Maternal bereavement in the antenatal period and oral cleft in the offspring


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**STUDY QUESTION:** Is maternal bereavement (emotional stress) due to loss of a close relative in the antenatal period associated with the risk of oral cleft in the offspring?

**SUMMARY ANSWER:** Our study suggests prenatal maternal bereavement is associated with an increased risk of oral cleft in the offspring, especially when the bereavement was due to a sudden death or death of a child.

**WHAT IS KNOWN ALREADY:** The aetiology of oral cleft is unknown but includes both genetic and environmental causes.

**STUDY DESIGN, SIZE AND DURATION:** We performed a population-based cohort study based on several national registers in Denmark from 1978 to 2008.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Our final study population consisted of 1,771,663 children. Of these 35,118 (2%) were born to mothers who experienced bereavement in the exposure window from 1 year before pregnancy to the end of the first trimester.

**MAIN RESULTS AND THE ROLE OF CHANCE:** In total, 3,043 children were diagnosed with a cleft; 968 with cleft lip, 1,206 with cleft lip and palate, and 869 with a cleft palate. For overall bereavement the prevalence was 1.7 per 1,000 live born in the unexposed children and 2.2 per 1,000 live born in the exposed children. Overall, maternal bereavement due to the death of a close relative from 1 year before conception to the end of the first trimester was associated with a significantly increased risk of oral cleft [odds ratio (OR): 1.28, 95% confidence interval (CI): 1.01; 1.61]. When mothers lost a relative due to a sudden death, the risk of oral cleft in the offspring was higher (OR: 1.76, 95% CI: 1.06; 2.94). Losing a relative in the time period before pregnancy and during the first trimester showed a tendency to an increased risk. The risk increase was 77% when the mother was bereaved due to sudden death and the estimation was robust in different analytical strategies.

**LIMITATIONS, REASONS FOR CAUTION:** It is a limitation that we only studied live born children, but most children with isolated oral cleft would survive their pregnancy and birth. Since oral cleft are rare and despite the large study population, we still had a relatively small number of cases, which results in limited power to detect small differences. We did not have actual measurements of the maternal cortisol concentration, but we believe that bereavement due to death of a close relative produces a strong stress reaction in most people. Also we did not have the opportunity to adjust for intake of folic acid and use of anti-depressant; however, analysis in a subset of the data showed no difference in these intakes between exposed and unexposed mothers.

**WIDER IMPLICATIONS OF THE FINDINGS:** With this study we add a large-scale human cohort study to the body of literature on stress and birth defects. Our study is in agreement with previously published results and can be generalized to similar populations like the native Danish population. Severe stress may be added to the list of potential causes for oral cleft.

**Key words:** bereavement / psychological stress / oral cleft / congenital abnormalities / pregnancy
Introduction

Oral cleft, including cleft lip, cleft lip and palate and isolated cleft palate, are among the most common non-fatal congenital anomalies. Oral cleft occur as a result of partial or complete lack of fusion of the maxillary prominence with the medial nasal prominence on one or both facial sides in the embryo (Sadler, 2010). The development of the face takes place in the embryo in Weeks 4–10 after conception. The upper lip is formed in Weeks 6–8 and the palate in Weeks 6–10, closing 1 week later in girls (Sadler, 2010). There is a critical exposure window just before Week 6 in the development of the upper lip and primary palate. The medial nasal processes merge with each other and with the maxillary processes to form the upper lip and the primary palate. Just before the completion of these processes there is a peak of cell division, which may make the cells susceptible and interference with this process in this window may lead to failure in the closing of the upper lip and palate (Mossey et al., 2009). Cleft lip and cleft palate have slightly different time windows of susceptibility and they need not share the same causal factors. Worldwide, the prevalence of oral cleft varies by race with populations of African descent having the lowest frequency and the highest is seen in Asians (Webby and Murray, 2010). However, Cooper et al. (2006) found the increased rate of oral cleft in Asian to be lower than previously reported. According to the European Surveillance of Congenital malformations the prevalence in Europe varied slightly from 1980 to 2010 with ~1.1–1.4 cases per 1000 births but in Denmark, a prevalence of ~1.4–1.5 per 1000 live births has remained constant from 1962 to 2001 (Bille et al., 2005). Oral cleft require surgical correction and care regarding infant feeding, dental care, speech and often have major personal and psychosocial consequences (Turner et al., 1998), which lead to hospitalization for all at some point in time.

The aetiology of oral cleft is unknown, but several risk factors have been identified including genetic factors (Grosen et al., 2010a; Grosen et al., 2010b; Grosen et al., 2011) with a few specific genes of small individual effect found by genome-wide association studies (Dixon et al., 2011), maternal smoking and alcohol consumption during the first trimester (Wyszynski et al., 1997; Lorente et al., 2000; Little et al., 2004; Mossey et al., 2009), and a higher birth order (Vieira and Orioli, 2002).

Emotional stress during the antenatal period has previously been associated with congenital anomalies (Carmichael and Shaw, 2000; Hansen et al., 2000; Carmichael et al., 2007) and oral cleft (Peer and Strean, 1956; Hultin and Ottosson 1971; Saxen, 1974; Blomberg, 1980; Montenegro et al., 1995; Nimby et al., 1999; Tan et al., 2009; Goenjian et al., 2011; Wallace et al., 2011). These findings are supported by animal studies (Montenegro et al., 1995). However, some human studies have found no association between maternal stress and oral cleft (McDonald, 1961; James, 1969; Fraser and Warburton, 1964).

Increased maternal cortisol levels due to bereavement in the antenatal period may result in higher cortisol concentrations in the fetus (Gitau et al., 1998) depending on the function of the enzyme 11B-hydroxysteroid dehydrogenase type 2 (11beta HSD2). This enzyme acts normally as a fetal–placental barrier to maternal glucocorticoids including cortisol, but may be down-regulated in response to high maternal stress (Welberg et al., 2005; O’Donnell et al., 2012), and it is not equally active throughout pregnancy. Bereavement due to death of a close relative is one of the most severe types of emotional stress (Goodkin, 2001; Pfeffer et al., 2007). We hypothesized sudden death of any relative and the death of a child to have a possible higher impact than expected deaths and death of other relatives. The objective of the current study was to investigate the associations between maternal bereavement due to loss of a close relative during the antenatal period and oral cleft in the offspring.

Methods

Study population and materials

We performed a cohort study based on the linkage of several national registries in Denmark. The detailed methodology of the cohort has been described elsewhere (Li et al., 2011). In short, we included all children born alive in Denmark from 1978 to 2008 with data available in the Danish Medical Birth Registry (n = 2 085 521) (Knudsen and Olsen, 1998) and their next of kin (mother, father, children, mother’s siblings and mother’s parents) from the Danish Civil Registration System (Pedersen et al., 2006). We linked data from the Danish Death Registry (Helweg-Larsen, 2011) in order to obtain the date and cause of death for any close relative of the mother. We also linked the Danish National Hospitalization Register (Andersen et al., 1999), the Danish Facial Cleft Database (Christensen and Fogh-Andersen, 1987; Christensen, 1999), Integrated Database for Labour Marked Research (Petersson et al., 2011), the Danish Prescription Registries (Gaist et al., 1997) and the Danish National Birth Cohort (Olsen et al., 2001). It was possible to identify information for the mother and the child in the different registers since all Danish inhabitants have a unique identification number assigned at birth, which is used in all registers.

Since some children with a cleft of the soft palate may not be diagnosed at birth, but later in relation to speech problems. We included children diagnosed with oral cleft up to 5 years after birth (n = 154) and excluded children diagnosed with an oral cleft as part of a syndrome or in connection with other anomalies (869). We also excluded children of mothers who were not born in Denmark due to missing values in many variables (312 835). The final study population consisted of 1 771 663 children.

Exposure

Maternal bereavement in the antenatal period was defined as the death of one of her close relatives [a child, partner (biological father), parent, sibling or grandparent] with the expected strongest exposure being the loss of an older child. All causes of death were considered and were divided into sudden deaths due to accidents, suicides and violence and expected deaths following a disease. The exposure window was 1 year before conception divided into 6 months intervals or during the first trimester of pregnancy and was defined according to the death of a close relative taking into consideration that severe stress exposure related to the date of the death often start before this event and continue after the death (allostatic load). Timing according to organogenesis was taken into account with the loss of a close relative in the first trimester hypothesized to present the highest risk. Gestational age of the fetus was based on the last menstrual period and the information came from the Medical Birth Registry.

Outcome

Information on oral cleft was obtained from the Danish Facial Cleft Database, which is based on surgical files from the Deaconess Hospital and the University Hospital of Copenhagen, where the treatment for oral cleft has been centralized since the mid-1930s, and from the National Institute
Covariates

We chose all covariates a priori according to previous literature and a directed acyclic graph (Pearl, 2009) to identify the best possible strategy to adjust for potential confounders (see Supplementary data file). Age of the mother and father, and parity, was obtained from the Danish Medical Birth Register. Parental age was included as a continuous measure and parity of the mother was categorized as one, two, three and four or more children. Parental age and birth order were included since they are risk factors for oral cleft (Veira and Orioli, 2002; Bille et al., 2005). Birth year of the offspring was divided into two time periods: 1978–93 and 1994–2008 according to the change in the classification of the diagnosis system from ICD-8 to ICD-10 and were included to take this change into account. Information about maternal education (primary, secondary, high), maternal income (four quartiles), cohabitation (married/partner or single) and place of residence (Copenhagen, cities or other) was obtained from the Integrated Database for Labour Marked Research. We chose to include these social variables to access any social gradient in the risk of oral cleft. Time of diagnosis was attained from the Danish National Hospitalization Register and from 1991 to 2007, information on maternal smoking was available in the Danish Medical Birth Register (Andersen et al., 1999).

Statistical analysis

Congenital anomalies are prevalence measures at the time of birth. We used logistic regression to estimate the prevalence odd ratios (ORs) for the association between bereavement and oral cleft. In the remaining part of the article, we will however use the term ‘risk’ instead of the prevalence odds to improve readability and the prevalence ORs may then be considered ‘relative risks’ since the disease is rare and that the exposure probably has no specific impact on fetal survival of fetuses with the malformation. We investigated four different outcomes: overall risk of oral cleft, cleft lip only, cleft lip and palate, and cleft palate only. All analyses were performed with and without stratification by sex since cleft lip occurs more frequently in boys (80% of all cleft lip) and isolated cleft palate occurs more often in girls (67% of all cleft palate) (Sadler, 2010). However, the sample size did not allow detailed sex specific outcome measures. We took into account that some mothers had more than one child in the cohort by using robust estimation in the statistical analysis. First, we examined the relations between overall loss at any time in the antenatal period and oral cleft. Secondly, we examined whether the association differed according to different types of losses. We compared mothers who lost a child with mothers who experienced no loss in the antenatal period. We also examined the disease risk according to three different time windows of importance (12-27 month and 6-0 months before pregnancy and first trimester of pregnancy). Finally, we examined the disease risk according to cause of death. All the different exposures were compared with the risk in offspring of mothers who experienced no loss at any time in the antenatal period.

We adjusted for maternal age, paternal age, parity, birth year and the social factors: maternal education, maternal income, maternal cohabitation and place of residence. In a sub-analysis we further adjusted for smoking from 1996 to 2008 where smoking data were available. All analyses were performed using STATA 11. ORs with 95% confidence intervals (95% CI’s) were reported. Since not all children were diagnosed at birth some children with oral cleft may die from other causes before being diagnosed. To account for this we also analysed the data using a Cox model with time to diagnosis of the different types of clefts as the outcome.

Ethical approval

All data were analysed at Statistics Denmark using encrypted identification numbers with no contact with the individuals, and principles for good epidemiological practice in Denmark were fulfilled. The study was approved by the Danish Data Protection Agency (J. NR. 2008-41-2680) and the local ethics committee (VEK, case number M-20100252) in a Central Denmark Region.

Results

Of the 1 771 663 children, 35 118 (2%) were born to mothers who experienced bereavement during the exposure window. In total, 3043 children were diagnosed with a cleft; 968 with a cleft lip, 1206 with a cleft lip and palate and 869 with a cleft palate. About 2% of the children with an oral cleft were related. Table I shows the characteristics of the study population. Mothers and fathers of exposed children tended to be older and exposed mothers also had a higher parity. Lower maternal education, lower income and residence outside larger cities were more common in exposed mothers, compared with unexposed mothers.

Overall, maternal bereavement due to the death of close relative, at any time point from 1 year before conception to the end of the first trimester was associated with a significantly increased risk of oral cleft (OR: 1.28, 95% CI: 1.01; 1.61). A statistically significant 45% increased risk of oral cleft was seen in boys but not in girls when adjusting for parental age, parity, birth year and social factors (Table II). When mothers lost a relative due to a sudden death, the risk of oral cleft in the offspring was increased (OR: 1.76, 95% CI: 1.06; 2.94). Regarding timing of the bereavement, losing a relative in the time period before pregnancy and during the first trimester showed a tendency to an increased risk. Regarding the results for the individual types of oral cleft, the risk of cleft lip after bereavement due to a sudden death showed the highest risk (OR: 2.20, 95% CI: 0.98;4.93) (Table III). Generally, the risk for cleft lip and palate in relation to all types of bereavement showed the same tendencies as over all clefts (Table IV). Concerning the cleft palate, bereavement after death of a child had an OR of 2.36 (95% CI: 1.09; 4.92) (Table V). When stratified on sex, boys generally showed a more consistent tendency to excess risk of oral cleft related to death of a child and a sudden death of bereavement before conception. For girls only sudden death of a close relative and exposure during the first trimester showed an increased risk for oral cleft but it should be noted that the
results from the stratified analysis on sex are based on a small number of cases. We therefore chose not to present ORs for these analyses.

We repeated all analyses including children with syndromes or more than one congenital malformation and found slightly attenuated associations, but with the same tendencies (results not shown). After adding smoking to the adjusted model \( n = 918\,831 \) in separate sub-analyses, the results were slightly strengthened regarding bereavement due to loss of a child, to sudden death of any relative and timing during the first trimester. Similar results were found when using the Cox regression to take into account the differences in the follow-up time. Moreover, we did sub-analysis regarding maternal alcohol intake on \( \approx 86\,000 \) children and found no higher use of alcohol in the exposed group compared with the unexposed group either before or during pregnancy. Equally we did analysis on 83 000 women regarding exposure to other types of stress such as feelings of anxiety, depression and stress and found a difference of 0.37% between the exposure groups. Likewise we did additional analysis on women who have used oral anti-depressant 6 month before pregnancy or during the first trimester and found no significant difference in the use between the exposed and unexposed women. Sub-analysis where we exclude the 2% of cases that are related to each other still were similar to those presented in the present paper.

We also tried broadening the exposure window by including the second and third trimester which takes into consideration that death is often preceded by severe stress related to the disease leading to death. The results from this analysis were very similar to the results for the period 12 month before pregnancy to the first trimester.

Furthermore, the absolute risk difference is small. For overall bereavement, the prevalence was 1.7 per 1000 live born in the unexposed children and 2.2 per 1000 live born in the exposed children.

**Discussion**

In this large population-based cohort study, we found a statistical significantly increased risk of oral cleft after overall maternal bereavement due to the death of a close relative. Bereavement due to a sudden death and the death of a child in the antenatal period showed an excess risk of oral cleft in the offspring. The association seemed stronger for boys compared with girls, though limited power does not allow any stronger conclusion.

Our study has several methodological advantages. First, the study is based on large national registers covering the entire Danish population from 1978 to 2008, which allowed a much larger study population compared with previously published studies. Secondly, the information

### Table I Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Exposed (%) (n = 35 118)</th>
<th>Unexposed (%) (n = 1 736 545)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>17 907 (51)</td>
<td>890 810 (49)</td>
</tr>
<tr>
<td>Girls</td>
<td>17 211 (51)</td>
<td>845 718 (49)</td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0)</td>
<td>17 (&gt;1)</td>
</tr>
<tr>
<td><strong>Maternal age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–26</td>
<td>10 710 (30)</td>
<td>614 643 (35)</td>
</tr>
<tr>
<td>27–30</td>
<td>10 413 (29)</td>
<td>545 977 (31)</td>
</tr>
<tr>
<td>31–35</td>
<td>8 538 (24)</td>
<td>374 956 (22)</td>
</tr>
<tr>
<td>36–39</td>
<td>4 679 (14)</td>
<td>174 773 (10)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>778 (2.2)</td>
<td>26 194 (1.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0)</td>
<td>2 (&gt;1)</td>
</tr>
<tr>
<td><strong>Paternal age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–26</td>
<td>5 960 (17)</td>
<td>343 952 (20)</td>
</tr>
<tr>
<td>27–30</td>
<td>9 014 (26)</td>
<td>491 843 (28)</td>
</tr>
<tr>
<td>31–35</td>
<td>9 388 (27)</td>
<td>450 319 (26)</td>
</tr>
<tr>
<td>36–39</td>
<td>6 807 (20)</td>
<td>290 347 (17)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>3 280 (9)</td>
<td>125 031 (7)</td>
</tr>
<tr>
<td>Missing</td>
<td>669 (2)</td>
<td>35 053 (2)</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>12 632 (40)</td>
<td>813 892 (47)</td>
</tr>
<tr>
<td>Second</td>
<td>14 266 (40)</td>
<td>664 356 (38)</td>
</tr>
<tr>
<td>Third</td>
<td>6 287 (17)</td>
<td>207 180 (12)</td>
</tr>
<tr>
<td>Fourth or above</td>
<td>1 933 (5)</td>
<td>50 937 (3)</td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0)</td>
<td>180 (&gt;1)</td>
</tr>
<tr>
<td><strong>Birth year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1978–1993</td>
<td>16 441 (45)</td>
<td>875 621 (50)</td>
</tr>
<tr>
<td>1994–2008</td>
<td>18 677 (54)</td>
<td>860 924 (50)</td>
</tr>
<tr>
<td><strong>Maternal education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>10 890 (31)</td>
<td>493 352 (28)</td>
</tr>
<tr>
<td>Secondary</td>
<td>11 500 (33)</td>
<td>576 986 (33)</td>
</tr>
<tr>
<td>Primary</td>
<td>12 146 (35)</td>
<td>623 224 (36)</td>
</tr>
<tr>
<td>Missing</td>
<td>582 (2)</td>
<td>42 983 (2)</td>
</tr>
<tr>
<td><strong>Maternal income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First quartile</td>
<td>571 (2)</td>
<td>35 411 (2)</td>
</tr>
<tr>
<td>Second quartile</td>
<td>7 322 (21)</td>
<td>352 837 (20)</td>
</tr>
<tr>
<td>Third quartile</td>
<td>13 694 (39)</td>
<td>664 923 (38)</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>13 195 (35)</td>
<td>652 805 (38)</td>
</tr>
<tr>
<td>Missing</td>
<td>336 (2)</td>
<td>30 569 (2)</td>
</tr>
<tr>
<td><strong>Maternal marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/partner</td>
<td>17 282 (50)</td>
<td>833 277 (48)</td>
</tr>
<tr>
<td>Single</td>
<td>17 500 (50)</td>
<td>872 704 (49)</td>
</tr>
<tr>
<td>Missing</td>
<td>336 (1)</td>
<td>30 564 (2)</td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen</td>
<td>8 187 (23)</td>
<td>415 796 (24)</td>
</tr>
<tr>
<td>Aarhus</td>
<td>4 138 (12)</td>
<td>209 412 (12)</td>
</tr>
<tr>
<td>Other</td>
<td>22 457 (64)</td>
<td>1 080 773 (62)</td>
</tr>
<tr>
<td>Missing</td>
<td>336 (1)</td>
<td>30 564 (2)</td>
</tr>
</tbody>
</table>
about oral cleft came from the Danish Facial Cleft Database with high diagnostic quality. Thirdly, we believe that our measurement of the exposure, bereavement due to the death of a close relative, is an almost universal valid measurement for maternal stress since most people have close bonds with their relatives and therefore will experience a severe reaction to loss of a close relative. This response is also well described in the literature (Goodkin, 2001; Pfeffer et al., 2007). It should be kept in mind that the reaction involves more than stress; grief and yearning are also part of the exposure. Finally, we were also able to adjust for a number of confounders in a cohort with virtually no loss to follow-up.

It is a limitation that we only had information on live born children, but most children with isolated oral cleft survive their pregnancy and birth (Mossey et al., 2009). Since oral cleft are rare and despite the large study population, we still had a relatively small number of cases, which results in reduced power to detect small differences. Our timing of the stress exposure often started earlier and ended later than the timing of the death we report. Relatives may have close bonds with their relatives and therefore will experience a severe reaction to loss of a close relative. This response is also well described in the literature (Goodkin, 2001; Pfeffer et al., 2007). It should be kept in mind that the reaction involves more than stress; grief and yearning are also part of the exposure. Finally, we were also able to adjust for a number of confounders in a cohort with virtually no loss to follow-up.

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83 000 mothers, which showed that exposed mothers were more likely to report more emotional stress of this type than unexposed mothers. Since we have several kinds of exposure and death of a child and sudden death have the strongest association with oral cleft, an overlap of 21% between these two exposures should be taken into consideration. Also sudden overlap with other death since part of the category other death contains diagnosis of stroke or heart attack which also may be of sudden nature. In addition, we did not have actual measurements of the maternal cortisol concentration, but we believe that bereavement due to the death of a close relative produces a strong biological response in most people (Goodkin, 2001; Pfeffer et al., 2007). Also we did not have the opportunity to adjust for folic acid intake preconception or in the first trimester. However, we did a sub-analyses on 86 387 women in the Danish National Birth Cohort and found that exposed and unexposed women were equally likely to take folic acid supplements.

Our results are consistent with the previously published results. In 2000 Hansen et al. showed that Danish children exposed to severe maternal stress while in utero had a higher risk of malformations of the organs from the neural cranial crest cells, which includes oral cleft [OR: 1.54, 95% CI: 1.05; 2.27] Also oral cleft have been correlated with stress exposure in the antenatal period and found positively associated by Carmichael et al. In the first study where questionnaires

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### Table II The prevalence OR for cleft according to bereavement.

<table>
<thead>
<tr>
<th>Cases exposed/ non-bereaved cases</th>
<th>Crude OR</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys and girls, all bereavement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind</td>
<td>1.30</td>
<td>1.28 (1.01;1.61)</td>
</tr>
<tr>
<td>Type of relative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death of a child</td>
<td>15/2965</td>
<td>1.40</td>
</tr>
<tr>
<td>Death of other relatives</td>
<td>63/2965</td>
<td>1.28</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudden</td>
<td>16/2965</td>
<td>1.90</td>
</tr>
<tr>
<td>Other</td>
<td>60/2965</td>
<td>1.18</td>
</tr>
<tr>
<td>Timing of bereavement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-7 month before conception</td>
<td>34/2965</td>
<td>1.41</td>
</tr>
<tr>
<td>6-0 month before conception</td>
<td>31/2965</td>
<td>1.18</td>
</tr>
<tr>
<td>First trimester</td>
<td>13/2965</td>
<td>1.35</td>
</tr>
<tr>
<td>All bereavement stratified by sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind, boys</td>
<td>53/1808</td>
<td>1.45</td>
</tr>
<tr>
<td>Any loss, any time, any kind, girls</td>
<td>25/1157</td>
<td>1.06</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, paternal age, parity, birth year, education, income, cohabitation and place of residence.

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### Table III The prevalence OR for cleft lip according to bereavement.

<table>
<thead>
<tr>
<th>Cases exposed/ non-bereaved cases</th>
<th>Crude OR</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys and girls, all bereavement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind</td>
<td>23/945</td>
<td>1.20</td>
</tr>
<tr>
<td>Type of relative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death of a child</td>
<td>4/945</td>
<td>1.17</td>
</tr>
<tr>
<td>Death of other relatives</td>
<td>19/945</td>
<td>1.21</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudden</td>
<td>6/945</td>
<td>2.24</td>
</tr>
<tr>
<td>Other</td>
<td>17/945</td>
<td>1.05</td>
</tr>
<tr>
<td>Timing of bereavement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-7 month before conception</td>
<td>11/945</td>
<td>1.43</td>
</tr>
<tr>
<td>6-0 month before conception</td>
<td>8/945</td>
<td>0.95</td>
</tr>
<tr>
<td>First trimester</td>
<td>4/945</td>
<td>1.10</td>
</tr>
<tr>
<td>All bereavement stratified by sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind, boys</td>
<td>16/599</td>
<td>1.33</td>
</tr>
<tr>
<td>Any loss, any time, any kind, girls</td>
<td>7/346</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, paternal age, parity, birth year, education, income, cohabitation and place of residence.

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**Downloaded from https://academic.oup.com/humrep/article-abstract/28/4/1092/649319 by guest on 13 March 2019**
Maternal bereavement and offspring oral cleft

were administered 3.7 years after delivery of the child they found an increased risk for oral cleft (OR: 1.50, 95% CI: 1.1–2.0) and in the second study with questionnaires administered no more than 6 weeks after birth, they found maternal stress to be associated with the risk of oral cleft (OR: 1.45, 95% CI: 1.03–2.06) (Carmichael and Shaw, 2000; Carmichael et al., 2007). Studies investigating the relation between maternal stress and catastrophes showed an increased prevalence of oral cleft in children after the catastrophe compared with before (Montenegro et al., 1995; Tan et al., 2009; Goenjian et al., 2011). However, exposure to other teratogens related to the disaster may have played a role. Other studies have also found a positive association between maternal stress in the antenatal period and oral cleft (Peer and Strean, 1956; Hultin and Ottosson, 1971; Blomberg, 1980; Nimby et al., 1999).

Previously, prenatal exposure to maternal stress or glucocorticoids has been linked to low birthweight, preterm birth, shortened gestational age and small for gestational age (Seckl and Meaney, 2004; Class et al., 2011). Our stress exposure may include both acute and chronic stress and alpha amylase has been related to psychosocial stress of a more acute nature (Rohleder et al., 2004). The physiologic consequences of repeated or chronic stress exposure involve the autonomic nervous system and the hypothalamic–pituitary–adrenal axis is often referred to as the allostatic load (Latendresse, 2009). Maternal chronic stress may increase the release of cortisol. An expected death is often preceded by a diagnosis of a lethal disease and the stress reaction may start before the actual date of death. The concentration of the enzyme 11beta HSD2, which transports blood cortisol over the placenta, is low early in pregnancy, and the fetus may subsequently be exposed to high levels of maternal cortisol in that time period (Gitau et al., 1998; Welberg et al., 2000; Fowden et al., 2006), possibly affecting the development of the organs in the fetus. In an animal study of rats exposed to a chronic prenatal stressor from Days 14 to 19 of gestation and then an acute stressor Welberg et al. showed that the capacity to up-regulate the 11beta HSD2 activity in the fetoplacental barrier was reduced by 90% when the rats were exposed (Welberg et al., 2005). Also in humans maternal stress was associated with a down-regulation of 11beta HSD2 gene expression in late pregnancy with altered placental function thereby exposing the fetus to excess levels of cortisol (O’Donnell et al., 2012).

The association between overall prenatal maternal bereavement and risk of oral cleft in the offspring was statistically significant and the most severe bereavement, due to a sudden death of a close relative or death of a child was associated with an almost 2-fold increased risk. With this study we add a large scale human cohort study to the

Table IV The prevalence OR for cleft lip and palate according to bereavement.

<table>
<thead>
<tr>
<th>Cases exposed/ non-bereaved cases</th>
<th>Crude OR</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys and girls, all bereavement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind</td>
<td>32/1174</td>
<td>1.35</td>
</tr>
<tr>
<td><strong>Type of relative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death of a child</td>
<td>4/1174</td>
<td>0.94</td>
</tr>
<tr>
<td>Death of other relatives</td>
<td>28/1174</td>
<td>1.44</td>
</tr>
<tr>
<td><strong>Cause of death</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suddenb</td>
<td>5/1174</td>
<td>1.50</td>
</tr>
<tr>
<td>Other death</td>
<td>26/1174</td>
<td>1.29</td>
</tr>
<tr>
<td><strong>Timing of bereavement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-7 month before conception</td>
<td>13/1174</td>
<td>1.36</td>
</tr>
<tr>
<td>6-0 month before conception</td>
<td>14/1174</td>
<td>1.34</td>
</tr>
<tr>
<td>First trimester</td>
<td>5/1174</td>
<td>1.31</td>
</tr>
<tr>
<td>All bereavement stratified by sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind, boys</td>
<td>23/818</td>
<td>1.40</td>
</tr>
<tr>
<td>Any loss, any time, any kind, girls</td>
<td>9/356</td>
<td>1.24</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, paternal age, parity, birth year, education, income, cohabitation and place of residence.

bTwo cases have missing exposure status.

Table V The prevalence OR for cleft palate according to bereavement.

<table>
<thead>
<tr>
<th>Cases exposed/ non-bereaved cases</th>
<th>Crude OR</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys and girls, all bereavement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind</td>
<td>23/846</td>
<td>1.35</td>
</tr>
<tr>
<td><strong>Type of relative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death of a child</td>
<td>7/846</td>
<td>2.29</td>
</tr>
<tr>
<td>Death of other relatives</td>
<td>16/846</td>
<td>1.14</td>
</tr>
<tr>
<td><strong>Cause of death</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suddenb</td>
<td>5/846</td>
<td>2.08</td>
</tr>
<tr>
<td>Other death</td>
<td>17/846</td>
<td>1.17</td>
</tr>
<tr>
<td><strong>Timing of bereavement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-7 month before conception</td>
<td>10/846</td>
<td>1.46</td>
</tr>
<tr>
<td>6-0 month before conception</td>
<td>9/846</td>
<td>1.20</td>
</tr>
<tr>
<td>First trimester</td>
<td>4/846</td>
<td>1.46</td>
</tr>
<tr>
<td>All bereavement stratified by sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any loss, any time, any kind, boys</td>
<td>14/391</td>
<td>1.78</td>
</tr>
<tr>
<td>Any loss, any time, any kind, girls</td>
<td>9/455</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*Adjusted for maternal age, paternal age, parity, birth year, education, income, cohabitation and place of residence.

bTwo cases have missing exposure status.
body of literature on stress and birth defects. Severe and acute stress exposure in the antenatal period may be added to the list of potential causes of oral cleft.

### Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

### Acknowledgements

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### Authors’ roles

The current study was designed by K.G.I, J.O., J.L., E.A.N. and B.H.B. and conceptualized by K.G.I, H.L., J.O., E.A.N., B.H.B. and C.S.W. The oral cleft data were provided by K.C. The data were analysed by K.G.I, H.L. and C.S.W. and the manuscript was drafted by K.G.I, J.O., J.L., E.A.N. and B.H.B. H.L. All authors interpreted the results, critically revised the article and approved the final version of the manuscript.

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### Conflict of interest

None declared.

### References


