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# Surveillance of multiple congenital anomalies; searching for new associations

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**Article**

**Keywords:** Multiple congenital anomalies, Associations, surveillance

**Posted Date:** March 13th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-2565779/v1

**Additional Declarations:** There is no duality of interest

**Version of Record:** A version of this preprint was published at European Journal of Human Genetics on December 5th, 2023. See the published version at https://doi.org/10.1038/s41431-023-01502-w.

## Abstract

Many human teratogens are associated with a spectrum of congenital anomalies rather than a single defect, and therefore the identification of congenital anomalies occurring together more frequently than expected may improve the detection of teratogens. Thirty-two EUROCAT congenital anomaly registries covering 6,599,765 births provided 123,566 cases with one or more major congenital anomaly (excluding chromosomal and genetic syndromes) for the birth years 2008 to 2016. The EUROCAT multiple congenital anomaly algorithm identified 8,804 cases with two or more major congenital anomalies in different organ systems, that were not recognised as part of a syndrome or sequence. For each pair of anomalies, the odds of a case having both anomalies relative to having only one anomaly was calculated and the p-value estimated using a two-sided Fisher's exact test. The Benjamini-Hochberg procedure adjusted p-values to control the false discovery rate and pairs of anomalies with adjusted p-values < 0.05 were identified. A total of 1,386 combinations of two anomalies were analysed. Out of the 31 statistically significant positive associations identified, 20 were found to be known associations or sequences already described in the literature and 11 were considered "potential new associations" by the EUROCAT Coding and Classification Committee. After review of the literature and detailed examination of the individual cases with the anomaly pairs, six pairs remained classified as new associations. In summary, systematically searching for congenital anomalies occurring together more frequently than expected using the EUROCAT database is worthwhile and has identified six new associations that merit further investigation.


## Introduction

Identifying new teratogens is one of the main goals of a congenital anomaly registry. As many known human teratogens are associated with a spectrum of congenital anomalies rather than an isolated anomaly (1–5), identification of cases with multiple congenital anomalies that occur together more frequently than would be expected due to chance alone is likely to be more sensitive in the detection of teratogens. Around three out of four fetuses with a congenital anomaly have an isolated anomaly (6). Among the remaining fetuses many of these anomalies are due to a chromosomal anomaly (for example Down syndrome), a single gene defect (for example Noonan syndrome) or a known teratogen (for example cytomegalovirus infection), but some fetuses have more than one major anomaly without known aetiology. Multiple congenital anomalies may also occur as a consequence of a single primary anomaly (for example, the Potter sequence resulting from renal agenesis and with secondary lung hypoplasia and clubfoot). It is therefore important to identify cases with two or more congenital anomalies in different organ systems, where the pattern of anomalies has not been recognised as part of a syndrome, known association or sequence as these could indicate unknown teratogens or new associations. Around 2% of all births have a congenital anomaly, but multiple congenital anomalies occur in around 16 per 10,000 births, with specific combinations of anomalies being even rarer (7). Therefore, it is necessary to analyse the data from large datasets with sufficient cases.

The aim of this study was to analyse data from the EUROCAT network of congenital anomaly registries collected from births between 2008 and 2016. A method to automatically identify all pairs or triplets of anomalies occurring more frequently than would be expected due to chance alone was developed. Once such pairs/triplets were identified, the literature was searched to determine if such pairs/triplets of anomalies had already been identified as being part of an association or sequence. Any new pairs of anomalies were examined in greater detail by the registries to determine if any genetic test results were available.

## Subjects And Methods

The first step in the identification of multiple anomalies is correct case classification. The EUROCAT multiple congenital anomaly algorithm has been developed in collaboration between EUROCAT Central Registry and the Coding and Classification Committee and continuously improved since 2004 [6]. The members of the Coding and Classification Committee are geneticists and paediatricians. The algorithm classifies cases into different groups based on ICD-10/British Paediatric Association (BPA) codes. The aim of the algorithm is to classify congenital anomaly cases into:

(a) Chromosomal syndromes: All cases where an unbalanced chromosomal anomaly has been diagnosed, irrespective of types of anatomically defined anomalies.

(b) Genetic and environmental syndromes: All cases due to a single gene defect or a known environmental teratogen, irrespective of types of anatomically defined anomalies. This includes skeletal dysplasias and hereditary skin disorders.

(c) Isolated anomalies: All cases with one congenital anomaly/ anomalies occurring in only one organ subgroup or with a known sequence where multiple congenital anomalies cascade as a consequence of a single primary anomaly.
(d) Multiple congenital anomalies: Cases with two or more major congenital anomalies in different organ systems, where the pattern of anomalies has not previously been recognised as part of a syndrome or sequence.

Papers published in 2011 and 2014 describe the methodology and results of the first 2 years of data(6, 7). The computer algorithm allocates 90% of all EUROCAT cases into classification groups (a), (b) or (c). Approximately 10% of cases are classified by the computer as potential multiple cases and these cases were reviewed by three EUROCAT geneticists to reach agreement for classification as true multiple congenital anomaly cases (d) or allocation to another group. A web-based system for review of cases has been developed, which allows easy and fast review of many cases and transfer of the final decision back to the central database. If two geneticists agreed on a case classification, this was considered the final decision. If all three geneticists disagreed or one of them classified the case for query, the moderator made the final decision.

Thirty-two full member registries covering 6,599,765 births provided 154,154 cases with one or more major congenital anomalies born between 2008 to 2016. Cases with chromosomal and genetic syndromes, skeletal dysplasias or hereditary skin disorders were excluded resulting in 123,566 cases for inclusion in this analysis.

## Statistical Methods

Sixty EUROCAT congenital anomaly subgroups were used in the analysis (Table A, Annex); 57 specific congenital anomaly subgroups and three more general congenital anomaly subgroups (neural tube defects (NTDs), congenital heart defects (CHD) and Severe CHD(8)).

## Analysis Of Multiple Congenital Anomaly Cases Only

All cases classified above as multiple congenital anomaly cases were analysed as follows. For each pair of anomalies (say A and B) the odds of a case having anomaly B given that it had anomaly A relative to the odds of a case having anomaly B given that it did not have anomaly A was calculated and the associated p-value estimated using a two-sided Fisher's exact test. (Note that the odds ratio for anomaly A given anomaly B is identical to the odds ratio for anomaly B given anomaly A – so only one test was performed for each anomaly pair). The relative odds were not calculated for pairs of anomalies included in the same organ or system (for instance ventricular septal defect (VSD) and any other cardiac anomaly). They were also not calculated for clubfoot with spina bifida or renal dysplasia as clubfoot is considered to arise as a result of the occurrence of spina bifida or renal dysplasia. Finally, they were not calculated for situs inversus and any cardiac anomaly as this association is part of the heterotaxy spectrum.

Multiple testing procedures were carried out using the Benjamini-Hochberg procedure to control the false discovery rate. This gave a corrected overall p-value to determine statistical significance and thus adjusted p-values were calculated. Pairs of anomalies with adjusted p-values < 0.05 were examined further. The analysis was repeated for males and females separately as hypospadias is only present in males (33 cases of indeterminate sex and 489 cases with missing sex were excluded).

Logistic regression models were used to examine associations between three anomalies. Each anomaly in turn was regressed on two other anomalies and the interaction term provided an estimate of the odds ratio for all three anomalies given any of

the other two anomalies. As before, sets of anomalies known to be related were excluded, and the Benjamini-Hochberg procedure to control the false discovery rate was applied to obtain adjusted p-values.

## Analysis Of All Cases With An Anomaly

The above analysis was repeated on the population of all anomaly cases (n = 123,566), not just those with multiple anomalies. The number of cases with both anomalies remains the same, but the number of cases with each individual anomaly increases due to the inclusion of cases with only one anomaly. The estimated relative odds were therefore inflated, and the p-values reduced. We therefore only examined pairs of anomalies with adjusted p-values < 0.01, rather than the < 0.05 cut-off used above.

## Results Of The Statistical Analysis

The EUROCAT multiple congenital anomaly algorithm followed by review by three EUROCAT geneticists identified 8,804 (7.1%; 95% confidence interval (CI): 7.0-7.3) cases for the nine years with two or more anomalies out of all 123,566 cases without a genetic disorder. The proportion of multiple anomaly cases was greater in males (7.1%; 95%CI: 6.9–7.3) than in females (6.8%; 95%CI 6.5-7.0), though not statistically significant.

A total of 31 statistically significant positive associations between two EUROCAT subgroups were found when analysing the cases with multiple congenital anomalies only and judging significance from an adjusted p-value < 0.05. These results are very similar to those obtained by analysing all cases with a congenital anomaly (rather than just those with multiple anomalies) and selecting those associations with an adjusted p-value of < 0.01 (Tables 1 and 2). The results were also similar when males and female were analysed separately.

Table 1
Identification of 20 known associations amongst the statistically significant positive associations between two EUROCAT subgroups

| | | | | Analysing multiples only | | Analysing all anomaly cases | |
|---|---|---|---|---|---|---|---|
| **Anomaly 1** | **Anomaly 2** | **Results of review by EUROCAT Coding and Classification Committee** | **No. of cases with both anomalies** | **Odds Ratio** | **Adjusted P Value** | **Odds Ratio** | **Adjusted P Value** |
| Omphalocele | Limb reduction | Limb-body-wall complex | 46 | 1.59 | 0.0477 | 5.35 | < 0.0001 |
| Encephalocele | Limb reduction | Limb-body-wall-complex | 15 | 2.3 | 0.0408 | 2.29 | 0.012 |
| Ano-rectal atresia / stenosis | Bladder exstrophy | OEIS Complex | 43 | 9.53 | < 0.0001 | 31.66 | < 0.0001 |
| Omphalocele | Bladder exstrophy | OEIS Complex | 43 | 17.68 | < 0.001 | 38.14 | < 0.001 |
| Spina bifida | Bladder exstrophy | OEIS Complex | 24 | 11.77 | < 0.0001 | 5.04 | < 0.0001 |
| VSD | Oesophageal atresia | VACTERL association | 165 | 1.53 | 0.0003 | 2.01 | < 0.0001 |
| Ano-rectal atresia / stenosis | Limb reduction | VACTERL association | 79 | 1.63 | 0.0028 | 8.14 | < 0.0001 |
| Ano-rectal atresia / stenosis | Renal Dysplasia | VACTERL association | 66 | 2.81 | < 0.0001 | 6.65 | < 0.0001 |
| Ano-rectal atresia / stenosis | Bilateral renal agenesis | VACTERL association | 42 | 5.03 | < 0.0001 | 15.55 | < 0.0001 |
| Tetralogy of fallot | Oesophageal atresia | VACTERL association | 40 | 2.49 | < 0.0001 | 5.77 | < 0.0001 |
| PDA only CHD in term infant | Diaphragmatic hernia | Associated anomalies | 22 | 2.3 | 0.0095 | 1.78 | 0.033 |
| Arhinencephaly / holoprosencephaly | Anophthalmos / microhpthalmos | Associated anomalies | 15 | 8.42 | < 0.0001 | 25 | < 0.0001 |
| Neural tube defects | Omphalocele | Known association | 111 | 5.65 | < 0.0001 | 5.23 | < 0.0001 |
| Anencephalus & similar | Omphalocele | Known association | 56 | 8.47 | < 0.0001 | 5.96 | < 0.0001 |
| Spina bifida | Omphalocele | Known association | 45 | 3.97 | < 0.0001 | 3.92 | < 0.0001 |
| Neural tube defects | Bladder exstrophy | Known association | 24 | 5.17 | < 0.0001 | 2.4 | 0.001 |
| Bilateral renal agenesis | Limb reduction defect | Known association | 18 | 2.12 | 0.0408 | 1.6 | 0.001 |
| Posterior urethral valve | Clubfoot | Sequence | 15 | 2.44 | 0.0325 | 0.65 | 0.195 |
| Ano-rectal atresia / stenosis | Posterior urethral valve | Mainly Prune Belly Sequence (11/14) | 14 | 2.33 | 0.0497 | 3.99 | < 0.0001 |

VSD = ventricular septal defect, PDA = Patent ductus arteriosus, CHD = congenital heart defects

| | | | | Analysing multiples only | | Analysing all anomaly cases | |
|---|---|---|---|---|---|---|---|
| **Anomaly 1** | **Anomaly 2** | **Results of review by EUROCAT Coding and Classification Committee** | **No. of cases with both anomalies** | **Odds Ratio** | **Adjusted P Value** | **Odds Ratio** | **Adjusted P Value** |
| Hydrocephalus | Anophthalmos / micropthalmos | Explained by coding errors | 21 | 2.45 | 0.0056 | 6.57 | < 0.0001 |
| VSD = ventricular septal defect, PDA = Patent ductus arteriosus, CHD = congenital heart defects | | | | | | | |

Table 2
Conclusions after literature reviews and individual case review by the EUROCAT Coding and Classification Committee for the remaining 11 statistically significant positive associations between two EUROCAT anomaly subgroups (ordered by odds ratios)

| | | | No. of cases with both anomalies | Analysing multiples only | | Analysing all anomaly cases | | |
|---|---|---|---|---|---|---|---|---|
| **Anomaly 1** | **Anomaly 2** | **Comments and conclusions after literature and individual case review by EUROACT Coding and Classification Committee** | | **Odds Ratio** | **Adj P Value[1]** | **Odds Ratio** | **Adj P Value[1]** | **% with karyotype known[2]** |
| Encephalocele | Anophthalmos / micropthalmos | 7 TOPFA; 6 occipital encephalocele & 4 nasofrontal encephalocele. Very few cases are reported in the literature: New association | 10 | 6.74 | 0.0001 | 13.15 | < 0.0001 | 70 |
| Ebstein's anomaly | Cleft lip | Association reported in literature for 2 cases with genetic diagnoses. Some of the 5 cases here may have an undiagnosed genetic disorder. In addition, significance is borderline: **Weak evidence for new association.** | 5 | 5.12 | 0.0437 | 0.61 | 0.556 | 75 |
| Microcephaly | Congenital Cataract | 10 cases reported with dysmorphic features or suspected genetic anomaly. **Not a new association** | 12 | 4.17 | 0.0013 | 3.12 | < 0.0001 | 67 |

[1] P value adjusted from multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (FDR)

[2]Norway excluded

AVSD = atrioventricular septal defect, CHD = congenital heart defect, NTD = neural tube defect

| Anomaly 1 | Anomaly 2 | Comments and conclusions after literature and individual case review by EUROACT Coding and Classification Committee | No. of cases with both anomalies | Analysing multiples only | | Analysing all anomaly cases | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Odds Ratio | Adj P Value[1] | Odds Ratio | Adj P Value[1] | % with karyotype known[2] |
| Anophthalmos / micropthalmos | Cleft lip | Majority cases diagnosed prenatally. 16 cases had associated cerebral anomalies (hydrocephaly, encephalocele or anomalies of corpus callosum). The association has not been found in the literature: **New association** | 27 | 3.62 | < 0.0001 | 4.43 | < 0.0001 | 64 |
| Anencephalus | Gastroschisis | See (12)NTD and gastroschisis below: (13)<br><br>**Weak evidence for new association.** | 9 | 3.36 | 0.0205 | 0.39 | 0.005 | 38 |
| Hydrocephaly | Hypoplastic right heart | All cases were male. 1 miscoded Hypoplastic left heart. Remaining cases had other anomalies (6 renal/genital). Literature describes a few cases with this association (mainly pulmonary valve stenosis), but with a genetic background(14, 15) Association of hydrocephaly and variable heart and other defects is also seen in VACTERL-H syndrome(16), but genital anomalies are not part of this. **New association** | 10 | 3.34 | 0.0178 | 2.27 | 0.039 | 70 |

[1] P value adjusted from multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (FDR)

[2]Norway excluded

AVSD = atrioventricular septal defect, CHD = congenital heart defect, NTD = neural tube defect

| Anomaly 1 | Anomaly 2 | Comments and conclusions after literature and individual case review by EUROACT Coding and Classification Committee | No. of cases with both anomalies | Analysing multiples only | | Analysing all anomaly cases | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Odds Ratio | Adj P Value[1] | Odds Ratio | Adj P Value[1] | % with karyotype known[2] |
| AVSD | Duodenal atresia / stenosis | See severe CHD and Duodenal atresia/ stenosis below: **New association** | 11 | 2.65 | 0.0382 | 4.81 | < 0.0001 | 50 |
| Tetralogy of fallot | Duodenal atresia / stenosis | See severe CHD and Duodenal atresia/ stenosis below: **New association** | 11 | 2.53 | 0.0476 | 2.89 | 0.006 | 22 |
| Encephalocele | Cleft lip | 2 incorrect codes. 10 cases TOPFA. Both anterior and posterior encephalocele observed. Few cases with encephalocele and cleft lip reported in literature (17, 18). Authors of case report of child with holoprosencephaly, orofacial cleft and fronto-nasal encephalocele discuss if it is a new syndrome (19). **Weak evidence for new association.** | 14 | 2.41 | 0.0412 | 0.95 | 1.0 | 50 |

[1] P value adjusted from multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (FDR)

[2]Norway excluded

AVSD = atrioventricular septal defect, CHD = congenital heart defect, NTD = neural tube defect

| Anomaly 1 | Anomaly 2 | Comments and conclusions after literature and individual case review by EUROACT Coding and Classification Committee | No. of cases with both anomalies | Analysing multiples only | | Analysing all anomaly cases | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Odds Ratio | Adj P Value[1] | Odds Ratio | Adj P Value[1] | % with karyotype known[2] |
| Neural tube defects | Gastroschisis | Association with NTD and omphalocele is well-described (12). Only 1 report in literature describing cases with NTD and gastroschisis (13). Due to the potential misclassification of omphalocele and gastroschisis, diagnosis of gastroschisis was checked and only 1 was incorrect. Only 8 cases with NTD and gastroschisis as the only anomalies: **Weak evidence for new association.** | 18 | 2.33 | 0.0182 | 0.32 | < 0.0001 | 50 |
| Severe CHD | Duodenal atresia / stenosis | Association of non-genetic duodenal atresia and Tetralogy of Fallot or AVSD seems new, but 50% missing karyotype information may explain this finding. There is little published literature, but (20) discuss a familial disorder with duodenal atresia and tetralogy of Fallot: **New association** | 41 | 2.02 | 0.0043 | 1.59 | 0.022 | 51 |

[1] P value adjusted from multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (FDR)

[2]Norway excluded

AVSD = atrioventricular septal defect, CHD = congenital heart defect, NTD = neural tube defect

There were no combinations of three anomaly subgroups that were statistically significantly more likely to occur than any of the combinations of two anomalies.

## Results Of The Review By The Eurocat Coding And Classification Committee

The list of the 31 significant associations was reviewed by the EUROCAT Coding and Classification Committee to determine if the pairs identified were potential unrecognised associations or were part of a known association or sequence or had occurred due to other reasons. Nineteen associations were known associations or sequences already described in the literature (Table 1). Associations were thought to be part of limb body wall complex, OEIS complex (omphalocele-exstrophy-imperforate anus-spinal defects), VACTERL association or sequences like Prune Belly Sequence. One association was explained by coding errors of the anomalies included in the association and related to a known association (also Table 1).

# Potential new associations

Eleven associations were determined “unknown” and were selected for literature reviews and reviews of the individual cases with the association by the Coding and Classification Committee members in collaboration with the local registries (Table 2). For some associations such as atrioventricular septal defect and duodenal atresia the registries checked for the most recent karyotype testing that may have been performed after the case was notified to the registry. Details of these investigations including proportion of cases with known karyotype are given in Table 2 ordered according to the odds ratios.

One anomaly pair, microcephaly and congenital cataract, was judged not to be a new association because most of these cases were suspected as having an undiagnosed genetic disorder. Four anomaly pairs were judged to have weak evidence in the literature of an association: Ebstein’s anomaly and cleft lip; anencephalus and gastroschisis; NTDs and gastroschisis; encephalocele and cleft lip. Six anomaly pairs were judged to have evidence of a new association and will be investigated in further detail during annual surveillance in EUROCAT: encephalocele and anophthalmos/micropthalmos; cleft lip and anophthalmos/microphthalmos; hydrocephaly and hypoplastic right heart; atrioventricular septal defect and duodenal atresia/stenosis; tetralogy of Fallot and duodenal atresia/stenosis; severe CHD and duodenal atresia/stenosis.

## Discussion

This study examined 1,386 different combinations of two anomalies occurring in the same case and identified six pairs of anomalies that occurred more frequently than would be expected due to chance alone and were not part of any known association or sequence. The EUROCAT surveillance on these six anomaly pairs will be continued as part of the routine surveillance for clusters and trends.

Other studies have used similar approaches to identify new associations. For example the Co-occuring defect analysis approach recommended by Benjamin et al (2019)(9) and used by Howley et al (2022)(10), is based on a modified observed-to-expected (O/E) ratio of co-occurring birth defects (congenital anomalies) that was originally proposed by Khoury, James, and Erickson (1990)(11). The method adjusts for the tendency of birth defects to cluster with other major malformations. The data analysed in our study firstly only compared the occurrence of a pair of anomalies within cases that had at least two anomalies and therefore the tendency to cluster did not need to be adjusted for in the first set of analyses. The second analyses did compare pairs of anomalies to cases with only one anomaly and as expected the odds ratios were higher. However, when adjusted p-values were calculated, a similar set of anomalies was statistically significant at $p < 0.01$. A second important difference between the method adopted by Benjamin et al (2019)(9), was that in this analysis we excluded any cases with known chromosome or genetic anomalies. We wanted to identify any new anomaly clusters – we were not interested in identifying known associations.

The strength of this study was that it was based on data from 32 EUROCAT full member congenital anomaly registries covering 6,599,765 births between 2008 to 2016. EUROCAT has standardised methods of coding and data cleaning which are adopted by all member registries and the data quality is monitored by the use of data quality indicators. The EUROCAT multiple congenital anomaly algorithm identifies all cases with two or more non-genetic major congenital anomalies in different organ systems, where the pattern of anomalies has not been recognised as part of an association or sequence. A limitation of the study was that it was not possible to obtain detailed genetic information on all cases – the researchers were

dependent on the data that had been provided to the registry as the individual cases could not be contacted for more information.

In summary, most associations found by the statistical analysis were known associations already described in the literature. However, there were six new associations that need continued investigation and will be followed by the annual EUROCAT surveillance system.

# Declarations

**No funding was obtained to perform this study**

### Data Availability

The data analysed during this study belong to the individual EUROCAT congenital anomaly registries. Applications to analyse these data will be considered by the JRC-EUROCAT management committee and if they are considered of high scientific merit, permissions for the sharing of the data will be sought by the JRC from the relevant registries.

### Code Availability

The R data analysis programs are available on reasonable request from JM, the corresponding author.

### Acknowledgements.

We would like to acknowledge the work of all the people involved in the EUROCAT Congenital Anomaly Registries for ensuring the availability of such high quality surveillance data. In addition, we would like to acknowledge the work of James Densem in creating software to enable the examination of individual cases with multiple anomalies.

### Author Contributions

JEB,IB,DW,DT,EG : Members of the EUROCAT Coding and Classification Committee and all have contributed to the creation of the algorithm for identifying which are multiple Cas,  have been involved in determining the final classification of the congenital anomaly cases and have performed literature reviews investigating the new multiples identified. They all provided feedback on the draft manuscript.

JM : Performed the statistical analysis and wrote the first draft and all subsequent drafts

EL : Performed the statistical analysis and provided feedback on the draft manuscript

MCA,CCC,CMD,ESD,LJEG,MG,KK,NL,KL,AMK,VN,AN,IP,AP,HR,JR,AR,FR,GS,WW,AKO : Provided the data and feedback on the draft manuscript

**Funding:** No financial assistance was received in support of the study. Each EUROCAT registry is responsible for its own funding for surveillance.

**Ethical Approval:** Ethical approval was not required, as this was an analysis of an existing anonymous data set.

**Competing Interests:** All authors declare that they have no competing financial interests in relation to the work described.

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