TITLE PAGE

Title: Epidemiology of Pierre-Robin sequence in Europe: a population-based EUROCAT study

Running title: Epidemiology of Pierre-Robin sequence

List of the Authors:

Michele Santoro[1], Alessio Coi[1], Ingeborg Barišić[2], Anna Pierini[1,3], Marie-Claude Addor[4], Silvia Baldacci[1], Elisa Ballardini[5], Ljubica Boban[2], Paula Braz[6], Clara Cavero-Carbonell[7], Hermien E K de Walle [8], Elizabeth S Draper[9], Miriam Gatt[10], Martin Haeusler[11], Kari Klungsøyr[12,13], Jennifer J Kurinczuk[14], Anna Materna-Kiryluk[15], Monica Lanzoni[16], Nathalie Lelong[17], Karen Luyt[18], Olatz Mokoroa[19], Carmel Mullaney[20], Vera Nelen[21], Mary T. O'Mahony[22], Isabelle Perthus[23], Hanitra Randrianaivo[24], Judith Rankin[25], Anke Rissmann[26], Florence Rouget[27], Bruno Schaub[28], David Tucker[29], Diana Wellesley[30], Nataliia Zymak-Zakutnia [31], Ester Garne[32]

[1] Unit of Epidemiology of Rare diseases and Congenital anomalies, Institute of Clinical Physiology, National Research Council, Pisa, Italy.

[2] Children's Hospital Zagreb, Centre of Excellence for Reproductive and Regenerative Medicine, Medical School University of Zagreb, Zagreb, Croatia.

[3] Fondazione Toscana Gabriele Monasterio, Pisa, Italy.

[4] Department of Woman-Mother-Child University Medical Center CHUV Lausanne Switzerland

[5] Neonatal Intensive Care Unit, Paediatric Section, IMER Registry (Emilia Romagna Registry of Birth Defects), Dep. of Medical Sciences, University of Ferrara, Italy

[6] National Institute of Health Doutor Ricardo Jorge, Epidemiology Department, Lisbon, Portugal.

[7] Rare Diseases Research Unit, Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, Valencia, Spain.

[8] University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands.

[9] Department Health Sciences, College of Life Sciences, University of Leicester, Leicester, UK.

[10] Malta Congenital Anomalies Registry, Directorate for Health Information and Research, Malta.

[11] Medical University of Graz, Graz, Austria

[12] Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway.

[13] Division of Mental and Physical Health, Norwegian Institute of Public Health, Bergen, Norway.

[14] National Perinatal Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, UK

[15] Department of Medical Genetics, Poznan University of Medical Sciences, Poland

[16] European Commission, Joint Research Centre (JRC), Ispra, Italy

[17] Université de Paris, Epidemiology and Statistics Research Center - CRESS, INSERM, Obstetrical, Perinatal and Pediatric Epidemiology research team (EPOPé), F-75004 Paris, France

[18] South West Congenital Anomaly Register, Bristol Medical School, University of Bristol, Bristol, UK

[19] Public Health Division of Gipuzkoa, Biodonostia Research Institute, Donostia-San Sebastian, Spain.

[20] Department of Public Health, HSE South East, Lacken, Kilkenny, Ireland

[21] Provincial Institute of Hygiene, Antwerp, Belgium.

[22] Department of Public Health, HSE South (Cork & Kerry), Ireland

[23] Auvergne registry of congenital anomalies (CEMC-Auvergne), Department of clinical genetics, Centre de Référence des Maladies Rares, University Hospital of Clermont-Ferrand, France

[24] Register of Congenital Malformations Isle of Reunion Ilsland, CHU St Pierre, la Reunion, France

[25] Population Health Sciences Institute, Newcastle University/National Congenital Anomaly and Rare Disease Registration Service (NCARDRS), Public Health England, UK.

[26] Malformation Monitoring Centre Saxony-Anhalt, Medical Faculty Otto-von-Guericke University Magdeburg, Germany

[27] Brittany Registry of congenital malformations, CHU Rennes, Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France

[28] French West Indies Registry, Registre des Malformations des Antilles (REMALAN), Maison de la Femme de la Mère et de l'Enfant, University Hospital of Martinique, Fort-de-France, France

[29] Congenital Anomaly Register & Information Service for Wales (CARIS), Public Health Wales, Swansea, UK.

[30] Wessex Clinical Genetics Service, University Hospitals Southampton, UK

[31] OMNI-Net for Children and Khmelnytsky City Children`s Hospital, Khmelnytsky, Ukraine

[32] Paediatric Department, Hospital Lillebaelt, Kolding, Denmark

Correponding Author:

Michele Santoro

Unit of Epidemiology of Rare disease and Congenital anomalies, Institute of Clinical Physiology,

National Research Council, Pisa, Italy

email address: msantoro@ifc.cnr.it

SYNOPSIS

Study question

What is the epidemiologic profile of Pierre-Robin sequence (PRS) in Europe?

What's already known

PRS is a rare congenital anomaly. Prevalence has been estimated in few studies performed in single areas. Cases can be isolated or associated with other anomalies, and syndromic cases are considered to be more severe. Prenatal diagnosis is challenging.

What this study adds

We investigated the epidemiology of PRS using a large cohort of cases collected in 29 population-based registries in 17 different European countries. Proportion of prenatally diagnosed cases increased in the last decade, although it was still low. We observed that advanced maternal age was associated with an increased risk of PRS.

ABSTRACT

Background. Pierre Robin sequence (PRS) is a rare congenital anomaly. Respiratory disorders and feeding difficulties represent the main burden.

Objective. The aim of this study was to investigate the epidemiology of PRS using a cohort of cases from EUROCAT, the European network of population-based registries of congenital anomalies.

Methods. We analysed cases of PRS born in the period 1998-2017 collected by 29 population-based congenital anomaly registries in 17 different countries. We calculated prevalence estimates, prenatal detection rate, survival up to 1 week, and proportions of associated anomalies. The effect of maternal age was tested using a Poisson regression model.

Results. Out of 11,669,155 surveyed births, a total of 1,294 cases of PRS were identified. The estimate of the overall prevalence was 12.0 per 100,000 births (95% CI: 9.9, 14.5). There was a total of 882 (68.2%) isolated cases and the prevalence was 7.8 per 100,000 births (95% CI: 6.7, 9.2). A total of 250 cases (19.3%) were associated with other structural congenital anomalies, 77 cases (6.0%) were associated with chromosomal anomalies and 77 (6.0%) with genetic syndromes. The prenatal detection rate in isolated cases was 12.0% (95% CI 9.8, 14.5) and increased to 16.0% (95% CI 12.7, 19.7) in the sub-period 2008-2017. The prevalence rate ratio of non-chromosomal cases with maternal age >= 35 was higher than in cases with maternal age <25 for total (PRR 1.26, 95% CI 1.05, 1.51) and isolated cases (PRR 1.33, 95% CI 1.00, 1.64). Survival of chromosomal cases (94.2%) and multiple anomaly cases (95.3%) were lower than survival of isolated cases (99.4%).

Conclusions. This epidemiological study using a large series of cases of PRS provides insights into the epidemiological profile of PRS in Europe. We observed an association with higher maternal age, but further investigations are needed to test potential risk factors for PRS.

KEYWORDS

Pierre Robin sequence; rare congenital anomalies; epidemiology; prevalence; EUROCAT

MAIN TEXT

Epidemiology of Pierre-Robin sequence in Europe: a population-based EUROCAT study

BACKGROUND

Pierre Robin sequence (PRS) is a rare congenital anomaly commonly recognized by three main clinical signs: micrognathia, glossoptosis and obstruction of the upper airways.[1] Common co-morbidities include respiratory disorders and feeding difficulties with different levels of severity.[2] The inclusion of cleft palate in the definition of PRS is still under debate, but nowadays it is considered a common and additional feature.[1,3] The heterogeneous clinical definition of the PRS makes it difficult to provide accurate prevalence estimates.[3] Most of the studies on the birth prevalence of PRS have been based on data referring to population subgroups with cleft palate or a wider spectrum of congenital anomalies.[4] In Europe, prevalence estimates have been provided by population-based studies performed in some countries (i.e. Denmark, Germany and Netherlands) with values ranging from 7.1 to 17.7 per 100,000 live births.[4-6] ORPHANET, the European portal for rare diseases, reports a prevalence of isolated PRS of 5.0 per 100,000 births.[7] There have been a number of theories developed to explain the pathogenesis of PRS, but the most prevailing is that during embryonic development, intrinsic and/or extrinsic factors lead to micrognathia, which in turn causes failure of the tongue to drop from between the palatal shelves resulting in most cases in cleft palate. Recent studies show that micrognathia in PRS is primarily due to neural crest developmental abnormalities caused by defects in the migration, proliferation, and survival of cranial neural crest cells and their derivatives .[8] The PRS is thought to be due to diminished signalling in cranial neural crest cells leading to reduced proliferation and/or osteogenesis within the mandible.[9,10] There is also convincing evidence suggesting that dysregulation of the *SOX9* gene, a transcription factor that regulates neural crest development,

affects the development of facial structures and cartilage, leading to PRS.[11,12] Environmental factors identified as risk factors in pathogenesis of PRS, include maternal exposure to tobacco, alcohol and certain medications such as methadone.[13-15]

Cases of PRS can be isolated or associated with genetic syndromes, chromosomal anomalies or other structural anomalies. Syndromic cases are considered to be more severe due to systemic involvement, in particular neurological and cardiac comorbidities, and major respiratory complications. A distinction between isolated and associated cases is widely recommended also for a better clinical management of the patients.[16] PRS is usually diagnosed at birth. Prenatal diagnosis is challenging, but a few recent studies reported that it is possible from the 20th week of gestation by ultrasonography and fetal MRI.[17,18]

The aim of this study was to describe the epidemiology of PRS including prevalence, prenatal detection rate, associated anomalies, and the effect of maternal age, using a large cohort of cases collected by EUROCAT, the European network of population-based registries of congenital anomalies.

METHODS

We analysed cases of PRS collected by the population-based congenital anomaly registries of EUROCAT. The EUROCAT registries collect data on structural anomalies, monogenic and teratogenic syndromes, and chromosomal anomalies among live births (LB), fetal deaths with gestational age (GA) ≥ 20 weeks, and terminations of pregnancy for fetal anomaly (TOPFA) following prenatal diagnosis.[19,20] All cases are coded using the International Classification of Diseases with British Paediatric Association (ICD-BPA) one-digit extension. Minor anomalies are excluded according to the EUROCAT guidelines.[21] All full member registries send individual anonymous records of their cases annually to the JRC-EUROCAT Central Registry at the European

Commission's Joint Research Centre (JRC) in Ispra, Italy, which manages the EUROCAT Central Database.[19,22] All EUROCAT full member registries were invited to participate in this study and 29 registries in 17 countries agreed that their data could be included in the study. Cases of PRS born between 1 January 1998 and 31 December 2017 formed the study population. Anonymous individual data on PRS cases were extracted from the EUROCAT Central Database using the ICD10-BPA code Q8708 and the ICD9-BPA code 75603, and an additional search through the text description of the anomaly variables. All extracted cases were then confirmed by each registry as cases of PRS. Each registry confirmed also the possible association with cleft palate. We used the number of total live and stillbirths to mothers resident in the area covered by each registry, stratified by year and maternal age, as denominators.

Following the EUROCAT multiple flowchart classification, cases were classified into isolated, multiple congenital anomalies, associated with chromosomal anomalies, associated with genetic syndromes, and teratogenic syndromes.[21,23] Two clinicians (IB and EG) reviewed all the cases to confirm the classification.

Outcome

Cases of PRS in this study included live births, fetal deaths with gestational age ≥ 20 weeks, and terminations of pregnancy for fetal anomaly following prenatal diagnosis. Cases were collected by the population-based congenital anomaly registries of EUROCAT using multiple data sources. For this study data was extracted from the Central EUROCAT database using the ICD10-BPA code Q8708 and the ICD9-BPA code 75603. An additional procedure of validation was performed by each registry checking the medical records. Only confirmed cases at this stage were included in the study.

Statistical analysis

We calculated prevalence estimates (overall and by two sub-periods, 1998-2007 and 2008-2017), prenatal detection rate, and proportions of associated anomalies. Prevalence of total and isolated cases per 100,000 births by registry was calculated with 95% confidence intervals (95% CIs) based on Poisson distribution. Overall prevalence was estimated using Poisson regression with random effects models to account for potential heterogeneity across registries. A Poisson regression model was used to assess differences among maternal age-classes on non-chromosomal and isolated cases. Prevalence rate ratio referred to the maternal age baseline group (mothers < 25 years) and the trend across the maternal age classes was assessed. The effect of maternal age was also tested including only cases without any congenital anomalies reported in the family history. Survival up to 1 week of age for LB cases was assessed. Statistical analyses were performed using Stata version 16.0 (StataCorp LP, College Station, TX, USA).

Ethics approval

We used anonymized data obtained from EUROCAT registries which have their own ethics approval, thus no specific ethics approval for the study was required.

RESULTS

A total of 11,669,155 births were surveyed during 1998-2017. A total of 1,294 cases of PRS were identified and confirmed by the 29 participating registries. Twenty-three cases were not confirmed by the registries. The estimate of the overall prevalence was 12.0 per 100,000 births (95% CI 9.9, 14.5). The prevalence was higher in the most recent 10 - year period, 2008-2017 (Table 1).

A total of 882 out of 1,294 (68.2%) cases of PRS were isolated cases with a prevalence of 7.8 per 100,000 births (95% CI 6.7, 9.2). There were 250 cases (19.3% of total cases) associated with other

structural congenital anomalies. Seventy-seven were cases associated with chromosomal anomalies (6.0%) ranging from 0.0% to 16.7% by registry. A proportion of 6.0% was observed also for cases diagnosed with genetic syndromes with a range of 0.0-28.6% by registry. Eight cases were classified as teratogenic syndromes. The majority of cases were liveborn (94.1%). The proportion of cases resulting in TOPFA was higher among the non-isolated cases (Table 2).

There were major differences in prevalence among regions with the highest prevalence observed for the registry of Brittany, France (37.2 per 100,000 births) (Table 3). Considering only the isolated cases, the highest prevalence was observed in Brittany (14.3 per 100,000 births), Paris (13.4 per 100,000 births) and Wales (13.3 per 100,000 births).

The prenatal detection rate in isolated cases was 12.0% (95% CI 9.8, 14.5). It increased from 7.2% (95% CI 4.8, 10.4) in the sub-period 1998-2007 to 16.0% (95% CI 12.7, 19.7) in the sub-period 2008-2017.

The male-to-female ratio was 0.94. The mean maternal age for all cases was 30.1 (standard deviation (SD) 5.7) years and it was similar for isolated cases (30.0, SD 5.7). Focusing the analysis on non-chromosomal cases with available information on maternal age, the prevalence by maternal age was investigated. Prevalence rate ratio (PRR) increased with increasing maternal age classes both for total and isolated PRS (Table 4). The PRR in the class with maternal age >= 35 was higher than in the reference class (maternal age < 25) for total (PRR 1.26, 95% CI 1.05, 1.51) and isolated cases (PRR 1.33, 95% CI 1.00, 1.64). The trend among the maternal age classes for total and isolated cases was 1.08 (95% CI 1.02, 1.15) and 1.10 (95% CI 1.03, 1.17), respectively.

We performed the analysis also including all cases without any congenital anomalies reported in the family history identified among the cases with known information about family history (400 out of 558). Also for this group we observed a higher risk in cases with maternal age >=35 (PRR 1.39, 95% CI 1.01, 1.91) and a trend of 1.12 (95% CI 1.01, 1.23).

First week survival was 98.5% for all the LB cases. Survival of PRS cases occurring with chromosomal anomalies (94.2%) and multiple anomaly cases (95.3%) were both lower than survival of isolated cases (99.4%).

For 1,103 cases (85.2%, 95% CI 83.2, 87.1), a diagnosis of cleft palate was reported and confirmed. The proportion decreased from 88.6% (95% CI 85.6, 91.1) to 82.9% (95% CI 80.0, 85.5) over the two sub-periods. Association with cleft palate in chromosomal cases (77.9%) was lower than the proportion of isolated cases with cleft palate (86.2%).

Fifty-three percent of the multiple anomaly cases of PRS was associated with a congenital heart defect, 34.4% with an anomaly of limbs, 16.0% with an anomaly of the nervous system and 15.6% with an anomaly of the urinary system (Table 5). Apart from cleft palate, the most frequent associated structural anomalies were: ventricular septal defect, atrial septal defect, limb reduction defects and club foot/talipes equinovarus. The most common chromosomal anomalies were microdeletions (16 out of 77 chromosomal cases, 20.8%). Seven out of the 16 cases of microdeletion were 22q11.2 microdeletion. Eleven cases occurred with trisomy 18 (14.3%). Among the 77 cases associated with genetic syndromes, 24 (31.2%) had a diagnosis of Stickler syndrome and eight (10.4%) of Treacher Collins syndrome.

COMMENT

Principal findings

This population-based study analysed 1,294 cases of PRS cases in Europe including all birth outcomes. We estimated a total prevalence of 12.0 per 100,000 births (95% CI: 9.9, 14.5).

An increase of the prenatal detection rate during the 20-year study period was observed. We found that risk of non-chromosomal PRS increased with increasing maternal age.

Strengths of the study

The main strength of this multicenter study is that data were extracted from population-based registries of EUROCAT that use standardized procedures for data collection, evaluation and coding. The large series of cases allowed increasing the statistical power of the epidemiological investigation on a rare congenital anomaly, facilitating the comparison of outcomes between classification groups. Furthermore, the study was performed on a cohort specifically focusing on all the cases of PRS and not on a subgroup with cleft palate. Data were collected from 29 registries in 17 different countries in Europe.

Limitations of the data

We observed a large variability of the prevalence estimate at the registry level. This could be due to real differences in prevalence in different populations. However, we can not exclude variation due to coding practices, completeness of data sources, the access to post mortem foetopathological examination, and accuracy of the case description adopted by different centers. Another limitation is a possible under-reporting by those registries which are not able to collect cases diagnosed after the neonatal period, or follow–up of a suspected diagnosis at birth.

Interpretation

Our epidemiological study is, to our best knowledge, the largest series of cases of PRS to date. Data were derived from 29 population-based registries of congenital anomalies in Europe. We estimated an overall prevalence of PRS in Europe of 12.0 per 100,000 births. This estimate is similar to a German study[6] and slightly lower than a Dutch study.[4] An American study, based on hospital admission records, reported a higher prevalence, but the authors acknowledged that their

prevalence estimate may have been affected by the data source used for their study. [24] The prevalence estimated from the random effects model (12.0 per 100,000 births) did differ from that observed by calculating the prevalence ignoring registry of origin (11.1 per 100,000 births). However both estimates are consistent as the 95% confidence intervals are wide and the prevalence from the random effects model is judged to be the most appropriate due to the high variability of prevalence across registries ranging from 2.3 to 37.2 per 100,000 births. A high variability across different studies of PRS was underlined also in a systematic review.[4] In our cohort, we observed a particularly high prevalence in the area of Brittany (France). A recent study on 105 cases extracted from hospital data in a region of Scotland reports a high prevalence in the area and the authors do not exclude a possible role of genetic predisposition and environmental factors.[25] However, the result of Brittany was characterized by an extremely high proportion of TOPFA cases in the registry (34%). A plausible explanation is that in Brittany there is a very high rate of autopsy examinations for TOPFA, fetal and neonatal deaths, with very detailed description. In our study population the proportion of cases with the autopsy examination was 81.4% in Brittany against an average proportion of 61.8%. Brittany is also one of the registries with the highest proportion of chromosomal cases (15.4%). If we only consider isolated cases or LB cases, the prevalence of PRS in Brittany is similar to the other registries. We did not observe the increase of the prevalence in the most recent 10 - year period, 2008-2017 if we did not include data of the Registry of Brittany whose cases are collected since 2011.

To limit the effect of high variability in prevalence across the registries, we used a meta-analytical approach to produce an overall estimate of PRS. However, some signs of PRS can be difficult to detect at an early stage as well as for TOPFA and the collection of cases can be influenced by the time of the registration activity. Variability in diagnostic criteria and the consequent different

definition of the cases are reported in clinical practice and a standard definition is recommended in order to improve the epidemiological surveillance of PRS.[3]

Most of the studies on PRS were performed using subgroups of cases of cleft palate even if it is recognized that cleft palate is very frequently associated, but it is not a required clinical sign of PRS.[1] The proportion of cleft palate in PRS cases has been estimated to be between 66% and 90%.[26] In our study, cleft palate was confirmed in 85.2% of cases. There were also 13 cases with a diagnosis of another anomaly of palate, mostly of high arched palate. Among the cases with cleft palate, there were 26 cases associated with cleft lip. We observed a high variability in the proportion of cleft palate among the participating registries. The diagnosis of PRS can vary among different centres and it is not excluded that in some cases, coders did not report the cleft palate diagnosis considering it implicit in cases of PRS. However, we observed a decrease of the association with cleft palate over time, suggesting the adoption of a more accurate description of the cases of PRS over time.

Prenatal diagnosis of PRS is challenging because micrognathia, retrognathia, and glossoptosis are useful predictors, but not sufficient for a complete diagnosis of PRS, and they can be difficult to find in two dimensional ultrasound.[27,28] Furthermore, micrognathia may appear in association with many other syndromes and structural disorders.[29,30] Ultrasound examination is more efficient in suspected cases of PRS with a family history.[18] However, the improvement of radiology techniques is helping to increase the prenatal diagnosis of PRS that is becoming more frequent.[16-18,31,32] The increase over time of prenatally diagnosed isolated cases is confirmed in our study. A prenatal diagnosis facilitates and makes clinical management more efficient and enables planned treatment soon after birth.[27] Furthermore, prenatal diagnosis can help to address interventions aimed to improve the quality of life of the patients and support their parents.[16]

In our study, we observed that advanced maternal age was associated with an increased risk of non-chromosomal PRS. To our best knowledge, no other evidence of association of PRS with maternal age was reported in literature. An association of cleft palate with advanced maternal age has been reported in a few studies.[33,34] Possible explanations could be related to a process of chromosomal alteration, a cumulative exposure to environmental agents, a higher susceptibility of placenta to teratogenic agents, or to the effects or treatment of chronic diseases.[33] However, other studies and evidence are needed to support the specific association of PRS with maternal age.

About 6% of the cases of PRS occurred as part of a genetic syndrome and Stickler syndrome was the most common. Other associated syndromes described in our cohort (e.g. Treacher Collins syndrome, Oculo-auriculo-vertebral spectrum) have also been reported previously.[35] Among the cases of PRS associated with a chromosomal anomaly, about 20% were represented by microdeletions, with 22q11.2 microdeletion the most commonly occurring (7 out of 16 microdeletion cases). A low association of PRS cases with 22q11 microdeletion was also reported in other recent studies.[36-38] Association of PRS with a chromosomal anomaly was confirmed as a risk factor for mortality. In our study, we observed that survival in chromosomal cases and multiple anomaly cases was lower than in isolated cases. This result is consistent with a longitudinal study on mortality.[39]

Conclusions

This population-based multi registry study is one of the largest epidemiological studies on PRS. We estimated an overall prevalence of 12.0 per 100,000 births (95% CI 9.9, 14.5). The prevalence of isolated cases was 7.8 per 100,000 births (95% CI 6.7, 9.2). Prenatal diagnosis of isolated cases was low, but increased during the 20-year study period, with a proportion of 16.0% in the last

decade. The survival at the first week of life was 98.5%, but was lower in cases associated with chromosomal and multiple anomalies. In our analysis we observed an impact of higher maternal age, but further investigation is needed to test the effect of environmental/teratogenic factors.

REFERENCES

1. Breugem CC, Evans KN, Poets CF, et al. Best Practices for the Diagnosis and Evaluation of Infants With Robin Sequence: A Clinical Consensus Report. JAMA Pediatr. 2016;170(9):894-902. doi:10.1001/jamapediatrics.2016.0796
2. Cole A, Lynch P, Slator R. A new grading of Pierre Robin sequence. Cleft Palate Craniofac J. 2008;45(6):603-606. doi:10.1597/07-129.1
3. Breugem CC, Courtemanche DJ. Robin sequence: clearing nosologic confusion. Cleft Palate Craniofac J. 2010;47(2):197-200. doi:10.1597/08-061_1
4. Paes EC, van Nunen DP, Basart H, et al. Birth prevalence of Robin sequence in the Netherlands from 2000-2010: a retrospective population-based study in a large Dutch cohort and review of the literature. Am J Med Genet A. 2015;167A(9):1972-1982. doi:10.1002/ajmg.a.37150
5. Printzlau A, Andersen M. Pierre Robin sequence in Denmark: a retrospective population-based epidemiological study. Cleft Palate Craniofac J. 2004;41(1):47-52. doi:10.1597/02-055
6. Vatlach S, Maas C, Poets CF. Birth prevalence and initial treatment of Robin sequence in Germany: a prospective epidemiologic study. Orphanet J Rare Dis. 2014;9:9. Published 2014 Jan 17. doi:10.1186/1750-1172-9-9
7. Orphanet Report Series - Prevalence of rare diseases: Bibliographic data - January 2020 - Number 1 http://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf accessed on 15th september 2020
8. Siismets EM, Hatch NE. Cranial Neural Crest Cells and Their Role in the Pathogenesis of Craniofacial Anomalies and Coronal Craniosynostosis. J Dev Biol. 2020 Sep 9;8(3):E18. doi: 10.3390/jdb8030018.

9. Parada C, Han D, Grimaldi A, Sarrión P, Park SS, Pelikan R, et al. Disruption of the ERK/MAPK pathway in neural crest cells as a potential cause of Pierre Robin sequence. Development. 2015 Nov 1;142(21):3734-45. doi: 10.1242/dev.125328.
10. Chen Y, Wang Z, Chen Y, Zhang Y. Conditional deletion of Bmp2 in cranial neural crest cells recapitulates Pierre Robin sequence in mice. Cell Tissue Res. 2019 May;376(2):199-210. doi: 10.1007/s00441-018-2944-5.
11. Gordon CT, Attanasio C, Bhatia S, Benko S, Ansari M, Tan TY, et al. Identification of novel craniofacial regulatory domains located far upstream of SOX9 and disrupted in Pierre Robin sequence. Hum Mutat. 2014 Aug;35(8):1011-20. doi: 10.1002/humu.22606.
12. Long HK, Osterwalder M, Welsh IC, Hansen K, Davies JOJ, Liu YE, et al. Loss of Extreme Long-Range Enhancers in Human Neural Crest Drives a Craniofacial Disorder. Cell Stem Cell. 2020 Sep 22:S1934-5909(20)30447-1. doi: 10.1016/j.stem.2020.09.001.
13. Honein MA, Rasmussen SA, Reefhuis J, Romitti PA, Lammer EJ, Sun L, Correa A et al. Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. Epidemiology. 2007 Mar;18(2):226-33. doi: 10.1097/01.ede.0000254430.61294.c0.
14. Meyer KA, Werler MM, Hayes C, Mitchell AA. Low maternal alcohol consumption during pregnancy and oral clefts in offspring: the Slone Birth Defects Study. Birth Defects Res A Clin Mol Teratol. 2003 Jul;67(7):509-14. doi: 10.1002/bdra.10057.
15. Cleary B, Loane M, Addor MC, et al. Methadone, Pierre Robin sequence and other congenital anomalies: case-control study. Arch Dis Child Fetal Neonatal Ed. 2020;105(2):151-157. doi:10.1136/archdischild-2019-316804
16. Giudice A, Barone S, Belhous K, et al. Pierre Robin sequence: A comprehensive narrative review of the literature over time. J Stomatol Oral Maxillofac Surg. 2018;119(5):419-428. doi:10.1016/j.jormas.2018.05.002
17. Cascone P, Papoff P, Arangio P, et al. Fast and early mandibular osteodistraction (FEMOD) in severe Pierre Robin Sequence. J Craniomaxillofac Surg. 2014;42(7):1364-1370. doi:10.1016/j.jcms.2014.03.027
18. Di Pasquo E, Amiel J, Roth P, et al. Efficiency of prenatal diagnosis in Pierre Robin sequence. Prenat Diagn. 2017;37(11):1169-1175. doi:10.1002/pd.5162
19. Tucker FD, Morris JK, JRC Management Committee, Neville A, Garne E, Kinsner-Ovaskainen A, Lanzoni M, Loane MA, Martin S, Nicholl C, Rankin J, Rissmann Ak. EUROCAT: an update on

its functions and activities. J Community Genet. 2018 Oct;9(4):407-410. doi: 10.1007/s12687-018-0367-3.

20. Boyd PA, Haeusler M, Barisic I, Loane M, Garne E, Dolk H. Paper 1: The EUROCAT network-organization and processes. Birth Defects Res A Clin Mol Teratol. 2011 Mar;91 Suppl 1:S2-15. doi: 10.1002/bdra.20780.
21. EUROCAT (2013). EUROCAT Guide 1.4: Instruction for the registration of congenital anomalies. EUROCAT Central Registry, University of Ulster. https://eu-rd-platform.jrc.ec.europa.eu/eurocat/data-collection/guidelines-for-data-registration_en#inline-nav-2; accessed 10th September 2020
22. Kinsner-Ovaskainen A, Lanzoni M, Garne E, et al. A sustainable solution for the activities of the European network for surveillance of congenital anomalies: EUROCAT as part of the EU Platform on Rare Diseases Registration. 2018 Sep;61(9):513-517. doi: 10.1016/j.ejmg.2018.03.008.
23. Garne E, Dolk H, Loane M, Wellesley D, Barisic I, Calzolari E, Densem J; EUROCAT Working Group. Paper 5: Surveillance of multiple congenital anomalies: implementation of a computer algorithm in European registers for classification of cases. Birth Defects Res A Clin Mol Teratol. 2011 Mar;91 Suppl 1:S44-50. doi: 10.1002/bdra.20777.
24. Scott AR, Mader NS. Regional variations in the presentation and surgical management of Pierre Robin sequence. Laryngoscope. 2014;124(12):2818-2825. doi:10.1002/lary.24782
25. Wright M, Mehendale F, Urquhart DS. Epidemiology of Robin sequence with cleft palate in the East of Scotland between 2004 and 2013. Pediatr Pulmonol. 2018;53(8):1040-1045. doi:10.1002/ppul.24038
26. Cohen SM, Greathouse ST, Rabbani CC, et al. Robin sequence: what the multidisciplinary approach can do. J Multidiscip Healthc. 2017;10:121-132. Published 2017 Mar 27. doi:10.2147/JMDH.S98967
27. Evans KN, Sie KC, Hopper RA, Glass RP, et al. Robin sequence: from diagnosis to development of an effective management plan. Pediatrics. 2011;127(5):936-948. doi:10.1542/peds.2010-2615
28. Kaufman MG, Cassady CI, Hyman CH, et al. Prenatal Identification of Pierre Robin Sequence: A Review of the Literature and Look towards the Future. Fetal Diagn Ther. 2016;39(2):81-89. doi:10.1159/000380948

29. Bronshtein M, Blazer S, Zalel Y, Zimmer EZ. Ultrasonographic diagnosis of glossoptosis in fetuses with Pierre Robin sequence in early and mid pregnancy. Am J Obstet Gynecol. 2005;193(4):1561-1564. doi:10.1016/j.ajog.2005.03.052
30. Mouthon L, Busa T, Bretelle F, et al. Prenatal diagnosis of micrognathia in 41 fetuses: Retrospective analysis of outcome and genetic etiologies. Am J Med Genet A. 2019 Dec;179(12):2365-2373. doi: 10.1002/ajmg.a.61359. Epub 2019 Sep 11.
31. Kooiman TD, Calabrese CE, Didier R, et al. Micrognathia and Oropharyngeal Space in Patients With Robin Sequence: Prenatal MRI Measurements. J Oral Maxillofac Surg. 2018;76(2):408-415. doi:10.1016/j.joms.2017.07.163
32. Kruse T, Neuschulz J, Wilhelm L, et al.. Prenatal Diagnosis of Robin Sequence: Sensitivity, Specificity, and Clinical Relevance of an Index for Micrognathia. Cleft Palate Craniofac J. 2020 Nov 24:1055665620972297. doi: 10.1177/1055665620972297. Epub ahead of print.
33. Herkrath AP, Herkrath FJ, Rebelo MA, Vettore MV. Parental age as a risk factor for non-syndromic oral clefts: a meta-analysis. J Dent. 2012 Jan;40(1):3-14. doi: 10.1016/j.jdent.2011.10.002. Epub 2011 Oct 13. PMID: 22019990.
34. Luo YL, Cheng YL, Gao XH, Tan SQ, Li JM, et al. (2013) Maternal Age, Parity and Isolated Birth Defects: A Population-Based Case-Control Study in Shenzhen, China. PLoS ONE 8(11): e81369. doi:10.1371/journal.pone.0081369
35. Izumi K, Konczal LL, Mitchell AL, Jones MC. Underlying genetic diagnosis of Pierre Robin sequence: retrospective chart review at two children's hospitals and a systematic literature review. J Pediatr. 2012;160(4):645-650.e2. doi:10.1016/j.jpeds.2011.09.021
36. Basart H, Paes EC, Maas SM, et al. Etiology and pathogenesis of robin sequence in a large Dutch cohort. Am J Med Genet A. 2015;167A(9):1983-1992. doi:10.1002/ajmg.a.37154
37. McDonald-McGinn DM, Sullivan KE, Marino B, Philip N, Swillen A, et al. 22q11.2 deletion syndrome. Nat Rev Dis Primers. 2015 Nov 19;1:15071. doi: 10.1038/nrdp.2015.71.
38. Karempelis P, Hagen M, Morrell N, Roby BB. Associated syndromes in patients with Pierre Robin Sequence. Int J Pediatr Otorhinolaryngol. 2020 Apr;131:109842. doi: 10.1016/j.ijporl.2019.109842.
39. Logjes RJH, Haasnoot M, Lemmers PMA, et al. Mortality in Robin sequence: identification of risk factors. Eur J Pediatr. 2018;177(5):781-789. doi:10.1007/s00431-018-3111-4

Acknowledgements

We thank Prof. Joan Morris (St. George's University of London) for her helpful suggestions. We thank JRC-EUROCAT Central Registry, European Commission, Joint Research Centre (JRC), Ispra, Italy, for the data management and selection of cases included in the study. We also thank the many people throughout Europe involved in providing and processing information, including affected families, clinicians, health professionals, medical record clerks, and registry staff.

FUNDING

There was no specific funding for this study

TABLES

Table 1. Prevalence (per 100,000 births) of total and isolated cases of Pierre Robin sequence, by study periods

		Total cases		Isolated cases	
Period	**Total births**	**N**	**Prevalence per 10,000 (95% CI)**	**N**	**Prevalence per 10,000 (95% CI)**
1998-2007	5,219,231	534	10.9 (8.9, 13.3)	405	8.0 (6.6, 9.6)
2008-2017	6,449,924	760	12.1 (9.8, 14.8)	477	7.6 (6.3, 9.2)
1998-2017	11,669,155	1,294	12.0 (9.9, 14.5)	882	7.8 (6.7, 9.2)

Prevalence values do not correspond to the ratio between cases and births as they are estimated using Poisson regression with random effects models (see methods)
95% CI = 95% Confidence Interval

Table 2. Distribution of cases of Pierre Robin sequence by birth outcomes and classification

	Total	Live births		TOPFA		Fetal deaths	
		N	%	N	%	N	%
Isolated	882	871	98.8	9	1.0	2	0.2
Multiple congenital anomalies	250	224	89.6	24	9.6	2	0.8
Chromosomal	77	55	71.4	20	26.0	2	2.6
Genetic syndromes	77	61	79.2	15	19.5	1	1.3
Teratogenic syndromes	8	7	87.5	1	12.5	0	0.0
Total	1294	1218	94.1	69	5.3	7	0.5

TOPFA= terminations of pregnancy for fetal anomaly

Table 3. Number and prevalence (per 100,000 births) of total, livebirths and isolated cases of Pierre Robin sequence in 29 EUROCAT registries

Registry	Years included	Total births	Total		Livebirths		Isolated	
			N	Prevalence per 100,000 (95% CI)	N	Prevalence per 100,000 (95% CI)	N	Prevalence per 100,000 (95% CI)
Brittany (France)	2011-2017	244,690	91	37.2 (29.9, 45.7)	59	24.1 (18.4, 31.1)	35	14.3 (10.0, 19.9)
Auvergne (France)	1998-2017	268,338	56	20.9 (15.8, 27.1)	52	19.4 (14.5, 25.4)	32	11.9 (8.2, 16.8)
Wales (UK)	1998-2017	668,205	124	18.6 (15.4, 22.1)	124	18.6 (15.4, 22.1)	89	13.3 (10.7, 16.4)
Paris (France)	1998-2017	561,416	104	18.5 (15.1, 22.5)	93	16.6 (13.4, 20.3)	75	13.4 (10.5, 16.8)
Cork & Kerry (UK)	1998-2017	181,756	31	17.1 (11.6, 24.2)	31	17.1 (11.6, 24.2)	23	12.7 (8.0, 19.0)
Antwerp (Belgium)	1998-2016	370,959	59	15.9 (12.1, 20.5)	59	15.9 (12.1, 20.5)	39	10.5 (7.5, 14.4)
NCARDRS/Northern England (UK)	2000-2017	575,155	87	15.1 (12.1, 18.7)	72	12.5 (9.8, 15.8)	58	10.1 (7.7, 13.0)
South East Ireland	1998-2017	137,175	20	14.6 (8.9, 22.5)	20	14.6 (8.9, 22.5)	14	10.2 (5.6, 17.1)
Saxony Anhalt (Germany)	1998-2017	336,187	48	14.3 (10.5, 18.9)	47	14.0 (10.3, 18.6)	31	9.2 (6.3, 13.1)
Vaud (Switzerland)	1998-2017	155,522	22	14.2 (8.9, 21.4)	22	14.2 (8.9, 21.4)	18	11.6 (6.9, 18.3)
French West Indies (France)	2009-2017	85,250	11	12.9 (6.4, 23.1)	10	11.7 (5.6, 21.6)	5	5.9 (1.9, 13.7)
Valencian Region (Spain)	2007-2016	489,361	60	12.3 (9.4, 15.8)	60	12.3 (9.4, 15.8)	36	7.4 (5.2, 10.2)
Basque Country (Spain)	1999-2016	351,271	43	12.2 (8.9, 16.5)	43	12.2 (8.9, 16.5)	29	8.3 (5.5, 11.9)
Malta	1998-2017	83,668	10	12.0 (5.7, 22.0)	10	12.0 (5.7, 22.0)	6	7.2 (2.6, 15.6)
North Netherlands	1998-2017	360,762	42	11.6 (8.4, 15.7)	41	11.4 (8.2, 15.4)	28	7.8 (5.2, 11.2)
Isle de Reunion (France)	2002-2017	232,043	25	10.8 (7.0, 15.9)	24	10.3 (6.6, 15.4)	15	6.5 (3.6, 10.7)
NCARDRS/Thames Valley (UK)	1998-2017	429,945	44	10.2 (7.4, 13.7)	44	10.2 (7.4, 13.7)	32	7.4 (5.1, 10.5)
Emilia Romagna (Italy)	1998-2017	690,381	63	9.1 (7.0, 11.7)	57	8.3 (6.3, 10.7)	51	7.4 (5.5, 9.7)
Funen (Denmark)	2000-2015	81,392	7	8.6 (3.5, 17.7)	6	7.4 (2.7, 16.1)	3	3.7 (0.8, 10.8)
OMNI-Net (Ukraine)	2005-2016	360,948	31	8.6 (5.8, 12.2)	31	8.6 (5.8, 12.2)	20	5.5 (3.4, 8.6)
NCARDRS/South West England (UK)	2005-2017	641,971	53	8.3 (6.2, 10.8)	52	8.1 (6.1, 10.6)	32	5.0 (3.4, 7.0)
Styria (Austria)	1998-2016	199,998	16	8.0 (4.6, 13.0)	16	8.0 (4.6, 13.0)	10	5.0 (2.4, 9.2)

NCARDRS/East Midlands & South Yorkshire (UK)	1998-2012; 2016-2017	1,143,462	89	7.8 (6.3, 9.6)	88	7.7 (6.2, 9.5)	69	6.0 (4.7, 7.6)
Wielkopolska (Poland)	1998-2017	741,725	51	6.9 (5.1, 9.0)	51	6.9 (5.1 , 9.0)	37	5.0 (3.5, 6.9)
NCARDRS/Wessex (UK)	1998-2017	570,130	38	6.7 (4.7, 9.2)	37	6.5 (4.6, 9.0)	34	6.0 (4.1, 8.3)
Zagreb (Croatia)	1998-2017	123,473	7	5.7 (2.3, 11.7)	7	5.7 (2.3, 11.7)	7	5.7 (2.3, 11.7)
Norway	1999-2009	650,709	32	4.9 (3.4, 6.9)	32	4.9 (3.4, 6.9)	29	4.5 (3.0, 6.4)
South Portugal	1998-2017	366,939	17	4.6 (2.7, 7.4)	17	4.6 (2.7, 7.4)	16	4.4 (2.5, 7.1)
Tuscany (Italy)	1998-2017	566,324	13	2.3 (1.2, 3.9)	13	2.3 (1.2, 3.9)	9	1.6 (0.7, 3.0)

95% CI = 95% Confidence Interval

Table 4. Prevalence Rate Ratio (95% confidence interval) of non-chromosomal and isolated cases of Pierre Robin sequence by maternal age, 1998-2017

	Total non-chromosomal cases		Isolated cases	
Maternal age	**N**	**PRR (95% CI)**	**N**	**PRR (95% CI)**
<25 (Reference)	212	1.00	149	1.00
25-29	316	1.07 (0.90, 1.27)	231	1.11 (0.90, 1.36)
30-34	383	1.17 (0.99, 1.39)	275	1.20 (0.98, 1.46)
35+	270	1.26 (1.05, 1.51)	199	1.32 (1.01, 1.64)
trend		1.08 (1.02, 1.15)		1.10 (1.03, 1.17)

PRR = Prevalence Rate Ratio
95% CI = 95% confidence Interval

Table 5. Most frequent major structural anomalies, chromosomal anomalies and genetic syndromes associated with Pierre Robin sequence

Structural anomaly	N	% of multiple cases (n=250)
Nervous system	40	16.0
Malformation /agenesis of corpus callosum	13	5.2
Severe microcephaly	7	2.8
Other reduction deformities of brain	7	2.8
Congenital hydrocephalus	6	2.4
Other specified congenital malformations of brain	4	1.6
Eye	11	4.4
Ear, face and neck	14	5.6
Congenital absence, atresia and stricture of auditory canal (external)	5	2.0
Heart	134	53.6
Ventricular septal defect	63	25.2
Atrial septal defect	41	16.4
Patent ductus arteriosus (only livebirths >=37 weeks)	20	8.0
Congenital insufficiency of aortic valve	8	3.2
Coarctation of aorta	8	3.2
Other congenital malformations of heart	7	2.8
Pulmonary valve stenosis	5	2.0
Pulmonary valve atresia	5	2.0
Tetralogy of Fallot	5	2.0
Other congenital malformations of aorta	4	1.6
Other congenital malformations of tricuspid valve	4	1.6
Peripheral pulmonary artery stenosis >37 weeks	4	1.6
Respiratory	8	3.2
Choanal atresia	4	1.6
Other congenital malformations of larynx	4	1.6
Digestive system	25	10.0
Diaphragmatic hernia	6	2.4
Ano-rectal atresia and stenosis	4	1.6
Urinary	39	15.6
Congenital hydronephrosis	14	5.6
Renal agenesis, unilateral	8	3.2
Horseshoe kidney	4	1.6
Genital	21	8.4
Hypospadias	13	5.2
Limb	86	34.4
Clubfoot, (talipes equinovarus)	30	12.0
Syndactyly	17	6.8
Hip dislocation and/or dysplasia	8	3.2
Polydactyly	7	2.8
Arthrogryposis multiplex congenita	5	2.0
Other specified congenital musculoskeletal deformities	5	2.0
Other anomalies		
Craniosynostosis	6	2.4

Other congenital malformations of spine, not associated with scoliosis	5	2.0

Chromosomal anomaly	N	% of chromosomal cases (n=77)
Microdeletions	16	20.8
Trisomy 18	11	14.3
Other deletions of part of a chromosome	9	11.7
Triploidy and polyploidy	6	7.8
Minor partial trisomy	5	6.5
Duplications with other complex rearrangements	3	3.9
Other specified trisomies and partial trisomies of autosomes	3	3.9
Trisomy 13	3	3.9
Trisomy 21	2	2.6
Major partial trisomy	2	2.6
Other deletions from the autosomes	2	2.6
Deletion of short arm of chromosome 4	2	2.6
Trisomy and partial trisomy of autosomes, unspecified	2	2.6
Duplications seen only at prometaphase	2	2.6

Genetic syndrome	N	% of genetic syndrome, skeletal dysplasia and monogenic disorder (n=77)
Stickler syndrome	24	31.2
Treacher Collins syndrome	8	10.4
Achondrogenesis Type II	3	3.9
Spondyloepiphyseal dysplasia	3	3.9
Oculo-auriculo-vertebral spectrum (OAVS)	3	3.9
Cerebrocostomandibular syndrome	2	2.6
Meckel Gruber syndrome	2	2.6
Fragile X syndrome	2	2.6
Ehlers-Danlos syndrome	2	2.6
Diastrophic dysplasia	2	2.6
Hanhart syndrome	2	2.6