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# Ammonia, ammonium, and the risk of asthma: A register-based case–control study in Danish children

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**Background:** Evidence concerning the health effects of exposure to fine particulate matter less than 2.5 µm in diameter ($PM_{2.5}$) and particulate components such as ammonium ($NH_4^+$) is limited. We aimed to investigate gaseous ammonia ($NH_3$), particulate ammonium ($NH_4^+$), the total concentration of these pollutants ($NH_x$), and $PM_{2.5}$ and their association with asthma in Danish preschool children.

**Methods:** Estimates of incidence rate ratios associated with exposure to air pollution were calculated for children followed for the development of childhood asthma (n = 335,629) from their first to their sixth birthday during the time period 2006–2012 using a time-matched case–control design by combining register information. Modeled average concentrations of $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ (5.56 km × 5.56 km grid resolution) during the past 3 months prior to the date of first diagnosis of asthma for cases.

**Results:** We identified 12,935 incident cases of asthma. The base adjusted hazard ratio of incident asthma was 1.74 for children exposed to the highest decile of $NH_3$ exposure (95% confidence interval = 1.60, 1.89) compared to children exposed to the lowest decile of $NH_3$ exposure during the past 3 months prior to first diagnosis. Similar hazard ratios were found for the highest levels of $NH_4^+$ (2.33; 2.04, 2.65) and $NH_x$ (1.82; 1.68, 1.96). The positive associations were slightly attenuated, when adjusting for socioeconomic status, but disappeared when additionally adjusting for region, except in a two-pollutant model including $NH_4^+$ and $PM_{2.5}$. No association was found with $PM_{2.5}$. The findings were robust when using different exposure time windows.

**Conclusions:** Increased $NH_4^+$ exposure may be a risk factor for the onset of asthma in preschool children.

**Keywords:** Asthma, Ammonia, Ammonium, $PM_{2.5}$ components, Air pollution, Register-based epidemiological study

## Introduction

Asthma is the most prevalent disease in children worldwide.[1] Concern is growing regarding the impact of outdoor ambient air pollution exposure on the risk of pediatric asthma. Several studies have shown that fine particulate matter less than 2.5 µm in diameter ($PM_{2.5}$) is associated with respiratory symptoms, exacerbation, and new-onset asthma in children.[2,3] Previous studies have focused almost exclusively on health effects associated with transport- and industrial-related $PM_{2.5}$ exposure, whereas surprisingly little attention has been paid to agricultural air pollutants and the role of particulate precursors and components such as gaseous ammonia ($NH_3$) and ammonium ($NH_4^+$), although $NH_4^+$ accounts for a substantial proportion of the $PM_{2.5}$ mass.[4] More information on the physical and chemical properties of $NH_3$ and $NH_4^+$ is given in the online data supplement; http://links.lww.com/EE/A13.

Results of the few existing epidemiological studies showed an association of $NH_4^+$ concentrations and ambient ammoniumbisulphate exposure from lags of 0–4 days and increased respiratory hospitalizations in adults[5,6] and increased emergency department visits due to upper respiratory infections in children.[7] Furthermore, high ambient $NH_3$ exposure during the last day has been linked with declining lung function, but not asthma symptoms,[8] whereas annual average $NH_3$ exposure has neither been associated with increased use of asthma medication nor asthma[9] in 8- to 12-year-old children. A review of controlled human exposure studies on inorganic $NH_4^+$ salts concluded that there was no evidence of acute adverse pulmonary health effects from sulfate or nitrate salts at environmentally relevant levels, but at concentrations well

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### What this study adds

In this large-scale study of 336,648 children, we report that $NH_3$ and $NH_4^+$ exposure may be risk factors for the onset of asthma before their sixth birthday. Our findings support the need for the continuous monitoring of air pollution policies, given not only the potential detrimental health effects of $NH_3$ and $NH_4^+$ exposure but also the fact that these pollutants contribute substantially to the formation of secondary fine particulate matter that is recognized as a significant burden of disease and mortality.

above ambient levels, changes in pulmonary function might be occurring.[10] Similarly, high $NH_3$ and $NH_4^+$ concentrations in occupational settings and rodent experiments have been associated with airway irritation and increased inflammatory responses.[11] However, so far, no studies have investigated the effect of long-term ambient $NH_3$ and $NH_4^+$ exposure on the risk of asthma.

The present study differs from earlier studies in that (1) It applies atmospheric model calculations, enabling an investigation of the effect of long-term ambient exposure on a nation-wide basis while accounting for both spatial and temporal concentration variations on a regional and local level; (2) The use of Danish population registers provides an excellent opportunity to link environmental exposure via residential coordinates with information on personal characteristics and diagnosis history; (3) The focus is exclusively on children, who are possibly more susceptible than adults because their lungs are developing and they generally spend more time outdoors. The main objective of our study was to investigate the effect of gaseous $NH_3$, particulate $NH_4^+$, the total concentration of $NH_3$ and $NH_4^+$ ($NH_x$), and $PM_{2.5}$ on incident asthma in a large population of preschool-aged Danish children. We hypothesized that exposure to high levels of $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ during the past 3 months increase the risk of asthma.

## Methods

### Information base: The Danish Civil Registration System

The Danish Civil Registration System[12] is a register established in 1968 of all people alive and living in Denmark, currently 5.7 million persons. The system includes information on personal identification number, sex, date and place of birth, continuously updated information on vital status and individual and longitudinal information on place of residence. The personal identification number is used in all national registers, enabling accurate linkage between registers. The source population of the present study consisted of all persons who were born in Denmark from January 1, 2000, to December 31, 2011, and whose parents were both born in Denmark (642,075 persons). The residential longitudinal database obtained from the Danish Civil Registration System was linked with the Danish official standard addresses and coordinates to obtain exact information on the longitudinal geographical residential coordinates of all Danish residents from 1978 onwards.

### Modeling of $NH_3$, $NH_4^+$, and $PM_{2.5}$ concentrations

Concentrations of pollutants were modeled on a daily basis with a spatial resolution of 5.56 km × 5.56 km for all of Denmark covering the period January 1, 2005, to December 31, 2012. Primary pollutants of interest comprised $NH_3$, $NH_4^+$, total inorganic ammonia (NHx), and $PM_{2.5}$. Secondary pollutants comprised $O_3$, $NO_2$, and $SO_2$. Concentrations were shown for Denmark's five administratively and geographically disjoint regions (north Denmark, central Denmark, south Denmark, capital region, Zealand). Location and characteristics of the Danish regions are shown in Figure e1; http://links.lww.com/EE/A13 (see supplementary information). All were obtained from the Danish Eulerian Hemispheric Model (DEHM),[13] which is part of the Danish Ammonia Model System (DAMOS) previously described in detail.[14,15] Briefly, the coupling of DEHM and DAMOS compose a three-dimensional Eulerian atmospheric chemistry transport model (CTM) with a horizontal domain covering the Northern Hemisphere. This allows extracts of hourly average concentrations to grid cells covering Denmark based on the regional and local contribution.[14]

### Assessment of asthma

Asthma cases within the cohort were identified from the Danish National Patient Register.[16] This register contains data on all admissions to public hospitals in Denmark from 1977 onwards. All Danish residents are entitled to free national health care, and the Danish National Patient Register has virtually complete data on hospital visits. Information on outpatient visits was included from 1995 and onwards. The diagnostic system used was the *International Classification of Diseases*, 10th revision (ICD-10-CM). Cohort members were classified with asthma (ICD-10: J45 or J46) if they had been admitted to a public hospital or had been treated as an outpatient and had received their first diagnosis of asthma as the final diagnosis of the visit (release diagnosis). The date of onset was defined as the first day of the first visit (in- or outpatient) when the diagnosis of asthma was made. Childhood asthma was defined as children who were first diagnosed with asthma between the ages of one and six. The reason for choosing this age group is that early onset asthma is largely based on inflammation and to a minor degree allergy, and we wanted to study this in relation to air pollution since air pollution may induce inflammatory changes in the airways.

### Study design and statistical analyses

We used survival analyses techniques to follow all children from their first to their sixth birthday between 2006 and 2012 for the risk of asthma, using exposure to air pollutions during the past 3 months as the exposure of interest. From the outset of the study, we hypothesized that past exposure to air pollution during the past 3 months influences the risk of asthma. The rationale behind the choice of exposure time window was based on an assumption that asthma develops over time and possibly not due to a single peak in exposure. Incidence rate ratios were estimated using an individually time-matched case–control design. For each child first diagnosed with asthma, we selected at random 25 control children who shared the same sex and birthday, who were alive, and who were not diagnosed with asthma when the case was included (n = 322,694). For cases and their individually matched controls, we calculated the average exposure during the past 3 months before the case was diagnosed, accounting for residential changes for both cases and controls. Average pollutant concentrations were first categorized into deciles using the lowest decile as the reference category. Secondary analyses treated the average pollutant concentrations as a continuous variable measuring the risk for children exposed to the highest decile compared to children exposed to the lowest decile, while utilizing information on all intermediate deciles. Incidence rate ratios were estimated using conditional logistic regression, with each case–control set forming separate strata. The following adjustment scenarios were used: (1) age, sex, date of birth, calendar year (base adjustment), (2) base adjustment + socioconomic status (SES), (3) base adjustment + region, and (4) base adjustment + region + parental SES. The latter was measured by maternal educational level and paternal income that may be risk factors for asthma in children. The rationale for including region is several: regions are administrative responsible for registration of asthma diagnosing, regions may differ in meteorological factors and unknown factors that affect the air quality overall, and region and residential area can serve as a proxy for SES. We performed sensitivity analyses, considering the past 6- and 12-month exposure time-windows, considering different age groups (1, 2–3, and 4–5 years), and considering consistency of the results in the five geographical regions by performing a log likelihood test for interaction between the effect of each pollutant and region. Statistical analysis was conducted using SAS statistical software (SAS Institute Inc., Cary, NC).

## Results

### Study population characteristics

During the period 2006–2012, 12,935 persons born in Denmark were diagnosed with asthma for the first time in their life before

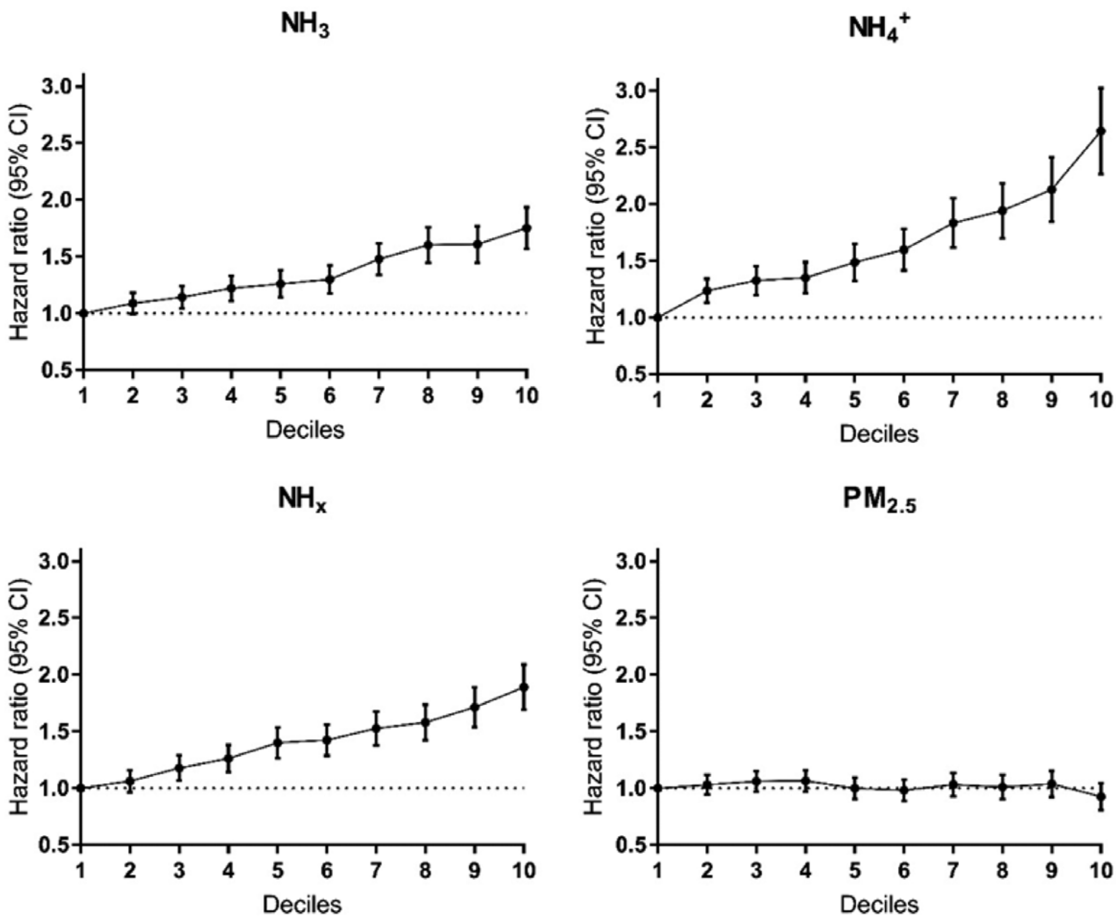


**Figure 1.** Effects of $NH_3$, $NH_4^+$, $NH_x$ and $PM_{2.5}$ concentrations on the risk of asthma with base adjustment. $NH_3$, ammonia; $NH_4^+$, ammonium; $NH_x$, $NH_3 + NH_4^+$; $PM_{2.5}$, particulate matter less than 2.5 μm in aerodynamic diameter.

their sixth birthday. Table e1; http://links.lww.com/EE/A13 (see supplementary information) shows characteristics of the cases and their individually matched controls (recorded at the time each case was first diagnosed) comprising 335,629 in total. By design, cases and controls were matched by age and sex. Study participants ranged in age from 1 to 6 years; the majority of cases occurred among children less than 3 years old, and 61% of the children were boys (Table e1; http://links.lww.com/EE/A13). The asthma incidence was lower in the capital region compared with the other Danish regions, with the region of south Denmark having the highest hazard. High SES was associated with a lower hazard (Table e1; http://links.lww.com/EE/A13).

### *Concentrations and distribution of ammonia and ammonia-related pollutants*

Figure e2; http://links.lww.com/EE/A13 maps pollutant concentrations averaged over 2008 in the study area. The annual mean (SD) was 1.19 ppb (0.39) for $NH_4^+$, 1.99 pbb (1.35) for $NH_3$, 3.19 pbb (1.40) for $NH_x$, and 7.10 μg/m$^3$ (1.66) for $PM_{2.5}$. Concentrations of $NH_3$ were highest in the southwestern part of Denmark, generally higher over the mainland of Denmark (Jutland) and lowest over Zealand. Concentrations of $NH_4^+$ showed a strong southwest north-east gradient ranging from 0.30 to 0.77 ppb. $PM_{2.5}$ concentrations ranged from 7.20 to 10.00 μg/m$^3$ and were slightly elevated over the largest cities, especially over the capital city of Copenhagen. While highly positive correlations were found between concentrations of $NH_3$ and $NH_x$ ($r = 0.96$) and $NH_4^+$ and $PM_{2.5}$ ($r = 0.91$), a moderate correlation was shown between $NH_4^+$ and $NH_x$ ($r = 0.28$), low positive correlations were found between $NH_x$ and $PM_{2.5}$ ($r = 0.09$), whereas low negative correlations were found between $NH_3$ and $PM_{2.5}$ ($r = -0.17$). No correlation was found between $NH_3$ and $NH_4^+$ ($r = -0.002$).

### *$NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ concentrations and asthma morbidity*

Figure 1 depicts effects of $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ concentrations and risk of asthma. A clear positive exposure–response association between the level of $NH_3$ and a later risk of asthma is shown. The children exposed to the highest level of $NH_3$ had a 1.74 (95% confidence interval = 1.60, 1.89)-fold increased risk of asthma compared to children exposed to the lowest level (Table e1; http://links.lww.com/EE/A13). $NH_4^+$ and $NH_x$ showed nearly identical patterns as shown in Figure 1 and Table 1. On the other hand, $PM_{2.5}$ exposure was not associated with asthma (adjusted hazard ratio; 95% confidence interval 0.96; 0.86–1.06; Table 1). Similar results were found for $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ when using 6- and 12-month exposure time windows (Table e2; http://links.lww.com/EE/A13) and consistently across different age groups (Table e3–e5; http://links.lww.com/EE/A13).

When we adjusted effects sizes for SES, the positive associations were slightly attenuated (Table 1), but the associations for $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ with asthma incidence were negative when adjusting for region. Correspondingly, a similar pattern of association was shown when we performed the analyses stratified on geographical regions (Table e6; http://links.lww.com/EE/A13). Finally, we performed all analyses adjusting for base characteristics (sex, date of birth, age, and calendar year), region, and SES and additionally for any other pollutant by including

**Table 1**

**Effects of $NH_3$, $NH_4^+$, $NH_x$ and $PM_{2.5}$ concentrations and risk of asthma with different adjustments**

| | Base Adjustment[a] HR (95% CI) | Second adjustment[b] HR (95% CI) | Third adjustment[c] HR (95% CI) | Fourth adjustment[d] HR (95% CI) |
|---|---|---|---|---|
| Pollutants | | | | |
| $NH_3$ | 1.74 (1.60–1.89) | 1.55 (1.42–1.68) | 0.76 (0.67–0.86) | 0.73 (0.64–0.83) |
| $NH_4^+$ | 2.33 (2.04–2.65) | 2.14 (1.89–2.44) | 0.96 (0.80–1.15) | 0.90 (0.75–1.08) |
| $NH_x$ | 1.82 (1.68–1.96) | 1.61 (1.49–1.75) | 0.80 (0.71–0.89) | 0.76 (0.68–0.85) |
| $PM_{2.5}$ | 0.96 (0.86–1.06) | 1.07 (0.97–1.19) | 0.75 (0.65–0.88) | 0.78 (0.67–0.91) |

[a]Base adjustment for sex, date of birth, age, and calendar year.

[b]Second adjustment for sex, date of birth, age, and calendar year, and socioeconomic status.

[c]Third adjustment for sex, date of birth, age, calendar year, and region.

[d]Fourth adjustment for sex, date of birth, age, calendar year, region, and socioeconomic status.

95% CI, 95% confidence interval; HR, hazard ratio; $NH_3$, ammonia; $NH_4^+$, ammonium; $NH_x$, $NH_3 + NH_4^+$; $PM_{2.5}$, particulate matter less than 2.5 μm in aerodynamic diameter. Conditional logistic regression results are presented as HRs and 95% CIs describing the effects of $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ on the risk of asthma treating the pollutants as trend variables showing the risk of the highest exposed children (10th decile) compared to the lowest exposed children (1st decile).

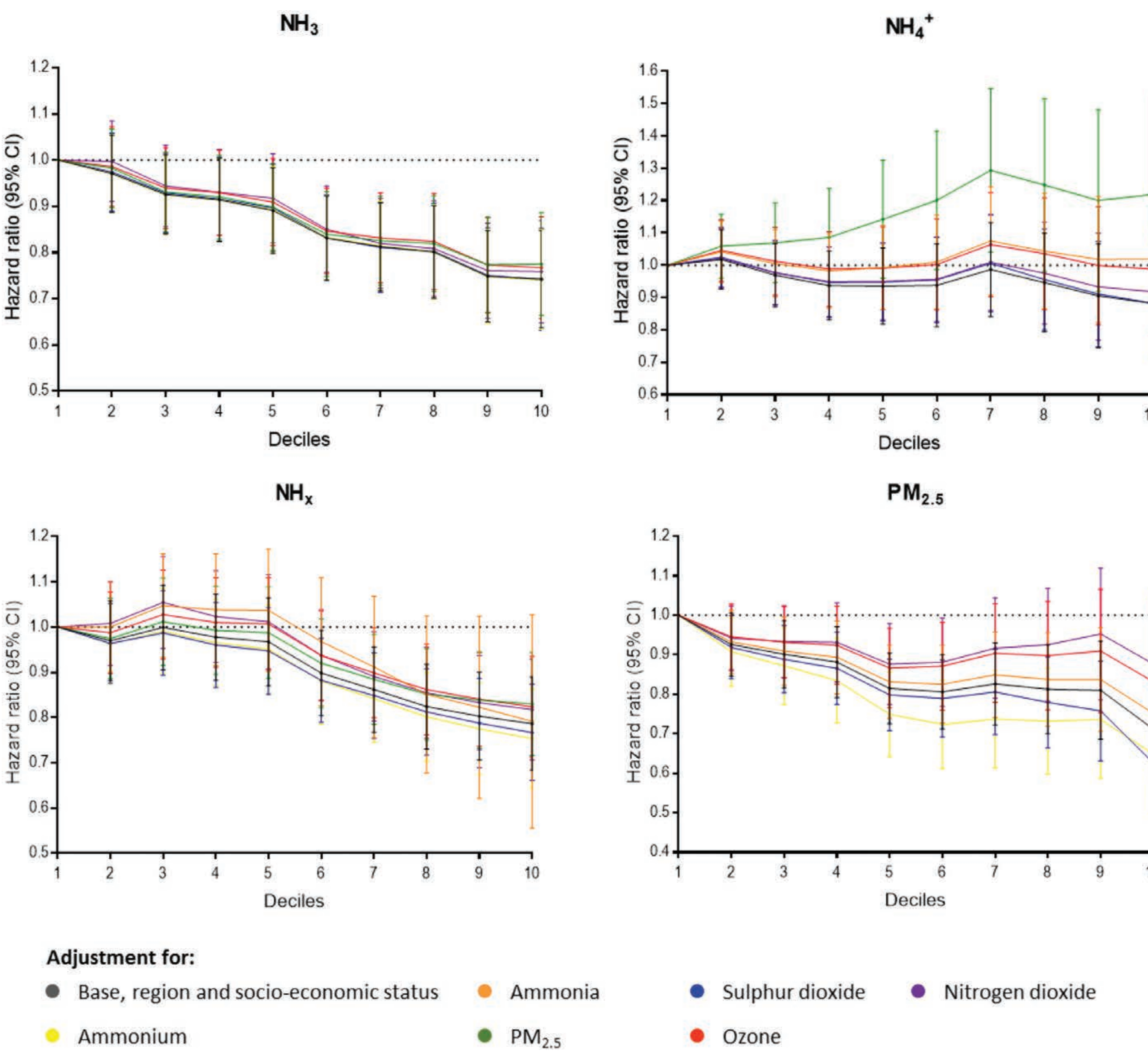


**Figure 2.** Effects of $NH_3$, $NH_4^+$, $NH_x$, and $PM_{2.5}$ concentrations on the risk of asthma with base, region, and socioconomic status (SES) adjustment and additional adjustment for other pollutants. $NH_3$, ammonia; $NH_4^+$, ammonium; $NH_x$, $NH_3 + NH_4^+$; $PM_{2.5}$, particulate matter less than 2.5 μm in aerodynamic diameter.

one pollutant simultaneously in each logistic regression analysis in a two-pollutant model (Figure 2). The negative association for $PM_{2.5}$, $NH_3$, and $NH_x$ with asthma remained, whereas the positive association persisted for $NH_4^+$ when adjusting for $PM_{2.5}$.

## Discussion

### Main findings

The present study is unique as it allowed us to potentially link concentrations of specific components of particulate air pollution with incident cases of asthma, thus, to investigate whether gaseous $NH_3$, particulate $NH_4^+$, and $PM_{2.5}$ are risk factors for asthma. The basic adjusted analyses revealed a positive association between $NH_3$, $NH_4^+$, and the sum $NH_x$ exposure and asthma diagnosis but showed no association between $PM_{2.5}$ and asthma. In a second step, the positive associations of $NH_3$ and $NH_4^+$ exposure with asthma were slightly attenuated, when adjusting for SES, whereas the associations disappeared when adjusting for region in a third step. Finally, in a fourth step, we adjusted additionally for any other pollutant in a two-pollutant model, where the positive association remained for $NH_4^+$ when adjusting for $PM_{2.5}$, which was made possible by the virtue of the study size. These findings indicate that early life exposure to $NH_4^+$ may be a risk factor for onset asthma, but we cannot rule out confounding by region or other factors covarying with $NH_3$, $NH_4^+$, and $PM_{2.5}$. If regional confounding exists, this means that the regions either differ in diagnostic practises, population characteristics, concentrations of other pollutants, or in some other unknown factor(s) related to the region that may be the underlying cause of asthma and hereby introduced ecological fallacy. Additionally, it can be argued that stratification for and adjustment for region could have caused an over-adjustment due to the evident regional distribution of pollutants causing a lack of exposure contrast within regions. Furthermore, although $PM_{2.5}$ followed the regional sections, associations of $PM_{2.5}$ with asthma were generally inconsistent and negative when adjusting for region, not supporting earlier findings showing a positive association for $PM_{2.5}$ with childhood asthma.[2,3,17] The high correlation between $NH_4^+$ and $PM_{2.5}$ ($r = 0.91$) and uneven distribution of the pollutants may thus be the reason for the change in association between $NH_4^+$ and asthma when adjusting for region. The adjustment for $PM_{2.5}$ can be viewed as an adjustment for traffic and heating of houses by wood burning in the cities, unmasking the effect of $NH_4^+$ to be observed in more urban areas, as can be seen in Figure e2; http://links.lww.com/EE/A13.

### Comparison with other studies

To our knowledge, this large-scale study is the first to explore ambient $NH_3$ and $NH_4^+$ exposure and the risk of developing pediatric asthma from a nation-wide perspective. Among the studies performed with children, results showed no association with self-reported asthma symptoms and medication use,[8,9] whereas an inverse association was found between $NH_3$ concentrations with forced expiratory volume in 1 second.[8] Moreover, some studies have suggested an increased risk of respiratory hospitalizations linked with high $NH_4^+$ concentrations in adults in Atlanta (USA)[5] and New York (USA).[6] Similarly, one study found an association of $NH_4^+$ exposure with increased emergency department visits due to upper respiratory infections, but not due to pneumonia or bronchiolitis, in a large population of 1- to 4-year-olds in Atlanta (USA).[7] Generalizations of these results are problematic as the studies explored the respiratory health effects related to $NH_3$ and $NH_4^+$ exposures either in small restricted areas, or during short time-periods, or in small-scale study populations, which increases the risk of bias affecting the results.

The positive associations between $NH_3$, $NH_4^+$, and asthma may be causal relations or, alternatively, it may be that $NH_3$ and $NH_4^+$ serve as markers for other farming exposures associated with asthma—for example, organic dust exposure that has previously been linked to respiratory symptoms and asthma,[18] also in combination with NH.[3,19] Other studies on respiratory morbidity relied on proxies as "living on or near a farm" due to the known large agricultural contribution of local and regional emissions of $NH_3$ and $NH_4^+$. Some studies have indicated detrimental health effects in adults in terms of declined lung function,[20] increased respiratory symptoms,[18] and doctor-diagnosed asthma.[21] Beneficial effects of farm contact have also been reported in terms of a lower prevalence of sensitization and atopic asthma.[22] Studies have shown that children exposed to farming environments suffer more from asthma and respiratory symptoms[23,24] and less of allergy[1] than their counterparts. However, most farming studies use "general farming exposures" as the exposure variable, and therefore these findings cannot be specifically related to either $NH_3$ or $NH_4^+$ exposures.

Although our findings indicated that only $NH_4^+$ is a risk factor for onset asthma early in life, emissions of $NH_3$ are essential as $NH_3$ is a precursor to $NH_4^+$ that is formed in a reaction between $NH_3$ and acid aerosols. The spatial pattern of $NH_3$ concentrations showed a south-west, north-east gradient and $NH_4^+$ showed a south-west north-east gradient. A validation study on the DEHM and DAMOS models concluded that these gradients are caused partly by atmospheric transport of primarily $PM_{2.5}$ from northern Europe and by local and regional ammonia emissions south of Denmark,[15] thus emphasizing transboundary movement of pollutants. In a previous study on European $NH_3$ emissions, the $NH_3$ emissions varied considerably with the month of the year, in which Danish emissions were low to moderate in February but moderate to high in April due to manure application. However, we did not observe an increase in asthma diagnoses in the 3-month period following April (data not shown).

### Strengths and limitations

***Design.*** A major strength of our study is the large sample size that was possible owing to the register-based design. This design secures high completeness of data, prospective data collection and eliminates selection bias. Therefore, the study is representative of preschool children of parents born in Denmark, regardless of their place of residence, SES, sex, and age, but precludes children and parents that are emigrants to avoid handling the complexicity of different cultures and ethnicities that are known to influence on asthma. Additionally, as the exposure time window was the same for cases and their individually matched controls, the study design and the adjustment for sex, date of birth, age, and calendar year preclude confounding by time-invariant characteristics of participants. In our study, some covariates were found to be risk factors of onset asthma, including children's mothers having a short education compared to a longer education and fathers having a low income compared to a higher income. A shortcoming of our study, however, is the lack of information on other potential confounders that might have played a role in our study, especially smoking and dampness in the children's home, two factors known to greatly influence the incidence of childhood asthma.[25] Information on smoking is of particular importance because tobacco smoke, on the one hand, is a strong predictor of asthma, and on the other hand, $NH_3$ is an endogenous part of tobacco smoke chemistry.[26,27] Furthermore, it would have been informative to know whether the children lived on or near a farm and spent time in enclosed livestock facilities to compare the risk of highly exposed children with low-exposed children.

***Exposure assessment.*** Another strength of this study was the nationwide estimation of exposure that provides information on modeled $NH_3$ and $NH_4^+$ exposure during the past year for all participants, comprising both long-term exposures with a high grid resolution and providing satisfying exposure contrasts. Although the validation of the DAMOS model has shown satisfying agreement between modeled and measured concentrations of $NH_3$,[28] a limitation of the model appears to be related to the performance of the wet deposition of $NH_3$, which can potentially be improved by better estimation of precipitation.[14] A clear advantage of this study is that it contained enough spatial variation in pollutants to enable us to explore the change in effects when adjusting for any other pollutant. Still, the complexity of pollutant mixtures like $PM_{2.5}$ makes it difficult to identify causal components and study interactions in those mixtures. Also the high correlations between pollutants often makes it difficult to disentangle their individual effects on health. This may also explain why previous studies on $PM_{2.5}$ constituents and health effects have been inconsistent in their results and $PM_{2.5}$ mass persists as the best predictor of adverse health outcomes.[29]

We used modeled pollutant concentrations linked to each child's residential address as proxy for each child's exposure. Besides being at home, most Danish children attend daycare centers that most often are located in the same area as their residential address. Therefore, we assumed that the children remain in the same area of their home address the majority of their time.

An alternative to exposure modeling by residential address would be the measurement of exposures at residential addresses or personal air monitoring. However, although these exposure assessments are favored, it is impractical in a large study population over time. Validation of the DAMOS model has shown satisfying agreement between modeled and measured concentrations of $NH_3$ (correlation coefficients for five monitoring stations, range 0.51–0.78) based on the daily mean values averaged over measurement stations as time series and annual mean values, as well as the daily maximum values as scatter plots.[28]

***Outcome assessment.*** In epidemiological studies, various definitions of pediatric asthma are used, and therefore outcome misclassification is often a concern, especially in children with less distinctive symptoms. It is a strength of our study that we used information on doctor-diagnosed asthma derived from the Danish National Patient Register, which limits misclassification since diagnosis is based on validated criteria. However, the register most likely only reflects cases of severe asthma and leaves out children with mild to moderate asthma (or easy treatable asthma) diagnosed from general practitioners. Most children in our study were diagnosed with asthma before 3 years of age. These children may be defined as early wheezers as in early ages, it can be unclear whether respiratory symptoms are part of asthma development or attributable to respiratory virus infections. As a result, effect estimates may therefore bias associations towards the null. Our stratified analyses in different age groups revealed, however, similar results, although the findings were most consistent in the 2- to 3 year-olds. It should be noted though that asthma is a heterogeneous disease, and the time of diagnosis and progress may reflect different asthma phenotypes as recently suggested.[30] Unfortunately, we had no information on atopy, lung function, inflammatory markers, and disease progress and prognosis, and therefore, we cannot distinguish further between phenotypes.

## Conclusions

Our results indicate that high levels of $NH_4^+$ may be associated with an increased risk of developing asthma in preschool children causing an important public health problem affecting a significant number of children. Therefore, there is a continuous need to pay attention to air pollution policies and highly exposed populations of children to protect these children from developing asthma that may affect them and society throughout their lives. Further exploration in areas with a high agricultural activity is needed to confirm our result.

## Conflict of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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Data availability Health and Welfare, NordicWelfAir: Data are not available due to a strict data-sharing agreement. Data on individual characteristics were requested through Statistics Denmark from the Danish Civil Registration System and data on asthma from the Danish National Patient Register. Environmental data can be requested for payment from the Department of Environmental Sciences, Aarhus University. The overall link of information on residential coordinates with exposure assessment, personal characteristics, and diagnosis history was performed within Statistics Denmark that requires all users to have permission to access data and carry out analyses.

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