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Title

Twins' Risk of Childhood Asthma Mediated By Gestational Age and Birth Weight

Condensed title for running head

Twins' Asthma Risk Mediated By Perinatal Factors

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Key words

Child; risk factors; epidemiology; population-based birth cohort; twinship, gestational age, birth weight.

Abstract

Background

Children born with low gestational age (GA) or low birth weight (BW) are at increased risk of asthma. Twins as compared to singletons are on average more likely to be born with lower GA and BW, and have been hypothesized to comprise a high risk population for asthma. Many previous studies have not accounted for potential confounders or mediators.

Objective

To investigate the association between twinship and childhood asthma or early life wheeze and identify potential mediators, such as GA/BW.

Methods

The study population consisted of two cohorts including all children born in Sweden from January 1st 1993 to June 1st 2001 (n=756,363 singletons, n=22,478 twins) and July 1st 2005 to December 31st 2009 (n=456,239 singletons, n=12,872 twins). Asthma was defined using validated register-based outcomes of diagnosis or medication. The data were analysed using logistic (older cohort) and Cox regression (younger cohort). Adjusted models incorporated potential confounding or mediating factors including gestational age and birth weight.

Results

In the younger cohort, the crude hazard ratio (HR) of asthma medication after 1.5 years of age was 1.12 (95% CI 1.01-1.23), and fully adjusted HR 0.80, 95% CI 0.72-0.89. Crude HR of asthma diagnosis in the same age group was 1.14 (95% CI 0.99-1.30), fully adjusted 0.78 (0.68-0.98). Adjusted analyses in the older group yielded similar results.

Conclusions

Twins were at significantly higher unadjusted risk of asthma or early life wheeze compared to

singletons in the younger, but not in the older cohort. Associations attenuated following adjustment for GA/BW suggesting that GA/BW mediates the effect of twinship on asthma risk. After adjustments twins were at lower risk of asthma outcomes, possibly due to unmeasured confounding.

Introduction

Asthma is a worldwide contributor to childhood morbidity and the most frequent non-communicable disease among children [1]. It is a disease of many phenotypes, and several longitudinal studies have attempted to describe the natural course of asthma-related symptoms throughout childhood [2-4]. Genetic, environmental and early life risk factors all contribute to the childhood asthma panorama [5-7]. The first environmental impressions that affect human health are made in utero; and several known risk factors for asthma are of intrauterine origin. Low birth weight [8-10] and shorter gestation [8-11] are associated with childhood asthma in twins [8, 9] as well as singletons [10, 11].

Twin studies can provide valuable insights on the relative contribution of genetic and environmental factors to disease development [12]. However, there could be concerns regarding generalisability to singletons from such studies. Twins share the limited intrauterine space with another person: their co-twin. Consequently, twins are smaller on average at birth compared to singletons and twin pregnancies are shorter [13]. This has led to the hypothesis that twins could be at higher risk of developing asthma compared to singletons, which would have implications for twin studies investigating asthma by suggesting that twins comprise a high-risk population, possibly with different underlying mechanisms for disease. Previous studies testing this have presented conflicting conclusions, some reporting a decreased risk of asthma in twins compared to singletons [10, 14-16] whereas others observe no significant differences [17-20]. These studies display important methodological differences particularly in terms of analytical methods. A central aspect of these differences is the selection of covariates, as many of the previous investigations have not adjusted their statistical models for potential confounding factors [18-20]

or have chosen not to include birth weight or gestational age in the models [17]. The selection of covariates becomes particularly important when aiming to distinguish between direct effects and those acting through known potential mediators, as failure to include a covariate implies that its role in the causal pathway cannot be assessed [21].

The aim of our study is to estimate the association between twinship and asthma outcomes or early life wheeze in a large population-based birth cohort study based on Swedish health registers, while adjusting for mediating factors such as birth weight, gestational age and other perinatal factors. The study is carried out in one older and one younger group of children to illustrate possible differences related to age.

Methods

Study design and data sources

In Sweden, large population-based longitudinal birth cohort studies linking data from several different registers are made possible through the unique personal identification number [22]. In this study, several such registers were combined to connect birth characteristics and early life exposures to child health outcomes from hospitals and pharmacies. The national registers included the Medical Birth Register (MBR, covering >97% of births in Sweden [23]), the Migration Register (up to December 31st, 2009), the Cause of Death Register (up to December 31st, 2010), the longitudinal integration database for health insurance and labour market studies (LISA by Swedish acronym), the Multi-generation Register (MGR), the National Patient Register, NPR (up to December 31st, 2009) and the Prescribed Drug Register, PDR (started July 1st, 2005; data available up to May 31st, 2011). The linkage between the population-based registers has been approved by the Regional Ethical Review board in Stockholm (Dnr 2013/862-31/5) and did not require consent from participants. All data was anonymised and de-identified prior to analyses, and kept on a secure server.

Study population

The study participants were identified through the MBR and selected based on their birth dates. All live-born twin and singleton children were eligible for the study. Our study population consisted of two groups; the older cohort (born January 1st 1993 to June 1st 2001) and the younger cohort (born July 1st 2005 to December 31st, 2009). Birth date cutoffs in the older cohort were chosen to ensure that all study participants were covered for at least five years in the PDR before their 18th birthday. Those who were deceased, had an emigration event not followed by a

return to Sweden before the start of the PDR or emigrated during follow-up were excluded from the older cohort. The younger cohort included all children born after the start of the PDR for whom information was available in the MBR. For both cohorts, the follow-up period took place between birth and December 31st, 2009 (for asthma diagnosis) and between July 1st 2005 (or birth for the younger cohort) and May 31st 2011 (for dispensed asthma medication).

Between January 1993 and June 2001, 804,774 twin and singleton children were born in Sweden. Of these, 4,328 died during childhood and 21,605 emigrated without returning to Sweden before July 1st, 2005. This left n=778,841 individuals in the older cohort. From July 2005 to December 2009, n=469,111 twin and singleton children were born into the younger cohort.

Variables

Information on twin or singleton status was collected from the MBR. This was also the source of information on gestational age, birth weight, child's Apgar score at 5 minutes after delivery, mode of delivery, maternal smoking during pregnancy, involuntary childlessness and maternal body mass index (BMI). Maternal and paternal ages at the child's birth were calculated based on parental birth dates retrieved from the MGR, which was also the source of information on the presence of older siblings sharing the same mother and of mother's country of birth. The parents' highest educational level was extracted from the LISA register. The highest attained level within each parent pair during the birth year of the child was used.

Data on asthma outcomes were collected from the NPR (first asthma diagnosis in in- or outpatient care) and PDR (dispensed asthma medication). All diagnoses with an ICD-10 code J45 or J46 or ICD-9 code 493 were extracted from the NPR. For the older cohort, only diagnoses

given and medications prescribed between 5-18 years of age were included, indicating prevalent asthma. There was no age limit in the younger cohort, in contrast register coverage from birth allowed detection of incident asthma cases. Asthma medication use was assessed based on two pre-defined asthma medication outcomes, which have been validated against medical records with high positive predictive values for clinical asthma diagnosis [24], The first medication outcome was defined in the older cohort as ≥2 dispenses of inhaled corticosteroids, ICS (ATC code R03BA), fixed combinations of β2-adrenoreceptor agonists and ICS, β2-ICS (R03AK) and/or leukotriene receptor antagonists, LTRA (R03DC), independent of time between distributions. In the younger cohort, the same medications were used but time between dispenses had to be ≥14 days. For both cohorts, the second medication outcome was having ≥3 dispenses of, ICS, selective β2-adrenoreceptor agonists, β2 (ATC code R03AC), β2-ICS and/or LTRA within a year. In both cohorts overall asthma medication was defined as fulfilling either the first or the second medication outcome. Additionally, combined outcomes were constructed. In the older cohort, the combined outcome was defined as either asthma diagnosis or medication, whereas in the younger cohort both a stricter requirement of both asthma diagnosis and medication was applied to reduce potential misclassification due to early life wheeze.

Statistical methods

In the older cohort logistic regression was used, estimating crude and adjusted odds ratios (ORs) with 95% confidence intervals (CI). In the younger cohort, which was followed from birth, time to event or censoring was calculated, and Cox proportional hazard regression performed, estimating crude and adjusted hazard ratios (HRs) with 95% CIs. Attained age was used as the time scale. Exit from the study population was through either asthma outcome (for medication outcomes, the date of the first prescription in combination with dispenses fulfilling the criteria)

or censoring at emigration, death, or end of follow-up (May 31st, 2011). Descriptive characteristics of all covariates were presented for twins and singletons and for the older and younger cohorts. Selection of confounders or mediators was based on potential association with both exposure and outcome. Only covariates which fulfilled these criteria, but which were not considered possible colliders or common consequences of exposure and outcome, were included in the adjusted models. For example, gender was not included in the adjusted models since it is not associated with twinship. In addition to the crude model, four adjusted models were constructed. Adjusted models 1 and 2 included only gestational age and birth weight, respectively. The fully adjusted model included gestational age and birth weight, as well as mode of delivery, Apgar score, highest parental education level at birth, and presence of older siblings. Adjusted model 3 was a reduced version of the fully adjusted model not including gestational age and birth weight. Directed acyclic graphs (DAGs) were used to support this process [25]. All standard errors were estimated using a sandwich estimator to account for the clustering of observations within families (twins and other siblings). The proportional-hazards assumption of the Cox proportional hazard regression was tested on the basis of Schoenfeld residuals in combination with visual inspection of hazard curves. If significantly non-proportional hazards for the twinship variable, age-dependent HRs were estimated and presented, with cut-offs based on the hazard curves. If the inclusion of a covariate was found to induce non-proportional hazards, the Cox regression model was stratified by said covariate. In both cohorts individuals with missing data on a covariate were excluded from adjusted analyses including said covariate (complete case analysis). Statistical analyses were conducted in STATA/IC 12.1 (StataCorp, TX).

Results

Table 1 shows background characteristics of twins and singletons in the older and younger cohort. There was no difference in the proportion of males and females between twins and singletons in either cohort, but they displayed differences in distribution of all other variables. For example, 63.4 % of twins in the older cohort were born before 39 weeks of gestation, compared to 10.7% of the singletons, and twins in the older cohort were born with a birth weight lower than 2500 grams in 38.2% of births which was the case for only 3.0% of singletons. The same pattern was seen in the younger cohort.

A selection of parental background characteristics are presented in Table 2. Mothers of twins were slightly older compared to mothers of singletons. In the older cohort, 38.0% of mothers of twins were below 30 years of age, compared to 51.5% of the mothers of singletons. Smoking habits during pregnancy were similar between twin and singleton mothers. Differences between twins and singletons for any given variable tended to follow the same pattern in both age groups. Parents of twins had consistently attained a higher educational level compared to parents of singletons.

Results for the association between twinship and asthma outcomes in the older cohort are displayed in Table 3. In crude analyses, there was no significant difference between twins and singletons in terms of asthma diagnosis in the NPR (OR 1.04, 95% CI 0.98-1.11) or asthma medication (OR 1.04, 95% CI 0.99-1.10) between 5-18 years of age. When adjusting for gestational age, twins were at significantly lower odds of having any asthma diagnosis in the NPR (adjusted model 1, OR 0.83, 95% CI 0.78-0.88). This association was further emphasized

when also adjusting for birth weight, mode of delivery, Apgar score, parental education and presence of older siblings. In the model of all covariates except gestational age and birth weight, point estimates were similar as in the crude model but associations weaker. Similar findings were obtained for the asthma medication outcomes. Adjusting for birth weight yielded similar results as adjusting for gestational age for all asthma outcomes. The combined definition of asthma (either diagnosis or medication) also resulted in similar estimates, crude OR 1.03 (95% CI 0.99-1.09) and fully adjusted OR 0.83, 95 CI 0.79-0.87), not displayed in table.

In the younger cohort, the proportional hazards assumption of the Cox regression models was not fulfilled. Hazard ratios are therefore estimated age-dependently. A summary of the results of the Cox regression analyses in the younger cohort is presented in Table 4. Between 0 and 1.5 years of age, the HR of receiving any asthma diagnosis in twins compared to singletons was significantly increased at 1.42 (95% CI 1.29-1.55) with similar HR for asthma medication outcomes. Similar to the older cohort, adjusting the analyses for gestational age reversed the direction of the associations, although a statistically significant reduced rate was only seen for asthma medication (HR 0.91, 95% CI 0.85-0.98). Models adjusted for birth weight resulted in that the association between twinship and asthma outcomes could no longer be detected. In the fully adjusted models, twins were at significantly lower risk of an asthma outcome compared to singletons. After 1.5 years of age, twins were at significantly higher risk of asthma medication (HR 1.12, 95% CI 1.01-1.23), but not asthma diagnosis. Fully adjusted models consistently resulted in statistically significant reduced risk of twinship on asthma outcomes. As in the older cohort, the model including all covariates except gestational age and birth weight yielded estimates in between crude and fully adjusted values. For asthma outcome defined as both

diagnosis and medication, crude HR between 0 and 1.5 years of age was 1.48 (95% CI 1.34-1.63), whereas after 1.5 years of age it was 0.98 (95% CI 0.83-1.16). The fully adjusted HRs in the respective age groups were 0.85 (95% CI 0.76-0.94) and 0.68 (95% CI 0.57-0.81).

Sensitivity analyses for all crude models including only individuals with gestational age ≥37 weeks or birth weight between 2500-3500 grams resulted in similar results as the models adjusted for gestational age or birth weight, respectively (OR of asthma diagnosis between 5-18 years was 0.92, 95% CI 0.86-0.99 in individuals with gestational age ≥37 weeks, compared to OR adjusted for gestational age in the same age group in the full cohort 0.83, 95% CI 0.78-0.99).

Discussion

In this large population-based study, we found that twins were at increased risk of childhood asthma outcomes or early life wheeze compared to singletons in preschool ages, but not later in childhood. This finding was consistent across several definitions of asthma outcomes. We also saw that the distributions of gestational age and birth weight varied between twins and singletons. When gestational age was included in the statistical models, effect measures consistently showed significantly decreased risks of childhood asthma outcomes or early life wheeze in twins compared to singletons. Adjusting for birth weight had similar although slightly less pronounced effects. This indicates that the observed higher risk of childhood asthma in twins compared to singletons appears to be mediated by differences in gestational age and birth weight between these populations.

The issue concerning whether gestational age [26] and birth weight [27] should be adjusted for in analyses of associations between early exposures and disease outcomes has been debated. The principal argument against including these factors in a statistical model is that significant unmeasured confounding may be introduced. This could occur if reasons for singletons to be born at a low gestational age are different from twins', and that these reasons for prematurity are the real culprits resulting in observed lower risk of disease among twins. The notion that premature twins are better or at least not worse off than equally premature singletons in terms of neonatal outcomes is supported to some degree by the literature. Twins have been reported to be less severely affected by gestational hypertension [28] and diabetes [29]. Comparing twins and singletons with very low birth weight [30] or matched for growth restriction [31], previous studies could not show significant differences in terms of certain neonatal outcomes (including

sepsis and intraventricular haemorrhage). Twins and singletons matched for gestational age also did not display differences in incidence of adverse neonatal outcomes including respiratory distress syndrome, intraventricular haemorrhage [32, 33], sepsis, pneumothorax [33], and neonatal mortality [32, 34]. However, it remains possible that relevant differences exist. While the identity of these potential unmeasured confounders remains unknown, we argue that adjusting our analyses for gestational age and birth weight adds a new layer of information, although the results should not be used to infer a causal effect of twinship in itself. This implies that differences in prevalence between twins and singletons do not preclude generalisability from twin studies, but rather that the biological mechanisms relevant to the exposure-outcome association under study should be considered in each case [35].

Previous studies of twinship as a risk factor for asthma have come to diverging conclusions [10, 14-20]. Our study is the first to show an increased risk of asthma outcomes or early life wheeze in twins, and one of few to include gestational age and birth weight as covariates. Among the four previous studies which did not report significant difference in asthma risk between twins (sometimes including triplets and higher order multiples) and singletons [17-20], one (Huovinen and Kaprio) studied self-reported asthma in adults [19] and three (Huovinen and Kaprio, Davidson et al and Sicignano et al) presented only unadjusted statistical models [18-20]. Aspberg et al included a multivariate model, but did not adjust for birth weight or gestational age [17]. Two previous British studies showed a decreased risk of asthma in twins in unadjusted models [15, 16], although McKeever et al mentioned that adjusting for some potential confounders other than birth weight or gestational age did not significantly alter the effect estimates [16]. One previous literature review available on the subject [36] was based on papers studying the

prevalence of asthma in twins alone and does not contain direct comparisons between twin and singleton individuals in the same setting. Finally, two previous studies (Dik et al 2004 and Bråbäck and Hedberg 1998) showed a decreased risk of asthma in multiple birth individuals, adjusted for birth weight and gestational age [10, 14], which is in agreement with our adjusted findings. However, neither of these studies were carried out specifically in twins but also included other multiple birth individuals such as triplets, and one did not include children [14].

Ideally, comparisons between previous studies should be made based on both crude and adjusted estimates with relevant covariates. Different definitions of outcome between studies may pose additional problems in terms of comparisons. Whereas we applied a set of validated register-based outcomes from registers reflecting both in- and outpatient as well as general practice care in Sweden, previous studies used either self-reported diagnoses or symptoms [14, 19], diagnoses given only in connection with a hospital admission [15, 17, 18] or included those given also at other types of clinical visits [10, 16]. For these differences in definition of asthma to act in different directions between studies requires a process in which asthma is over- or underestimated in twins compared to singletons. This could be the case if an asthma diagnosis in one twin leads to the co-twin also being examined in some health care systems, but not in others, or if twins are more likely to self-report asthma if their co-twin also has the disease. Another possible mechanism could lie in a higher degree of health care consumption during childhood in twins compared to singletons due to other factors, which could lead to that milder cases of asthma would be discovered to a higher extent in twins. As hospital admission due to asthma is associated with more severe cases of the disease, however, the previous potential explanations should mainly be applicable when using outcomes typically related to less severe disease.

Strengths of our study are several. Firstly, we studied a large population with robust measures of exposure and validated register-based outcomes [24]. Secondly, we adjusted for confounders not included in previous studies, which enabled us to estimate direct effects, accounting for mediation by gestational age and birth weight as well as other potentially relevant factors. Thirdly, the possibility to study different age groups indicated that the associations shown are particularly pronounced on incident disease in early childhood. The study should also be interpreted in the context of its limitations. In general, coverage of the Swedish registers is high, however, the outpatient part of NPR is an exception with 80% coverage since 2001 [37]. As there is no reason to believe register coverage is different between twins and singletons, this should not lead to differential misclassification. Finally, an important concern in the younger cohort is that asthma is not the only condition featuring wheeze, and as a result the register-based outcomes are less specific in this age group, potentially also including children with infection-induced symptoms [38] . Based on the previous validation study of the PDR and NPR, about 1/4 of the cases classified as asthma before 4.5 years of age may have obstructive bronchitis or other respiratory symptoms [24]. However, we do not expect this issue to be more or less common among twins compared to singletons. The validity of the register-based outcomes for preschool children is improved when combining PDR and NPR data; when using both data sources 89% fulfilled criteria for asthma [24]. When we thus constringed the asthma definition in our analyses, we found that the patterns observed when studying the two outcomes separately remained.

In conclusion, we observed an increased risk of childhood asthma outcomes or early life wheeze in twins compared to singletons in preschool ages, which was largely consistent across asthma outcomes. The association between twinship and asthma appeared to be mediated by gestational age and birth weight; adjusting for these factors throughout childhood resulted in a significantly decreased risk of childhood asthma in twins, which may be due to unmeasured confounding. Further research on the nature of childhood asthma in twins compared to singletons could shed more light on this issue by highlighting the underlying biological mechanisms.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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