NHS, 2021). This was evident for fetuses/babies with isolated NTD cases and NTD cases with co-occurring anomalies. In terms of the status of the fetus/baby when the anomaly was discovered, approximately 9,665 fetuses/babies were alive. However, since 9,277 fetuses/babies resulted in TOPFA, it can be assumed that only 388 of these fetuses/babies were not terminated. This shows a significant confounding effect that prenatal screening exerts on the ascertainment of co-occurring anomalies.

As stated in the literature, several risk factors are associated with NTDs (CDC, 2020; WHO, 2020). Some of these risk factors are the number of babies/fetuses delivered, the number of malformed in multiple sets, maternal age, paternal age, sex, maternal family history, paternal family history, affected siblings, assisted conception, medication in the first trimester. It is seen that majority of the fetuses/babies delivered were singletons, with more fetuses/babies having isolated NTD cases. In terms of maternal and paternal age, we see that many parents were between the range of 26-35 years of age. EUROCAT's Paris centre reported the highest rate of pregnancies 30 years of age and above at 750 cases, followed by Dublin at 488, East Midlands and South Yorkshire (UK) at 464, and Norway at 464.

Many fetuses did not survive beyond one week of life, indicating the severity and complexity of the NTD. We found that females had a higher proportion of isolated NTDs than NTDs with co-occurring anomalies, like men. Females showed predominance for isolated NTDs, and co-occurring anomalies as compared to men (Seller, 1987). We found similar underlying patterns for maternal and paternal family history with higher reports of isolated NTD cases and NTDs with co-occurring anomalies on the maternal side. We found a higher proportion of both types of NTDs on the maternal side than on the paternal side (Seller, 1987). We also found that siblings were more likely to share isolated NTD diagnoses versus co-occurring anomalies. However, a significant portion of fetuses/babies did not have siblings with any anomalies. There is no significant difference between isolated cases versus co-occurring anomalies for the mothers who took medication during their first trimester. There is a more significant difference for those who did not take any medication with higher proportions of isolated NTD cases than NTD co-occurring anomalies.

In addition, a sensitivity analysis was done to assess the impacts of consanguinity and family history on NTD. Although consanguinity is shown to be a risk factor in the literature, we found a higher proportion of isolated NTD cases compared to NTDs with co-occurring anomalies (WHO, 2020). We found similar outputs for paternal and maternal family history but a drastic impact when consanguinity was controlled.

Lastly, the number of NTD cases is widespread across Europe. Most countries (except the UK) still do not have mandated folic fortification (Morris et al., 2021; Pachón et al., 2013). The protective effects of folate on NTDs are well documented within the literature (Dolin et al., 2018; RM, 2007). However, folate fortification mandates are still non-existent across Europe. Since fortification has not been mandated in Europe, no significant overall decline at the birth of NTDs was observed during the period 1991-2011, whereas in countries that mandated folic acid fortification of flours, a decrease in NTD prevalence was observed (Castillo-Lancellotti, Tur, & Uauy, 2013; Khoshnood et al., 2015). However, the total number of births has increased across Europe, particularly in the more developed parts of Europe. Although folate supplementation recommendations exist, less than 50% of women have blood folate levels considered optimal to prevent NTDs (FSAI, 2016). A possible explanation for an increase in the prevalence of NTDs in Ireland may be explained by the low blood folate levels, which is essential for red blood cell formation and healthy cell growth and function (Berg, 1999; Ebara, 2017). In addition, the EUROCAT centres in this dataset are scarce in Eastern Europe, resulting in fewer cases. The UK has six centres compared to the two centres in Eastern Europe. Northern Europe has had more centres operating since 1980, resulting in more data and case count. Out of 14,703 fetuses, 7,033 (47.8%) were in Northern Europe with 9 centres, 2,310 (15.7%) in Southern Europe 7 centres, 1,111 (7.6%) in Eastern Europe with 2 centres, and 4,249 (28.9%) in Western Europe with 10 centres. Western Europe had the most centres but yielded fewer cases than Northern Europe. The disparities between the European regions could be explained by the overall quality of life, socioeconomic status, dedicated funding towards healthcare, infrastructure, employment and education, and geographical and climate variation (Enjolras, 2016; Mcgill et al., 2016). The only European countries that have mandated folate fortification are Moldova and Kosovo (FFI, 2016). Another possible explanation for the high proportion of Northern Europe's NTD cases could be seasonal variations as seen in other countries (Rough, 2021; Vego and López-Cepero, 2009). Perhaps the cold winters and humid summers may impact NTD presentation, particularly in the frequency of anencephaly and spina bifida births (Maclean and Macleod, 1984).

4.1 Strengths and Limitations

This study has many strengths. Firstly, the dataset contained widespread surveillance data for up to 35 years from 28 registries, representing a total denominator population of 15.7 million births, and included data on fetuses from terminations of pregnancy after prenatal detection. The dataset provided both diagnostic codes (ICD9-BPA and ICD10-BPA) and supplementary text coding for all the anomalies. In addition, the EUROCAT 1.4 guide provided a code mapping scheme and guidelines for grouping congenital anomalies based on subgroups. This approach also allowed for categorizing anomalies into smaller subgroups for further analyses such as abdominal wall defects, congenital heart defects, limb defects, chromosomal defects, etc. Also, we were able to provide rates on multiple co-occurring congenital anomalies based on the NTD subgroup. Since CAs can occur as isolated or with multiple congenital anomalies (MCA), detailed surveillance of MCA cases is essential. They are more sensitive to teratogenic exposure than isolated cases (Calzolari et al., 2014). Lastly, this study employed two algorithms that include unique diagnostic codes, minimizing the chances of missing critical nuances while controlling for exclusions. The second algorithm also employs the steps highlighted in the EUROCAT 1.4 for categorizing isolated NTDs, NTDs with anomalies due to genetic syndrome or sequences, Anomalies secondary to NTDs, and associative anomalies to NTDs. This study is a first of its kind that has reported on and distinguished between isolated NTD cases with and without secondary anomalies, NTD cases with recognized genetic and unknown etiological syndromes, and NTDs with co-occurring anomalies. We were able to further break down the rates of co-occurring anomalies by anomaly subgroup and by NTD subtype.

There were also limitations in this study. The first limitation was handling the coding transition from ICD9 to ICD10. Although a mapping scheme was provided, there were ICD10 codes that did not map on ICD9 (e.g., Arnold Chiari syndrome). This could result in underreporting of case types. Secondly, the dataset had several variables that had more than 50% missing data. This is because of the nature of surveillance data and the sensitive nature of collecting information on pregnancies that were terminated because of the detection of a fetal anomaly. Therefore, missing data varied from 5% to 75%. This made it challenging to draw trends and inferences from certain variables, such as folic (use of folic acid during pregnancy). Text variables also varied in missing data making it difficult to verify diagnostic code entry for individual cases. This also varied between regions, and often, the language would be different in the text section (e.g., English versus French versus Italian). The language barrier or the changes in the syntax often proved challenging to decipher.

Lastly, although two algorithms were developed that followed the EUROCAT 1.4 guide's guidelines, there were limitations in ascertaining cases. Additions needed to be made to the algorithm steps in the EUROCAT 1.4 guide as it was incomplete and did not include ICD9 codes identical to ICD10. Due to the complexity of case presentations, certain assumptions had to be made, and anomalies had to be restricted to a maximum of two or three per individual. For individuals with more than 2 cases that an underlying genetic syndrome or sequence could not explain and was not considered secondary, they were grouped and reported as multiple co-occurring anomalies. Among these fetuses were individuals with combinations for isolated NTDs, but they were grouped under co-occurring anomalies because of the number of anomalies present. This could result in an underreporting of isolated NTD and NTDs associated with genetic syndromes and an overreporting of associative anomalies. However, this assumption was kept constant throughout the analysis and therefore, minimizing the skewing of results.

4.2 Future implications

As discussed, although NTD cases have increased in Europe over the past 35 years, the total population has also increased. With improvements in prenatal screening, certain factors (e.g., karyotyping) did affect ascertainment but data must be cautiously assessed due to proportion of missing data. The highest prevalence was in Eastern Europe. It is recommended to repeat this study with more EUROCAT centres and build upon the existing algorithm to improve case ascertainment. In addition, it would be beneficial to develop case definitions for how anomalies will be categorized with dysmorphologists. The study should also synthesize and highlight information on the impact of voluntary folic acid supplementation on NTD presentation across Europe. A study conducted by Morris and colleagues suggested that failure to implement mandatory folate fortification in 28 European countries is a significant factor for the increase in NTD cases (Morris et al., 2021). The folic acid policy in Europe has shown to be inadequate in its protective effects against NTD rates (BMJ, 2015). There is no clear evidence of a downward trend in neural tube defects in Europe despite seeing a downward trend in the USA (BMJ, 2015; Morris et al., 2021). The European Union (EU), in conjunction with federal health bodies, must strategically develop and implement policies that will mandate folate fortification in Europe. Policies and guidelines around voluntary folic acid consumption during pregnancy have failed to diminish NTD rates and instead have increased the burden on the healthcare system and economy (Antrim, 2003). European countries could increase population awareness about the benefits of starting folic acid supplementation before conception (Antrim, 2003). Since many pregnancies are unplanned, the EU can implement folate fortification to achieve more effective prevention of NTD (Antrim, 2003). This will also decrease any socioeconomic and cultural disparities that may exist due to financial constraints or lack of access. Lastly, regions of Europe, mainly Eastern Europe, require additional funding and a better healthcare system to target the severity of NTDs.

5.0 Conclusion

NTDs have been prevalent across Europe over the past 35 years. Isolated NTDs without secondary anomalies have been the most prevalent, followed by spina bifida with limb defects, congenital heart defects, and abdominal wall defects. It is seen that there are sex-specific impacts on NTD development as well as birth type, birthweight, karyotype, post-mortem examination, and condition at discovery consanguinity, Risk factors such as maternal age, medication use during pregnancy, and condition at discovery have significant impacts on the ascertainment of co-occurring anomalies. Policy recommendations around mandating folate fortification across Europe have been suggested. Further work is needed to highlight the trends in European countries that the current study did not have data on and improve case ascertainment for NTD subgroups. Lastly, the study should be repeated with data from more centres, particularly Eastern Europe to understand the trends and prevalence of NTDs by further developing and refining the algorithms to improve case ascertainment.

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Appendix

Table 9. Categories of anomalies

Category in analysis	***Type of anomaly***	***EUROCAT subgroup***	***ICD Code***	
			ICD10-BPA (2005-2015)	*ICD9-BPA (1980-2004)*
A. *Neural tube defects*				
Primary types of anomalies to be included*; analysis by presence of absence of anomalies of other types and by type of NTD*	*Neural tube defects*	*al3*	*Q00, Q01, Q05*	*740, 741, 742*
	Spina bifida	*al6*	*Q05*	*741*
	Encephalocele	*al5*	*Q01*	*742.0*
	Anencephaly	*al4*	*Q00*	*740*
	Iniencephaly		*Q00.2*	*740.2*
B. *Syndromes associated with NTDs*				
Exclusions from primary analysis*; document exclusions, by type of syndrome and by type of NTD*	*Chromosomal anomalies*	*al88*	*Q90-Q92, Q93, Q96-Q99*	*758.0-758.3, 758.5-758.9*
	Amniotic bands	*al76*	*Q79.80*	*762.80*
	OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects)		*Q64.10*	*759.890*

	Meckel-Gruber syndrome		*Q61.90*	*759.89*
	Other single-gene disorder that includes multiple malformations	*al105*	*Q44.71, Q74.84, Q75.1, Q75.4, Q75.81, Q87, Q93.6, D82.1*	*755.81, 756.01, 756.04, 759.8, 279.10*
***C.** Anomalies secondary to the presence of an NTD*				
Cases of NTDs with any of these anomalies will be classified as ***isolated cases***	*CNS anomalies such as Arnold-Chiari malformation, agenesis, or other anomalies of the corpus callosum, hydrocephaly, microcephaly, diastomatomyelia and similar spinal cord anomalies*		*Q04.0, Q07.0, Q03, Q02, Q06.2, Q06.1, Q06.3-Q06.9*	*742.48, 742.21, 742.3, 742.1, 742.52, 742.51, 742.53, 742.54, 742.58*
	Musculoskeletal anomalies such as hip dysplasia, club foot and other joint deformation or contractures, and spine anomalies that relate to the site of the lesion		*Q65, Q66.0, Q66.8, Q68.2, Q68.8, Q67.5*	*754.3, 754.50, 754.73, 755.6, 756.1*
	Urinary tract dysfunction leading to hydronephrosis or reflux		*Q62.0, Q62.7*	*753.20, 753.48*

	Adrenal gland hypoplasia in cases of anencephaly or other cranial NTD		*E25.0*	*759.10, 759.11, 255.20-255.29*
D. Minor anomalies				
NTDs with minor anomalies affecting one system only, and no other anomalies will be classified as isolated. NTDs with minor anomalies affecting more than one system will be classified as NTDs with anomalies of other systems	*Two lists have been in use. First, the "List of Minor Anomalies for Exclusion up to Birth Year 2004" and second, a list for births from 2005 onwards*	*The list in use for births up to 2004 appears to be shorter than that in use from 2005. We will maximize the harmonization of these lists, and report this.*	*Selected Q codes, or text, as specified in EUROCAT Guide 1.4, Section 3.2*	

Table 10. Previous population-based or multi-hospital studies of NTDs associated with malformations of other systems.

Study	**Location and period**	**Approach to dealing with anomalies secondary to NTD, and anomalies considered to be minor or unreliably reported**	**Total number of cases with NTDs**	**Number of cases with recognized syndromes or sequences**	**NTD cases excluding recognized syndromes or sequences**				**Subgroup analysis**
					NTDs with anomalies of other systems/total and %	*Anencephaly with anomalies of other systems/total and %*	*Spina bifida with anomalies of other systems/total and %*	*Encephalocele with anomalies of other systems/total and %*	
Hall et al., 1988	*Canada, British Columbia, Vancouver, and Victoria*	*Clinical review; included with isolated NTDs*	*512*	*0 (excluded by clinical review)*	*73/512 14.3%*	*30/163 18.4%*	*31/307 10.1%*	*9/30 30%*	*There were 12 cases of multiple NTDs, 3 with anomalies of other systems*
Dolk et al., 1991	*13 registries in Europe, 1980-7*	*Included with isolated NTDs*	*2319*	*70*	*342/2249* 15.2%*	*134/923 14.5%*	*139/1096 12.7%*	*42/191 22.0%*	*Proportion with additional anomalies slightly higher in Continental Europe (17.9%) than in the UK and*

									Ireland (14.6%)
Källén et al., 1998	Central-East France 1976-1994	Included with isolated NTDs	637	11	76/626 12.1%	13/78 16.7%	49/475 10.3%	14/73 19.2%	Site of lesion for spina bifida; lower spina bifida had lowest proportion of cases with additional anomalies; aborted fetuses vs. births – excess of body wall defects in aborted fetuses
	Sweden 1973-1993		1606	26	262/1580 16.6%	76/376 20.2%	123/1047 11.7%	63/157 40.1%	
	California 1983-1992		1643	79	521/1564 33.3%	166/558 29.7%	267/829 32.2%	88/177 49.7%	
McDonnell et al., 1999	East Ireland 1980-1994	Not specified (as EUROCAT registry, likely that included with isolated NTDs)	821	Not specified, but none listed in the table specifying the seven most freque	184/821 22.4%	Information by type of NTD not presented			16 were births from twin pregnancies

				nt additional anomalies					
Stevenson et al., 2004	*US, South Carolina, 1992-2002***	*Not specified, although commented on in discussion, with suggestion that abdominal wall defects, diaphragmatic hernia, Meckel diverticulum and cardiac defects could be secondary to lack of pleural and peritoneal space, and facial clefting and cardiac*	*564*	*51*	*47/513 9.1%*	*28/217 12.9%*	*12/232 5.2%*	*7/64 10.9%*	*Additional anomalies more likely in cases of anencephaly with craniorachischisis than in other cases, and in upper spina bifida; 21 cases (3.7% of 564) were from 20 multiple pregnancies*

		defects due to disturbance of cranial neural crest migration							
Bupp et al., 2015	*US, South Carolina, 1992-2012*	*Hydrocephalus, club foot and "insignificant" anomalies included in isolated cases* *Analysis limited to fetuses and births to 615 women who provided informat*	*606*	*25, but included in cases with anomalies of other systems*	*Mother reported using FA*				*By reported use of supplements containing folic acid (FA) for at least 3 months prior to conception*
					42/163 25.8%	*10/64 15.6%*	*23/79 29.1%*	*9/20 45.0%*	
					Mother did not report using FA				
					92/443 20.8%	*31/176 17.6%*	*34/200 17.0%*	*27/67 40.3%*	*Time trend graphs presented indicating decline in prevalence at birth of isolated*

		*ion on their pregnancies, including folic acid intake****							*NTDs, but not NTDs associated with other anomalies*
Stoll et al., 2011	*France, Bas-Rhin, 1979-2008*	*Club foot included in isolated cases*	*441*	*34, but included in cases with anomalies of other systems*	*90/441 20.4%*	*21/182 11.5%*	*48/202 23.8%*	*21/57 36.8%*	

Parker et al., 2014	*US and Canada, multicentre, 1976-2011* *It is stated that data on fetuses from terminated pregnancies were included, but not routinely collected; the proportion of cases that were fetuses from terminated pregnancies was 19.5% in the pre-fortification period,*	*Included with isolated spina bifida*	*Spina bifida only 1237*	*67*	*N/A*	*N/A*	*223/1164§ 19.1%*	*N/A*	*Proportion of cases with anomalies of other systems was 16.7% pre-fortification and 27.7% post-fortification; 19.8% when reported folic acid intake (diet and supplemental combined) ≥400µg per day, 19.0% when <400µg per day*

	15.7% in post-fortification period								

Sargiotto et al., 2015	*Argentina, multiple hospitals (covering 38.1% of all births), 2009-2013 Termination of pregnancy is not legal, and data on fetuses from terminated pregnancies not included*	*Included with isolated NTDs [It is stated that the classification of Rasmussen et al (PMID: 12797461) was used]*	*695*	*16*	*162/679 23.9%*	*48/212 22.6%*	*72/375 19.2%*	*42/92 45.7%*	*Fortification of wheat flour and its derivatives was mandated in 2002 Proportion of twin pregnancies among affected 5.1%*

**includes 39 cases with iniencephaly (27 of these had an anomaly of another system, 69%)*
***Cites and extends earlier study in South Carolina, 1992-8 (18). More recent analysis of Collins et al. (29) covers period 1992-2007, but does not provide number of cases with specific types of NTDs with recognized syndromes or sequences. In that analysis, there was a total of 916 NTDs, 76 with recognized syndromes or sequences – excluding these cases, the proportion with anomalies of other systems was 9.8% (82/840).*

****Partial overlap with Stevenson et al., 2004 (16). Nine cases excluded from analysis – no explanation that I can find in paper*
§ 1170 minus 6 cases for whom it was unknown whether the spina bifida was isolated or not

Table 11. The EUROCAT Guide table used to make the subgroups for co-occurring anomalies in the algorithms.

EUROCAT Subgroups	ICD10-BPA	ICD9-BPA	Comments	Excluded minor anomalies post-2005	Excluded minor anomalies pre-2005	Subgroup binary variable number (al)
All anomalies *	Q-chapter, D215, D821, D1810^, P350, P351, P371	74, 75, 27910, 2281^, 76076, 76280, 7710, 7711, 77121		Exclude all minor anomalies as specified in Guide 1.4, section 3.2	Exclude all minor anomalies as specified in Guide 1.2 (ICD9 and ICD10)	al1
Nervous system	Q00, Q01, Q02, Q03, Q04, Q05, Q06, Q07	740, 741, 742		Q0461, Q0782		al2
Neural Tube Defects	Q00, Q01, Q05	740, 741, 7420				al3
Anencephalus and similar	Q00	740				al4
Encephalocele	Q01	7420	Exclude if associated with anencephalus subgroup			al5
Spina Bifida	Q05	741	Exclude if associated with anencephalus or encephalocele subgroups			al6
Hydrocephalus	Q03	7423	Exclude hydranencephaly 74232. Exclude association with NTD subgroup			al7
Severe microcephaly	Q02	7421	Exclude association with NTD subgroup			al8
Arhinencephaly / holoprosencephaly	Q041, Q042	74226				al9
Eye	Q10-Q15	743		Q101-Q103, Q105, Q135	74365	al10
Anophthalmos / microphthalmos	Q110, Q111, Q112	7430, 7431				al11
Anophthalmos	Q110, Q111	7430				al12
Congenital cataract	Q120	74332				al13
Congenital glaucoma	Q150	74320				al14
Ear, face and neck	Q16, Q17, Q18	744		Q170-Q175, Q179, Q180-Q182, Q184-Q187, Q1880, Q189	74411, 74412, 7443, 74491	al15
Anotia	Q160	74401				al16

EUROCAT Subgroups	ICD10-BPA	ICD9-BPA	Comments	Excluded minor anomalies post-2005	Excluded minor anomalies pre-2005	Subgroup binary variable number (al)
Congenital Heart Defects	Q20-Q26	745, 746, 7470-7474	Exclude PDA with GA <37 weeks Exclude peripheral pulmonary artery stenosis with GA < 37 weeks	Q2111, Q250 if GA <37 weeks, Q2541, Q256 if GA<37 weeks, Q261	Q250, 7470 if GA <37 weeks **	al17
Severe CHD	Q200, Q201, Q203, Q204, Q212, Q213, Q220, Q224, Q225, Q226, Q230, Q232, Q233, Q234, Q251, Q252, Q262	74500, 74510, 7452, 7453, 7456, 7461, 7462, 74600, 7463, 7465, 7466, 7467, 7471, 74720, 74742	ICD9-BPA has no code for HRH and double outlet right ventricle			al97
Common arterial truncus	Q200	74500				al18
Double outlet right ventricle	Q201	No code				al109
Transposition of great vessels	Q203	74510				al19
Single ventricle	Q204	7453				al20
VSD	Q210	7454				al21
ASD	Q211	7455		Q2111		al22
AVSD	Q212	7456				al23
Tetralogy of Fallot	Q213	7452				al24
Triscuspid atresia and stenosis	Q224	7461				al25
Ebstein's anomaly	Q225	7462				al26
Pulmonary valve stenosis	Q221	74601				al27
Pulmonary valve atresia	Q220	74600				al28
Aortic valve atresia/stenosis	Q230	7463	ICD9-BPA has no code for atresia			al29
Mitral valve anomalies	Q232, Q233	7465, 7466				al110
Hypoplastic left heart	Q234	7467				al30
Hypoplastic right heart	Q226	No code				al31
Coarctation of aorta	Q251	7471				al32
Aortic atresia / interrupted aortic arch	Q252	74720				al111
Total anomalous pulm venous return	Q262	74742				al33
PDA as **only** CHD in term infants (GA +37 weeks)	Q250	7470	Livebirths only			al100
Respiratory	Q300, Q32-Q34	7480, 7484, 74850, 74852, 74858, 7486, 7488	Exclude Q336	Q320, Q331	Q309, 74819	al34
Choanal atresia	Q300	7480				al35
Cystic adenomatous malf of lung	Q3380	No code				al36

EUROCAT Subgroups	ICD10-BPA	ICD9-BPA	Comments	Excluded minor anomalies post-2005	Excluded minor anomalies pre-2005	Subgroup binary variable number (al)
Oro-facial clefts	Q35-Q37	7490, 7491, 7492	Exclude association with holoprosencephaly or anencephaly subgroups			al101
Cleft lip with or without cleft palate	Q36, Q37	7491, 7492	Exclude association with holoprosencephaly or anencephaly subgroups			al102
Cleft palate	Q35	7490	Exclude association with cleft lip subgroup. Exclude association with holoprosencephaly or anencephaly subgroups			al103
Digestive system	Q38-Q45, Q790	750, 751, 7566		Exclude Q381, Q382, Q3850, Q400, Q401, Q4021, Q430, Q4320, Q4381, Q4382	Q381, Q401, 7500, 7506	al40
Oesophageal atresia with or without trachea-oesophageal fistula	Q390-Q391	75030-75031				al41
Duodenal atresia or stenosis	Q410	75110	Exclude if also annular pancreas subgroup			al42
Atresia or stenosis of other parts of small intestine	Q411-Q418	75111-75112				al43
Ano-rectal atresia and stenosis	Q420-Q423	75121-75124				al44
Hirschsprung's disease	Q431	75130-75133				al45
Atresia of bile ducts	Q442	75165				al46
Annular pancreas	Q451	75172				al47
Diaphragmatic hernia	Q790	75661				al48
Abdominal wall defects	Q792, Q793, Q795	75671, 75670, 75679				al49
Gastroschisis	Q793	75671				al50
Omphalocele	Q792	75670				al51
Urinary	Q60-Q64, Q794	75261, 753, 75672		Q610, Q627, Q633		al52
Bilateral renal agenesis including Potter syndrome	Q601, Q606	75300	Exclude unilateral			al53
Multicystic renal dysplasia	Q6140, Q6141	75316				al54
Congenital hydronephrosis	Q620	75320				al55
Bladder exstrophy and / or epispadia	Q640, Q641	75261, 7535				al56
Posterior urethral valve and / or prune belly	Q6420, Q794	75360, 75672				al57

EUROCAT Subgroups	ICD10-BPA	ICD9-BPA	Comments	Excluded minor anomalies post-2005	Excluded minor anomalies pre-2005	Subgroup binary variable number (al)
Genital	Q50-Q52, Q54-Q56	7520-7524, 75260, 75262, 7527-7529		Q523, Q525, Q527, Q5520, Q5521	Q540, 75260#	al58
Hypospadias	Q54	75260			Q540, 75260	al59
Indeterminate sex	Q56	7527				al60
Limb	Q65-Q74	7543-7548, 755		Q653-Q656, Q662-Q669, Q670-Q678, Q680, Q6810, Q6821, Q683-Q685, Q7400	75432, 75452, 75460, 75473, 75481, 75560	al61
Limb reduction defects	Q71-Q73	7552-7554				al62
Club foot – talipes equinovarus	Q660	75450				al66
Hip dislocation and / or dyspasia	Q650-Q652, Q6580, Q6581	75430				al67
Polydactyly	Q69	7550				al68
Syndactyly	Q70	7551				al69
Other anomalies / syndromes						
Skeletal dysplasias	Q7402, Q77, Q7800, Q782-Q788,	No code				al104
Craniosynostosis	Q750	75600				al75
Congenital constriction bands / amniotic band	Q7980	76280				al76
Situs inversus	Q893	7593				al79
Conjoined twins	Q894	7594				al80
Congenital skin disorders	Q80-Q82	7571, 7573		Q825, Q8280	Q825, Q8280, Q8281, 75731, 75738	al81
VATER/VACTERL	Q8726	759895				al112
Vascular disruption anomalies	Q0435, Q411, Q412, Q418, Q710, Q712, Q713, Q720, Q722, Q723, Q730, Q793, Q795, Q7980, Q7982, Q8706	No code				al113
Laterality anomalies	Q206, Q240, Q3381, Q890, Q893	No code				al114
Teratogenic syndromes with malformations	Q86, P350, P351, P371	No code				al82
Fetal alcohol syndrome	Q860	76076				al83
Valproate syndrome	Q8680	No code				al84
Maternal infections resulting in malformations	P350, P351, P371	7710, 7711, 77121				al86

EUROCAT Subgroups	ICD10-BPA	ICD9-BPA	Comments	Excluded minor anomalies post-2005	Excluded minor anomalies pre-2005	Subgroup binary variable number (al)
Genetic syndromes + microdeletions	Q4471, Q6190, Q7484, Q751, Q754, Q7581, Q87, Q936, D821	75581, 75601, 75604, 7598, 27910	Exclude Associations and sequences Exclude Q8703, Q8704, Q8706, Q8708, Q8724, Q8726 Exclude 759801, 759844, 759895			al105
Chromosomal	Q90-Q92, Q93 , Q96-Q99	7580-7583, 7585-7589	Exclude microdeletions Q936			al88
Down syndrome	Q90	7580				al89
Patau syndrome / trisomy 13	Q914-Q917	7581				al90
Edwards syndrome / trisomy 18	Q910-Q913	7582				al91
Turner syndrome	Q96	75860, 75861, 75862, 75869				al92
Klinefelter syndrome	Q980-Q984	7587				al93

* All Anomalies = ALL cases of congenital anomaly, excluding cases with only minor anomalies as defined in Section 3.2 in Guide 1.4 for cases born post-2005. Cases with more than one anomaly are only counted once in the “All Anomalies” subgroup.

^ ICD10 code D1810 (ICD 9 code 2281) is the code for cystic hygroma

** The additional PDA exclusion (<2500 grams) listed in Guide 1.2 is not applied

The ICD9 code for hypospadias did not differentiate between the different types of hypospadias therefore minor cases of hypospadias (glandular I) are excluded at local registry level

Algorithm 1: Categorizing diagnostic codes into system groups

```
from openpyxl import load_workbook, Workbook
import os

col_range = ["DP", "DQ", "DR", "DS", "DT", "DU", "DV", "DW"]
# new_cols = ["FH", "FI", "FJ", "FK", "FL", "FM", "FN", "FO", "FP", "FQ", "FR", "FS", "FT"]
iso_cols = ["FV", "FW", "FX", "FY", "FZ", "GA", "GB", "GC", "GD"]

# Abdominal wall defects
l1 = ["Q792", "Q792-", "Q792__", "Q792___", "Q79200", "Q793", "Q793-", "Q795", "Q7950",
"Q79500",
     "Q7951", "75670", "75671", "756700", "7567000", "756701", "7567004", "7567010",
"7567019", "7567095", "756710",
     "7567104", "756711", "756790", "756791", "7567099", "7567119"]

# Chromosomal
l2 = ["Q900", "Q901", "Q910", "Q911", "Q912", "Q913", "Q914", "Q915", "Q916", "Q917",
"Q920", "Q921", "Q922", "Q923"
     , "Q924", "Q925", "Q926", "Q927", "Q928", "Q9293", "Q932", "Q933", "Q934", "Q935",
"Q937", "Q939", "Q91000",
     "Q960", "Q963", "Q980", "Q985", "Q986", "Q990", "Q998", "Q999", "75833", "758000",
"758231", "75838", "75854",
     "75858", "758600", "758880", "758110", "75820", "75860", "758371", "75853", "Q7581",
"Q870","Q870F","Q872","Q875", "Q878","Q879","Q87800","Q878-", "q936","Q936",
"D821","75989","7598989","759891",
 "75988","759800", "27910"]]

# Urinary
l3 = ["Q600", "Q60000", "Q601", "Q60100", "Q602", "Q603", "Q604", "Q605", "Q606",
"Q611", "Q611-", "Q613", "Q61300",
     "Q614", "Q61400", "Q6140", "Q6141", "Q61410", "Q6142", "Q6148", "Q618", "Q620",
"Q62000", "Q621",
     "Q6210", "Q6211", "Q6218", "Q622", "Q623", "Q6231", "Q6233", "Q624", "Q625",
"Q6250", "Q626", "Q628", "Q630",
     "Q6300", "Q6301", "Q63000", "Q631", "Q6310", "Q63100", "Q6318", "Q63180", "Q632",
"Q632__", "Q6320", "Q6322",
     "Q63200", "Q6328", "Q63280", "Q638", "Q639", "Q63900", "Q640", "Q641", "Q641-",
"Q6410", "Q64100", "Q642",
     "Q6420", "Q643", "Q6430", "Q6433", "Q6442", "Q645", "Q646", "Q647", "Q6473",
"Q6476", "Q64760", "Q648", "Q649", "75261", "753","75672","75300", "75316","7535",
"75360","75672","Q649"]

# Oro-facial
l4 = ["Q35", "Q350", "Q351", "Q351-", "Q3519", "Q35190", "Q353", "Q3530", "Q3539",
"Q355", "Q3559", "Q357", "Q359", "Q359-", "Q3599", "Q35900", "Q360", "Q361", "Q361-",
```

"Q369", "Q369-", "Q3690", "Q3699", "Q370", "Q370-", "Q371", "Q371-", "Q3710", "Q3713", "Q373", "Q374", "Q374-",
"Q37400", "Q375", "Q375___", "Q3750", "Q3759", "Q378", "Q37800", "Q379", "Q379-", "Q3790", "Q3799", "Q37900",
"749000", "749002", "74901", "7490100", "7490194", "74902", "749020", "749030", "749040", "74905", "74906",
"749060", "74907", "749070", "749072", "74908", "74909", "749090", "749095", "749099", "7490999", "7490900",
"7490904", "7490949", "74910", "74911", "74919", "749190", "74920", "749200", "7492095", "74921", "749210",
"749211", "749212", "74922", "74929", "749200", "749201", "749202", "749210", "7492100", "7492104", "749211",
"7492119", "74929", "749290", "7492900", "7492905", "749299", "7492999", "749990"]

\# ear, face, neck
l5 = ["Q160", "Q161", "Q162", "Q164", "Q165", "Q169", "Q183", "744242", "744000", "744010", "74423", "74424",
"744810", "744880", "744120", "744219", "7440100", "744210", "744240", "7442404", "7442429", "74421", "74428",
"744230", "74484", "74488", "7442384", "744238", "7445", "7448199"]

\# respiratory
l6 = ["Q300", "Q301", "Q308", "Q30800", "Q321", "Q324", "Q330", "Q333", "Q3380", "Q33800", "Q334",
"Q338", "Q339", "Q340", "Q3480", "Q349", "748000", "748120", "7481200", "748180", "7481015",
"748188", "7481", "74812", "74818", "748121", "748389", "74850", "74852", "748582", "748501", "74851", "7485100",
"7485106", "748510", "748580", "7485004", "7485104", "748519", "7485194", "7485199", "74858", "7485906",
"7486200", "74868", "74869", "748690", "7486900", "7488960", "7488900", "7488100", "7489299"]

\# eye
l7 = ["Q100", "Q107", "Q110", "Q111", "Q111__", "Q112", "Q112-", "Q113", "Q120", "Q130", "Q130-", "Q131",
"Q132", "Q134", "Q13400", "Q141", "Q148", "Q150", "Q158", "Q159", "743000", "74301", "7430195", "7430199",
"74310", "743010", "743100", "7431000", "743679", "743101", "7432100", "74332", "743320", "74363", "743630",
"7438629", "7436305", "74368", "743682", "74360", "7438", "7436824", "7439", "743900", "7439004", "7439999",
"7430908", "743419", "74300", "743448", "74380", "7439000", "743301", "74352"]

```
# Neuro
l8 = ["""Q00""","""Q000""","""Q000-
""","""Q0000""","""Q0000__""","""Q00000""","""Q0001""","""Q0009""","""Q001""",
      """Q001-
""","""Q001_""","""Q0010""","""Q00100""","""Q0012""","""Q002""","""Q0020""","""Q00200"
"","""Q0021""",

"""Q003""","""Q0039""","""Q0042""","""Q0053""","""Q00579""","""Q01""","""Q010""","""Q0
11""","""Q012""",
      """Q012-""","""Q0120""","""Q01200""","""Q018""","""Q018-
""","""Q0180""","""Q01800""","""Q0182""","""Q0189""",
      """Q019""","""Q019-""","""Q01900""","""Q01--
""","""Q0181""","""Q019_""","""Q010__""","""Q0102""","""Q012___""",
      """Q0183""","""Q02""","""Q020""","""Q02000""","""Q02X""","""Q02--
""","""Q030""","""Q030-""","""Q03000""",

"""Q031""","""Q038""","""Q039""","""Q03100""","""Q03900""","""Q03""","""Q0399""","""Q0
40""","""Q0400""",
      """Q041""","""Q042""","""Q042-
""","""Q043""","""Q0432""","""Q0433""","""Q0434""","""Q0435""","""Q046""",
      """Q048""","""Q04300""","""Q0430""","""Q048-
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# Heart
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Genital

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wb = load_workbook("Combine_A.xlsx")
ws = wb["Combined"]

# wb_iso = load_workbook("results_LATEST.xlsx")
# ws_iso = wb_iso["Combined"]

for row in range(2, 14705):
    for cl in new_cols:
        ws['{}{}'.format(cl, row)].value = 0
    for col in col_range:
        cell = ws['{}{}'.format(col, row)].value
        if cell != None and cell != '':
            if cell in l1:
                ws['FH{}'.format(row)].value = 1
            if cell in l2:
                ws['FI{}'.format(row)].value = 1
            if cell in l3:
                ws['FJ{}'.format(row)].value = 1
            if cell in l4:
                ws['FK{}'.format(row)].value = 1
            if cell in l5:
                ws['FL{}'.format(row)].value = 1
            if cell in l6:
                ws['FM{}'.format(row)].value = 1
            if cell in l7:
                ws['FN{}'.format(row)].value = 1
```

```
            if cell in l8:
                ws['FO{}'.format(row)].value = 1
            if cell in l9:
                ws['FP{}'.format(row)].value = 1
            if cell in l10:
                ws['FQ{}'.format(row)].value = 1
            if cell in l11:
                ws['FR{}'.format(row)].value = 1
            if cell in l12:
                ws['FS{}'.format(row)].value = 1
            if cell in l13:
                ws['FT{}'.format(row)].value = 1

wb.save("results.xlsx")

# for row in range(2, 14705):
#     for cl in iso_cols:
#         ws_iso['{}{}'.format(cl, row)].value = 0
#     for col in col_range:
#         cell = ws_iso['{}{}'.format(col, row)].value
#         if cell != None and cell != '':
#             if cell in i1:
#                 ws_iso['FV{}'.format(row)].value = 1
#             if cell in i2:
#                 ws_iso['FW{}'.format(row)].value = 1
#             if cell in i3:
#                 ws_iso['FX{}'.format(row)].value = 1
#             if cell in i4:
#                 ws_iso['FY{}'.format(row)].value = 1
#             if cell in i5:
#                 ws_iso['FZ{}'.format(row)].value = 1
#             if cell in i6:
#                 ws_iso['GA{}'.format(row)].value = 1
#             if cell in i7:
#                 ws_iso['GB{}'.format(row)].value = 1
#             if cell in i8:
#                 ws_iso['GC{}'.format(row)].value = 1
#             if cell in i9:
#                 ws_iso['GD{}'.format(row)].value = 1
#
# wb_iso.save("IsoResults.xlsx")
```

Algorithm 2: Generating Isolated NTD cases

```
import pandas as pd
from openpyxl import load_workbook
```

```
file = pd.read_excel('file://Users/aqeel/Desktop/results_LATEST-1.xlsx',
sheet_name='Combined', engine='openpyxl')
col_range = ["malfo1", "malfo2", "malfo3", "malfo4", "malfo5", "malfo6", "malfo7", "malfo8"]

fn = '/Users/aqeel/Desktop/results_LATEST-1.xlsx'
df = pd.read_excel(fn, engine='openpyxl', header=None)
df2 = pd.DataFrame({'Data': [1]})
df3 = pd.DataFrame({'Data': [0]})

subset = file[col_range]

def update_iso(x):
    writer = pd.ExcelWriter(fn, engine='openpyxl')
    book = load_workbook(fn)
    writer.book = book
    writer.sheets = dict((ws.title, ws) for ws in book.worksheets)
    df.to_excel(writer, sheet_name='Combined', header=None, index=False)
    for i in range(0, 14703):
        if x[i] == True:
            df2.to_excel(writer, sheet_name='Combined', header=None, index=False,
                startcol=176,startrow=i+1)
    writer.save()

# In[58]:
import re

step1 = '^Q9[0126789].*|^Q93[^6].*|^759.*'
step5 = '^Q0[015].*|^74[01].*|^7420.*'

def apply_rules_step5(x):
    exclude = False
    match = False

    x = x.dropna()

    if len(x) > 0:
        col1 = x[0]

        res = re.match(step5, col1)

        if res and len(x) == 1:
            match = True

    return match
```

```
res = subset.apply(apply_rules_step5, axis=1)

update_iso(res)

post_step5 = subset[res]
print(post_step5)

# In[63]:
step8col1A = '^Q0[015].*|^74[01].*|^7420.*'
step8col2A = '^Q3[567 ].*|^7490.*|^7491.*|^7492.*'


def apply_rules_step8(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1A = x[0]

        if len(x) == 2:
            col2A = x[1]
        else:
            return False

        col1_res = re.match(step8col1A, col1A)
        col2_res = re.match(step8col2A, col2A)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step8, axis=1)
update_iso(res)
post_step8A = subset[res]

print(post_step8A)

# In[64]:

step8col1 = '^Q3[567 ].*|^7490.*|^7491.*|^7492.*'
```

```
step8col2 = '^Q0[015].*|^74[01].*|^7420.*'


def apply_rules_step8(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step8col1, col1)
        col2_res = re.match(step8col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step8, axis=1)
update_iso(res)

post_step8B = subset[res]

print(post_step8B)

# In[65]:


step198col1A = step5
step198col2A = '^Q95.*|^7584.*'


def apply_rules_step19(x):
    match = False

    x = x.dropna()
```

```
    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step8col1A, col1)
        col2_res = re.match(step8col2A, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step19, axis=1)
update_iso(res)
post_step19A = subset[res]

print(post_step19A)

# In[67]:

step19col1 = '^Q95.*|^7584.*'
step19col2 = step5


def apply_rules_step19(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False
```

```
        col1_res = re.match(step8col1, col1)
        col2_res = re.match(step8col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step19, axis=1)
update_iso(res)
post_step19 = subset[res]

print(post_step19)

# In[69]:


step21col1 = '^741.*|^7545.*|^7423.*|^Q05.*|^Q66.*|^Q03.*'
step21col2 = '^741.*|^7545.*|^7423.*|^Q05.*|^Q66.*|^Q03.*'
step21col3 = '^741.*|^7545.*|^7423.*|^Q05.*|^Q66.*|^Q03.*'


def apply_rules_step21(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 3:
            col2 = x[1]
            col3 = x[2]
        else:
            return False

        col1_res = re.match(step21col1, col1)
        col2_res = re.match(step21col2, col2)
        col3_res = re.match(step21col3, col3)

        if col1_res and col2_res and col3_res:
```

```
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step21, axis=1)
update_iso(res)
post_step21 = subset[res]

print(post_step21)

# In[ ]:

step22col1 = '^740.*|^75911.*|^Q891.*|^Q00.*'
step22col2 = '^740.*|^Q00.*|^75911.*|^Q891.*'


def apply_rules_step22(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]

        else:
            return False

        col1_res = re.match(step22col1, col1)
        col2_res = re.match(step22col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step22, axis=1)
```

```
update_iso(res)
post_step22 = subset[res]

print(post_step22)

step23col1A = '^Q05.*|^Q070.*'
step23col2A = '^Q05.*|^Q070.*'


def apply_rules_step23A(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step23col1A, col1)
        col2_res = re.match(step23col2A, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step23A, axis=1)
update_iso(res)
post_step23A = subset[res]

print(post_step23A)

step23col1B = '^Q01.*|^Q070.*'
step23col2B = '^Q01.*|^Q070.*'


def apply_rules_step23B(x):
    match = False
```

```
    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step23col1B, col1)
        col2_res = re.match(step23col2B, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step23B, axis=1)
update_iso(res)
post_step23B = subset[res]

print(post_step23B)

step24col1 = step5
step24col2 =
'^Q02.*|^Q062.*|^Q061.*|Q063.*|^Q069.*|^74248.*|^74221.*|^7421.*|^74252.*|^74251.*|^7425
3.*|^74254.*|^74258.*'


def apply_rules_step24(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
```

```
        else:
            return False

        col1_res = re.match(step24col1, col1)
        col2_res = re.match(step24col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step24, axis=1)
update_iso(res)
post_step24 = subset[res]

print(post_step24)

step25col1 =
'^Q02.*|^Q062.*|^Q061.*|Q063.*|^Q069.*|^74248.*|^74221.*|^7421.*|^74252.*|^74251.*|^7425
3.*|^74254.*|^74258.*'
step25col2 = step5


def apply_rules_step25(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step25col1, col1)
        col2_res = re.match(step25col2, col2)

        if col1_res and col2_res:
            match = True
```

```
        return match
    else:
        return False


res = subset.apply(apply_rules_step25, axis=1)
update_iso(res)
post_step25 = subset[res]

print(post_step25)

step26col1 = step5
step26col2 =
'^Q65.*|^Q660.*|^Q668.*|^Q682.*|^Q688.*|^Q675.*|^7543.*|^75450.*|^75473.*|^7556.*|^7561.
*'


def apply_rules_step26(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step26col1, col1)
        col2_res = re.match(step26col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step26, axis=1)
update_iso(res)
post_step26 = subset[res]
```

```
print(post_step26)

step27col1 =
'^Q65.*|^Q660.*|^Q668.*|^Q682.*|^Q688.*|^Q675.*|^7543.*|^75450.*|^75473.*|^7556.*|^7561.
*'
step27col2 = step5


def apply_rules_step27(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step27col1, col1)
        col2_res = re.match(step27col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step27, axis=1)
update_iso(res)
post_step27 = subset[res]

print(post_step27)

step28col1 = step5
step28col2 = '^Q620.*| ^ Q627. * | ^ 75320. * | 75348. * '


def apply_rules_step28(x):
    match = False
```

```
    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step28col1, col1)
        col2_res = re.match(step28col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step28, axis=1)
update_iso(res)
post_step28 = subset[res]

print(post_step28)

step29col1 = '^Q620.*| ^ Q627. * | ^ 75320. * | 75348. * '
step29col2 = step5


def apply_rules_step29(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False
```

```
        col1_res = re.match(step29col1, col1)
        col2_res = re.match(step29col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step29, axis=1)
update_iso(res)
post_step29 = subset[res]

print(post_step29)

step30col1 = step5
step30col2 = '^E250.*|75910.*|^25520.*|25529.*'


def apply_rules_step30(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step30col1, col1)
        col2_res = re.match(step30col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False
```

```
res = subset.apply(apply_rules_step30, axis=1)
update_iso(res)
post_step30 = subset[res]

print(post_step30)

step31col1 = '^E250.*|75910.*|^25520.*|25529.*'
step31col2 = step5


def apply_rules_step31(x):
    match = False

    x = x.dropna()

    if len(x) > 0:

        col1 = x[0]

        if len(x) == 2:
            col2 = x[1]
        else:
            return False

        col1_res = re.match(step31col1, col1)
        col2_res = re.match(step31col2, col2)

        if col1_res and col2_res:
            match = True

        return match
    else:
        return False


res = subset.apply(apply_rules_step31, axis=1)
update_iso(res)
post_step31 = subset[res]

print(post_step31)
```