**Urban upbringing and childhood respiratory and allergic conditions: a multi-country holistic study.**

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**Abstract**

**Background:** We integratively assessed the effect of different indoor and outdoor environmental exposures early in life on respiratory and allergic health conditions among children from (sub-) urban areas.

**Methods:** This study included children participating in four ongoing European birth cohorts located in three different bio-geographical regions: INMA (Spain), LISAplus (Germany), GINIplus (Germany) and BAMSE (Sweden). Wheezing, bronchitis, asthma and allergic rhinitis throughout childhood were assessed using parental-completed questionnaires. We designed “environmental scores” corresponding to different indoor, green- and grey-related exposures (main analysis, a-priori-approach). Cohort-specific associations of exposure to environmental scores with the respiratory health outcomes were assessed using random-effects meta-analyses. In addition, a factor analysis was performed based on the same exposure information used to develop the environmental scores (confirmatory analysis, data-driven-approach).

**Results:** A higher early exposure to the indoor environmental score, increased the risk for wheezing and bronchitis within the first year of life (combined adjusted odds ratio: 1.20 [95% confidence interval: 1.13-1.27] and 1.28 [1.18-1.39], respectively). In contrast, there were inverse associations with asthma (0.93 [0.85-1.02]) and allergic rhinitis between 6 and 8 years (0.85 [0.79-0.92]). There were no statistically significant associations for the outdoor related environmental scores in relation to any of the health outcomes tested. The factor analysis confirmed the observed association with? the indoor environmental score.

**Conclusion:** Although higher suggested microbial load indoors through occupants was associated with an increased risk for wheezing and bronchitis within the 1st year, this might serve as a preventive and beneficial mechanism against later childhood allergic respiratory outcomes in urbanized environments.

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**INTRODUCTION**

The prevalence of asthma and allergic conditions is increasing worldwide (1) and has coincided with the rapid and ongoing increase in the population residing in urban areas (2). Urban areas are characterized by a network of non-natural built-up infrastructures (i.e. grey spaces) with limited green or natural environments (3). The higher prevalence of asthma and allergic conditions in urban areas compared to the rural areas suggests that urban-related environmental factors may contribute to the pathogenesis of these conditions (4). Previous efforts to evaluate such contributions have mainly focused on a single factor (while adjusting for other exposures), either in indoor or in outdoor environment.

In general, there is a plethora of evidence to suggest both positive and negative associations with various indoor and outdoor factors and respiratory health outcomes. Some environmental factors are of particular interest, demonstrating strong associations with respiratory outcomes (5). For instance, growing up on a farm and thereby higher associated exposure including contact to farm animals, animal feed or unprocessed cow’s milk have shown to protect children from asthma, hay fever and allergic sensitization (6). These associations have been explained by the ‘hygiene hypothesis’ (7); an early, more intense contact to microbial agents might modulate and program the developing of immune system towards a non-allergic response (8,9). Still, less is known regarding ‘beneficial’ exposure conditions in urban areas. Nevertheless, previous literature in populations from affluent countries suggests the existence of an inverse association between number of siblings and reported prevalence of allergy-prone diseases, such as hay fever in later childhood, possibly due to increased exposure to infections early in life as well as shedding and sharing microbial exposures through more frequent contact (10,11). Further, a recent study among adults observed that a higher proxy for biodiversity in inner city environments, represented by early childhood exposure to pets, day care, bedroom sharing and older siblings was related to less allergic sensitization (12). Moreover, early exposure to pets, in particular dogs has been repeatedly suggested to be associated with a reduced risk of (non-atopic) asthma outcomes (13), although overall, associations are not very consistent (14,15). In contrast, associations were rather consistent for exposure to moisture and mould damage at home in relation to increased risk for asthma and respiratory conditions among children worldwide (16–20). Harmful effects of early secondhand tobacco smoke (SHS) exposure in relation to these outcomes have also been documented in a number of studies among children (21–23).

In terms of the outdoor environment, it has been speculated that urbanization leads to a loss of beneficial natural environments which may promote a weakened tolerance against harmful allergens ubiquitous in natural surroundings among children growing up in cities (24) as compared to bringing up in rural environments (6,25). Moreover, urban environments are known to vary in their ‘grey’ surfaces, which comprise industrial, transport and urban-fabric characteristics, often accompanied by an increased exposure to traffic-related air pollution (26,27). Finally, urban settings are also highly dependent on their surrounding climate and geographical characteristics (26–28).

Focusing on only one or very few exposures inadequately captures the complex nature of interrelated environmental factors in real-life and their potentially synergistic/antagonistic impacts on asthma and allergic conditions. To our knowledge, no study has evaluated how a combination of indoor and outdoor environmental factors experienced in early life may affect later respiratory health in order to provide a holistic perspective over the role of urban upbringing in the pathogenesis of asthma and allergic conditions in different bio-geographic regions. As such, the aim of the present study was to disentangle and prospectively evaluate the association between indicators of urban-related indoor and outdoor environmental exposure characteristics, using a holistic concept, with respiratory and allergic health outcomes in young children from four different birth cohorts established in diverse bio-geographical regions in Europe. Towards this aim, we were particularly interested as to whether we could identify *beneficial* environmental conditions in *urbanized* environments.

**MATERIALS AND METHODS**

*Study population and study area*

The study population comprises four ongoing birth cohorts of different bio-geographical regions across southern, central, and northern Europe: INMA (Spain, N=2472), GINIplus (Germany, N=5991), LISAplus (Germany, N=3094), and BAMSE (Sweden, N=4089). For the included studies, approval by the local ethics committees and written consent from participants’ families were obtained. A detailed description of the prospective and population-based birth cohorts included is provided in the **Supplementary Information 1**.

*Exposure assessment*

We used three different environmental domains that describe the home as well as the surrounding built environment, identically defined and available in each of the participating birth cohorts. For the (1) *a-priori* approach (main analysis), exposure was defined as the Indoor, Grey and Green environmental score (hereafter referred to as “environmental scores”). For the (2) data driven approach (confirmatory analysis), the same exposure data was used in a factor analysis (FA) in order to confirm or falsify the subjectively built environmental scores.

1) A-priori approach (main analysis)

INDOOR environmental score

Based on Campbell and colleagues (12), the “indoor score” was composed of environmental characteristics associated with suggested higher microbial load (“biodiversity proxy”). These included *family size, number of children, sharing bedroom*, and *pets at home* (12)which are suggested to be associated with higher exposure to various microbial agents. The indoor score was calculated from answers to the following four survey questions in the time interval between birth and one year: (1) *“Are there currently pets at home?”* (1 if yes, 0 if no), (2) *“Are there (older) children at home (excluding the study child)?”* (=1 if ≥ 1, =0 if =0), (3) *“How many persons sleeping in one room together with the study child?”* (=1 if ≥ 1, =0 if =0), and (4) *“How many people live permanently in the household together with the study child (excluding the study child for INMA* (=1 if > 2, =0 if ≤2)*, including the study child for GINIplus, LISAplus, and BAMSE)?”* (=1 if > 3, =0 if ≤3). The combined effect (sum of these scores) was examined together as the cumulative “indoor score” (ranged from 0 to 4).

OUTDOOR-GREEN and OUTDOOR-GREY environmental scores

The outdoor-green and outdoor-grey environmental scores were based on information available and identical among all participating cohorts. The outdoor-green score was composed of first, immediate *residential surrounding greenness* (100 m buffer around the home address) and second, neighborhood *green/natural land use pattern* (300m buffer around the home address). The outdoor-grey score was based on information describing first, home surrounding *urban land use* patterns, second, exposure to *nitrogen dioxide* *(NO2)* and third, *distance to the nearest major road* with constant traffic. A more detailed description and assessment of the exposure characteristics in the individual cohorts as well as the creation of the green and the grey environmental score is provided in **Supplementary information 2** and **Supplementary information 3**.

2) Data-driven approach (confirmatory analysis)

The second approach (“confirmatory analysis”) was performed in order to evaluate the assessment of the environmental scores as well as the findings in relation to the health outcomes with a data-driven-approach by using a Factor Analysis (FA) (29). Hence, the same environmental exposure data as it was used for building the environmental scores was applied. A more detailed description of the data driven approach can be found in **Supplementary information 4 and 5**.

*Health outcome assessment*

We focused on parental completed questionnaire information on (presumably infectious) respiratory outcomes including *wheezing* and *bronchitis* within the 1st year, as well as on current allergy-prone respiratory outcomes *asthma* and *allergic rhinitis / hay fever* in later childhood (INMA: 7y, GINI/LISA south and north: 6y, BAMSE: 8y). For GINI/LISA South and North, as well as for BAMSE, there were further data available on aeroallergen sensitization (specific immunoglobulin E (IgE) > 0.35 kU/l) at 6 and 8 years, respectively. Detailed information of the health outcome assessment in the birth cohorts is provided in the **Supplementary Information 6**.

*Statistical analysis*

Cohort-specific logistic regression models (30) were applied to analyze the individual associations between (1) the environmental scores as well as the (2) identified dimensions of the Factor analysis with each of the respiratory and allergic health outcomes at age 1 and between 6-8 years, respectively. Random-effects meta-analysis (31) was used to calculate combined estimates to allow for potential between–cohort heterogeneity. The regression models of the main analysis (Environmental Scores) were adjusted for sex, maternal education, maternal allergy, maternal smoking during pregnancy, breastfeeding, exposure to environmental tobacco smoke at home (first year), dampness at home (first year) and cohort (INMA: Asturias, Gipuzkoa, Sabadell, Valencia, child belongs to either GINIplus or LISAplus). The regression models of the confirmatory analysis were mutually adjusted for the identified dimensions in addition to the variables mentioned above for the main analysis. All results are presented as odds ratios (OR) with corresponding 95% confidence intervals (95%-CI).

*Sensitivity and stratified analyses*

With respect to the main analyses, we first evaluated whether the effect of the respective exposure was more pronounced among atopic children with asthma or allergic rhinitis/hay fever compared to diseases not accompanied by specific allergic sensitization. Due to data availability, this was only possible in GINI/LISA South, GINI/LISA North and BAMSE. In addition, we added “dampness” (1=yes, 0=no) as a further source of possible microbial exposure to the indoor score for all cohorts. Moreover, for INMA and BAMSE, we also had information on “attending daycare” before the 2nd birthday (1=yes, 0=no), which we included as a potential source of higher microbial load indoors to the indoor score. Lastly, we performed another FA and additionally included “dampness” as well as “passive smoke” exposure during the first year of life in order to evaluate whether this changes the allocations to the dimensions as compared to the first FA.

All statistical analyses were performed using the statistical software R, version 3.4.0 (R

Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>) using FAmix within the “PCAmixdata” package for factor analysis .

**RESULTS**

*Study population and exposure characteristics*

According to **Table 1**, study population and exposure characteristics varied widely across the birth cohorts and the differences were statistically significant for nearly all characteristics but sex (data not shown).

 *Environmental scores*

The cohort-specific distribution of the environmental scores is shown in **Figure 1**. For the indoor environmental score, a bimodal distribution (with either a high proportion of low or high exposure) was especially evident for GINI/LISA South and BAMSE. The outdoor-grey and outdoor-green associated environmental exposure was differentially distributed among the study centres.

*Main analysis: Associations between environmental scores and health outcomes*

Overall, as displayed in **Figures 1A-B**, exposure to suggested higher microbial load indoors, as represented by the indoor environmental score, was found to increase the risk for wheezing and bronchitis outcomes within the first year of life in adjusted random-effects meta-analyses (adjusted Odds Ratio aOR, 95% Confidence Interval 95%CI: aOR 1.20 [1.13-1.27] and 1.28 [1.18-1.39], respectively). In contrast, we observed inverse associations between exposure to suggested higher microbial load indoors with asthma and statistically significant with allergic rhinitis in later childhood (0.93 (0.85-1.02) and 0.85 [0.79-0.92], respectively. For the remaining environmental scores, no statistically significant associations were observed.

There were no major differences in the results when the analyses were stratified by atopic status, except a slightly more pronounced inverse effect between exposure to the indoor score and allergic rhinitis in the atopic subgroup (0.85 [0.76-0.95]) compared to the non-atopic children. Further, including “dampness” and “daycare before the 2nd birthday” as additional sources of microbial exposure to the indoor score did not change the magnitude or the direction of the effect estimates for any of the outcomes tested (data not shown).

*Confirmatory analysis (Factor analysis)*

We found similar associations for the dimension “crowding” (high factor loadings for “number of people at home” and “number of (older) children” in relation to the health outcomes as it was observed with the indoor environmental score in mutually adjusted regression analyses (**Figure 2A-B**). “Crowding” at home significantly increased the risk for wheezing and bronchitis within the first year (1.20 [1.15-1.26] and 1.27 [1.19-1.36], respectively). Similar to the indoor environmental score results, there were statistically significant inverse associations between “crowding” with later asthma and allergic rhinitis (0.91 [0.85-0.98] and 0.87 [0.81-0.93], respectively). In contrast, the factor described by “outdoor exposure” was observed to significantly increase the risk for bronchitis within the first year (1.04 [1.00-1.07]), but there was no significant associations with asthma and allergic rhinitis in later childhood. Lastly, we further included “dampness within the first year” and “passive smoke exposure within the first year” to the factor analysis. The assignment of the environmental exposure as well as their associations with the health outcomes remained stable for “outdoor exposure” and “crowding”. There were further no statistically significant associations between the third dimension (“pets”) and any of the health outcomes tested (data not shown).

**DISCUSSION**

To the best of our knowledge, this study is the first to specifically consider early life environmental exposures in relation to respiratory and allergic outcomes using a holistic approach that integrates several relevant indoor and outdoor exposure characteristics across different geographical regions. We observed that a higher suggested microbial load indoors was associated with increased risk for more infection prone wheezing and bronchitis within the first year of life. This exposure, on the other hand, was associated with a decreased risk of allergic rhinitis in later childhood. As compared to the strong signal describing the indoor home environment, no consistent results in relation to respiratory health was observed for the outdoor related green and grey environmental scores. The results of the a-priori based indoor environmental score could be confirmed by using a data-driven approach, identifying a “crowding” dimension. As opposed to the grey and green environmental scores, the results of factor analysis indicated that the outdoor environment cannot be considered as isolated environmental dimensions in relation to health, but they are highly interrelated.

For farm and rural environments, studies have suggested that the associated lifestyle as well as early higher and diverse microbial exposure might play a role in lower risk of allergic immune response later in childhood and adulthood (32,33). The indoor and outdoor related microbial profile in *urban* environments might differ considerably from those in rural areas, in terms of levels, composition, and diversity (34), and therefore might also have different effects on allergic outcomes. Nevertheless and apart from bio-geographical conditions, lower prevalences of hay fever and allergic sensitization have also been consistently observed with a higher number of (older) siblings in urban areas (10,11). Family size or more frequent human contact in general is suggested to be a source of higher microbial and viral exposure through shedding and sharing among themselves (7,35). According to the “hygiene hypothesis” (7), this might have the potential to attenuate the harmful effects of increased hygienic conditions and lower xenogeneic pressure associated with a “Westernized” life style on the maturating immune system, resulting in increased risks for allergy prone diseases in urban environments. In fact, although we here consistently observed that a higher suggested microbial and viral load indoors through occupants around birth was strongly associated with a higher risk of infections during the first year of life, this association was reversed for asthma and allergic rhinitis later in childhood. Further, for two out of four participating birth cohorts, sizeable inverse associations with allergic rhinitis were also found when we additionally took daycare attendance before the second birthday into account in the indoor score. A recent urban birth cohort study in the U.S. observed a bi-directional relationship between cumulative early day care attendance with asthma, pointing out a reduced risk for asthma with increased duration of daycare attendance (> 1800 hours) (36). Further, previous studies looking at the health effects of early higher exposure to microbial components in *urban* settled house dust (most prominently floor and mattress dust) are also partly in line with our findings for asthma and allergic rhinitis. According to the available literature, higher and more diverse microbial loads indoors have been associated with lower risks for allergic outcomes in a few small-scale studies (37–39). Lastly, especially the combination of large family size and exposure to farming was associated with a remarkable decrease in hay fever in a previous study (40). However, it was not possible to disentangle the effects of bothprotective factors, suggesting two different biological mechanisms and pointing out the magnitude of both environmental determinants in relation to allergy prone diseases.

Our results indicate an important signal of *human* derived and transferred microbial and viral exposure in homes in relation to early respiratory infections as well as the development of allergic rhinitis in childhood and attenuate the effect of outdoor related exposure characteristics on these outcomes. Though “crowding” has been also suggested to be a risk factor for hospitalization in childhood and viral infections are the major cause of acute wheezing exacerbation in early life (41); yet they are very common and for most of the children, no negative impact in later life is expected – unless they are not impaired by host factors or deficiencies in the innate immune response to these agents (42). We also included “exposure to pets” in the indoor environmental score, however, “crowding”, as identified by the factor analysis, was exclusively based on person associated factors. Previous dust microbiome studies suggest that bacterial exposure in urban settings is generally largely dominated by occupants and to a lesser extent by pets, and not by outdoor sources (43–45). A study in over 500 children living in the inner city environments of Baltimore, Boston, New York, and St Louis, United States, observed that a concomitant high exposure to bacteria in dust (Firmicutes and Bacteriodetes) and allergens might reduce the risk for atopy and recurrent wheezing (39). On the other hand, a recent investigation among 189 children from the German LISAplus study was not able to confirm protective findings of bacterial exposure in relation to atopy and wheezing, but rather associations were found with a higher and more diverse fungal exposure assessed in living-room floor dust samples (38). Unfortunately, until now, our knowledge remains limited as to which microbial markers in dust may be associated with a decreased risk for asthma and allergic outcomes via a mechanism that involves greater family size or more frequent human contact.

It is assumed that indoor microbial communities are part of the closer neighborhood and built environment (46). Therefore, the simultaneous exposure to indoor and outdoor environmental exposure might play even a more important role for metropolitan areas compared to rural areas due to a presumably more heterogeneous exposure profile of coincident hazardous and protective factors (47). Nevertheless, while there remained a consistent strong inverse association of exposure to suggested higher microbial load indoors, as determined by the indoor score and “crowding”, on later asthma and allergic rhinitis outcomes in all sensitivity analyses, the associations were less coherent for the remaining environmental exposure constructs. In general, compared to natural surroundings, artificial green urban areas can also be potential sources for harmful allergen exposure (48–51). Fundamentally, it is likely that associations with respiratory and allergic health will depend on the allergenicity of the respective green exposure surrounding the participants (25,28,52). Moreover, the contextual factors describing the outdoor environment are highly area-specific and a more detailed exposure characterization would be desirable. Unfortunately, this was not possible for the current publication as the aim was to capture a wide geographical region and the exposure characteristics were restricted to a common available base. However, future studies in a local area with region-specific characteristics at a finer scale, the type and amount of air pollution from traffic and industries, vegetation types as well as the amount of native versus non-native vegetation and lastly, a more detailed description of the built environment are therefore recommended (53). In summary, the results of our study might underline the importance of early exposure to *indoor* related exposure over outdoor related exposures with respiratory and allergic health outcomes in urbanized residential surroundings.

A key strength of this study is its comprehensive approach, integrating several indoor as well as outdoor environmental exposures in relation to respiratory and allergic health outcomes, providing a comprehensive perspective over such a relationship. Other advantages of our prospective study were the large sample size of the birth cohorts, the harmonized exposure and health outcome assessment, the confounder information and the inclusion of diverse bio-geographic regions across north, center, and south of Europe. Limitations of the study include that we could not include further potentially relevant (built) environment factors such as walkability, neighborhood social status, or the school environment, all of which may act as additional sources for regular microbial contamination. In addition, although several other environmental exposure characteristics were available in each of the birth cohorts, we only included those which were available and identically assessed in every study population. In this context, we also did not have data on the *actual* microbial exposure, e.g. as determined in dust samples, associated with the respective environmental exposure domains, which otherwise would assist us in identifying potential causal agents for the observed effects. Lastly, for the indoor environmental domain in the main analyses, we only focused on suggested higher microbial load exposure and excluded potential harmful exposure such as dampness and passive smoke exposure. Nevertheless, all statistical models were adjusted for dampness and passive as well as *in utero* tobacco smoke exposure. Apart from that, including more sources related to hazardous exposure characteristics in the factor analysis did neither change the assignment to the “dimensions”, nor result in a respective coherent third exposure domain.

**Conclusion**

We evaluated associations between several well-established environmental exposures from the indoor as well as the closer urban built environment in relation to infectious and allergic health outcomes in urban environments using two different statistical approaches. Our study indicates that in particular early exposure to a suggested higher microbial load indoors is associated with an increased risk of presumably infection-prone wheezing and bronchitis in early childhood but with a decreased risk for asthma and allergic rhinitis later in childhood. As compared to that, there were no coherent findings for exposure to outdoor related environmental on the tested health outcomes, suggesting a major importance of indoor related exposure early in life over outdoor related sources in adjusted analyses. The assumed biological mechanism might be an early and more intense encounter with viruses and higher microbial load associated with greater family size. There is substantial potential for preventive actions if protective microbial markers factors associated with this specific exposure can be identified, e.g. obtained through dust samples in homes with greater family size or daycare centers.

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