**Household environmental microbiota influences early-life eczema development**

Short title: Household environmental microbiota and eczema

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**Originality-Significance Statement**

Our study is the only longitudinal birth cohort study that has collected and evaluated dust microbiota composition in the preconception, pregnancy and postnatal phase. We observed higher abundance of human-associated bacteria in house dust of subjects with eczema, suggesting their possible contributions in regulating host immunity and increasing the susceptibility to eczema. Our study is also the first to propose the possible protective effect of an environmental microbe *Planomicrobium* (*Planococcaceae* family) against eczema, which was observed to be significantly higher in house dust of controls. This study highlights the possible influence of early life environmental microbiota exposure from preconception to infancy on eczema development in early childhood.

**Summary**

Exposure to a diverse microbial environment during pregnancy and early postnatal period is important in determining predisposition towards allergy. However, the effect of environmental microbiota exposure on allergy during preconception, pregnancy and postnatal life on development of allergy in the child has not been investigated so far. In the S-PRESTO (Singapore PREconception Study of long Term maternal and child Outcomes) cohort, we collected house dust during all three critical window periods and analysed microbial composition using 16S rRNA gene sequencing. At 6 and 18 months, the child was assessed for eczema by clinicians. In the eczema group, household environmental microbiota was characterized by presence of human-associated bacteria *Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium* at all time points, suggesting their possible contributions to regulating host immunity and increasing the susceptibility to eczema. In the home environment of the control group, putative protective effect of an environmental microbe *Planomicrobium* (*Planococcaceae* family) was observed to be significantly higher than that in the eczema group. Network correlation analysis demonstrated inverse relationships between beneficial *Planomicrobium* and human associated bacteria (*Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium*). Exposure to natural environmental microbiota may be beneficial to modulate shed human associated microbiota in an indoor environment.

**Keywords:** early-life exposure, eczema, household environmental microbiota, *Planomicrobium*, preconception

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**INTRODUCTION**

The Developmental Origins of Health and Disease hypothesis postulates that early environmental stimuli may contribute to the onset of allergic diseases via epigenetic effects on fetal and neonatal immune regulation (Waterland and Michels, 2007). Evidence from epidemiological studies have reported the influence of environmental factors on maternal as well as child health from early preconceptional periods (Deng et al., 2016; El-Heis et al., 2017; Gao et al., 2019; Garcia-Larsen et al., 2018; Goudarzi et al., 2018; Huang et al., 2015).

Exposure to a diverse microbial environment during pregnancy and early postnatal period is important in determining predisposition towards allergy (Douwes et al., 2008; Steiman et al., 2020). Prenatal farm exposures to animals and associated endotoxin exposure have been found to protect the offspring against allergy and eczema development (Douwes et al., 2008; Ege et al., 2011; Lynch et al., 2014). Increased bacterial diversity and richness in household dust during the first year of life were associated with a reduced risk of atopy and atopic wheeze in the Urban Environment and Childhood Asthma (URECA) birth cohort (Lynch et al., 2014). The PARSIFAL (Prevention of Allergy — Risk Factors for Sensitization Related to Farming and Anthroposophic Lifestyle) cohort (Ege et al., 2011; Schram-Bijkerk et al., 2005) and GABRIELA (Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community [GABRIEL] Advanced Study) cohort demonstrated that asthma and other atopic diseases are less prevalent in children living on farms compared to children from suburban areas due to an increased exposure to the environmental microbiome present in farming homes (Ege et al., 2011; Müller-Rompa et al., 2018; Valkonen et al., 2015). Similarly, Steiman et al. reported farm related exposures to poultry, pigs and feed grain during pregnancy to be significantly associated with lowered prevalence of atopic dermatitis (AD) in offspring (Steiman et al., 2020). Additionally, Lee et al. found that the house dust microbiome of allergic adults differed from those of healthy adults, with a significantly lower bacterial diversity associated with atopy and hay fever (Lee et al., 2020).

The constant exposure to external microbial environment leads to the potential acquisition of environmental bacteria which may affect the skin and gut microbial communities and lead to interactions with environmental microbes, regulating host immunity. In support, the Canadian Healthy Infant Longitudinal Development study reported that certain bacterial groups co-occurred in paired dust and infant stool samples such as *Actinobacteria* and *Bacilli* (Konya et al., 2014). Additionally, it has been demonstrated that host-environment interactions resulting in allergic airway diseases can be influenced by the impact of environmental dust on gut microbiota and subsequently, host immunity (Fujimura et al., 2014; Hanski et al., 2012).

Interestingly, the effect of environmental microbiota exposure during all three critical window periods namely preconception, pregnancy and postnatal life on the development of allergy in the child has not been investigated so far. Studying the preconceptional period offers valuable insights on the earliest risk factors and precursors for disease development and provides a route of intervention to prevent the onset of disease**.** We hypothesized that constant exposure to certain environmental microbiota throughout these critical periods would influence eczema development.

Our study aimed to determine potential associations between environmental microbial exposure in Singapore homes from the prospective, pre-conception birth cohort “S-PRESTO” (Singapore PREconception Study of long Term maternal and child Outcomes) and eczema development in early life. To the best of our knowledge, this is the only longitudinal birth cohort study that has collected and evaluated dust microbiota composition in the preconception, pregnancy and postnatal phase.

**RESULTS**

**Demographics, lifestyle, and clinical characteristics**

There were 36 eczema subjects and 25 non-eczema control subjects included in this study. There were no differences in the demographic variables such as ethnicity, maternal history of allergy, gender and mode of delivery between the groups (**Table 1**). There were fewer multiparous mothers in the eczema group [1 (2.8%)] as compared to the control group [6 (24.0%), p<0.01], and there were fewer mothers in the eczema group [3 (8.3%)] who ate on the bed during pregnancy compared to the control group [8 (32.0%), p=0.04]. Households of subjects with eczema tended to change the bedsheet more frequently during all 3 time periods and clean the floor more frequently postnatally as compared to the controls albeit without statistical significance. Subgroup analysis of non-atopic eczema (NAE) and atopic eczema (AE) showed no significant difference in demographic variables compared to controls (**Supplementary Table S1**).

**16S rRNA gene sequencing summary**

There were 363 dust samples collected from the household of the 61 study participants over the three timepoints. A negative control - water (blank) yielded an average of 30,000 reads. Excluding samples with low yield of DNA concentration and lower read counts than negative control (<30,000 reads), 357 dust samples were analyzed. The quality passed reads for dust samples from houses of controls, non-atopic eczema subjects and atopic eczema subjects were 1,198,152 ± 1,018,772, 1,327,264 ± 1,008,326 and 1,371,918 ± 1,058,398 respectively. The rarefaction curve for number of observed OTUs at the family level are described in **Supplementary Figure S1**.

**Microbiota composition in bed and living room floor dust**

The overall microbial profiling of bed and living room floor dust, representing the top 30 bacterial families with relative abundance of at least 0.1%, is shown in **Figure 1**. The dominant human-associated bacteria families identified by relative abundance in both locations were *Corynebacteriaceae, Propionibacteriaceae, Staphylococcaceae, Streptococcaceae*, and *Micrococcaceae.* On the other hand, the major environmental bacteria identified were *Carnobacteriaceae*, *Planococcaceae*, *Rhodobacteraceae*, *Gemellaceae* and *Dermabacteraceae*. Bacterial composition of living room floor dust remained stable over time while that of the bed dust microbiota changed with time. As dust from the postnatal timepoint was collected from the parents’ bed if the infant slept with them or the infant’s cot if the infant slept alone, we conducted separate and combined analysis with the dust collected at each site. Results from the bed microbiota analysis remained the same with separated or combined analysis of the dust collected from the parents’ bed or infant’s bed hence we describe the combined data analysis in the manuscript. Description at family and genus level is further depicted in the Sankey plots (**Supplementary Figure S2**).

Overall Shannon diversity index was significantly lower in bed than in living room floor dust regardless of clinical outcome (p<0.01) (**Supplementary Figure S3**). PCoA of the overall composition for dust microbial community was plotted to investigate the differences in community composition between two locations and to determine the determinant of the community (**Figure 2**). At all timepoints, two distinct bacterial clusters were observed: (1) a bed bacterial community, (2) a living room floor dust bacterial community. In cluster (1), the bed bacterial community was driven by mainly human-associated bacteria including *Corynebacteriaceae Micrococcaceae, Prevotellaceae, Propionibacteriaceae*, *Streptococcaceae* and *Tissierellaceae*. In contrast, in cluster (2) a mixed of environmental bacteria (*Carnobacteriaceae*, *Dermabacteraceae*, *Planococcaceae* and *Nocardioidaceae*) and human-associated bacteria (*Acetobacteraceae, Actinomycetaceae, Moraxellaceae* and *Staphylococcaceae*) shaped the living room floor composition (PERMANOVA test, p<0.01).

**Differences of household environmental microbiota between eczema and control subjects**

We hypothesize that exposures to environmental microbiota during critical window periods including preconception, pregnancy and early postnatal life influence the risk of development of eczema. Hence, we analyzed and determined the bacteria with longitudinal differences between the groups across the three-time points.

There was no difference in overall Shannon diversity between controls and eczema (NAE, AE) at each timepoint (**Supplementary Figure S4, S5**).Trend analysis for bed dust showed that diversity in AE and controls increased from preconception to pregnancy and decreased at post-natal timepoints while for living room floor dust, diversity in all three clinical groups increased over time. Sub-analysis of bed dust from the parents’ bed and baby’s cot separately at the postnatal timepoint showed the same decrease in diversity (data not shown). The PCoA between eczema and control groups were performed in both bed and living room floor using all bacterial families. There were no distinct cluster between groups at individual timepoints or combined timepoints at both bed and living room floor (data not shown).

Further pairwise comparisons of individual bacterial genera were investigated to identify the bacterial composition signature of each clinical group. For both locations (bed and living room floor dust), human-associated bacterial genera namely *Finegoldia* (*Tissierellaceae* family); *Micrococcus* (*Micrococcaceae* family) and *Prevotella* (*Prevotellaceae* family) were longitudinally higher in eczema group than in controls (LMM, p<0.05). In contrast, environmental bacteria *Planomicrobium* (*Planococcaceae* family) were longitudinally higher in the control compared to the eczema group (LMM, p<0.05) **(Figure 3)**.

Additionally, for bed dust, three human skin-associated bacteria *Anaerococcus* (*Tissierellaceae* family), *Propionibacterium* (*Propionibacteriaceae* family) and *Actinomyces* (*Actinomycetaceae* family) were longitudinally higher in the eczema group than in the control group (LMM, p<0.05) **(Figure 3A)**. For the living room floor dust, *Acinetobacter* (*Moraxellaceae* family) were enriched in the eczema group (LMM, p<0.05) **(Figure 3B).** Additionally, the longitudinal trends of significant taxa identified in both bed and living room floor dust using absolute read counts remained the same (data not shown).

Of note, further subgroup analysis of NAE and AE showed that for the AE group, there was a significant depletion of *Planomicrobium* (*Planococcaceae* family) in both locations compared to NAE. There was enrichment of *Acinetobacter* (*Moraxellaceae* family); *Finegoldia* (*Tissierellaceae* family); *Micrococcus* (*Micrococcaceae* family) and *Prevotella* (*Prevotellaceae* family) only in living room floor dust collected from the AE group as compared to NAE group (LMM, p<0.05) **(Supplementary Table S2, S3).**

**Bacterial co-occurrence patterns in household environmental microbiota**

To further investigate the potential interactions between bacteria within each location, two sets of correlation networks for living room floor and bed dust were analyzed. Each set consisted of bacteria that were longitudinally different between the eczema and control groups. For bed dust, seven genera (*Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus*, *Planomicrobium, Prevotella* and *Propionibacterium*) were included. For living room floor dust, five genera (*Acinetobacter, Finegoldia, Micrococcus*, *Planomicrobium* and *Prevotella*) were included (**Figure 4**).

We identified *Planomicrobium* as the “hub” within the networks in both locations due to its involvement in the greatest number of interactions. Overall, in the eczema groups, compared to control, there was a decrease in negative interactions between *Planomicrobium* and other bacteria. There was an increase in number of positive interactions between bacteria which were enriched in eczema groups (*Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium*) as compared to the control group.

**Influence of demographic, lifestyle and environmental determinants on composition of bed and living room floor dust**

The effect of demographic (gender, parity and ethnicity), lifestyle (cleaning frequency, and eating habit on bed) and environmental (type of home, length of stay at the house, living near express ways and pet ownership) factors on overall composition of the major bacterial genera (relative abundance >0.1%) in bed and living room floor dust were evaluated in term of relative abundance (**Supplementary Table S4, S5**) and absolute read counts (data not shown). Only significant differences between groups were reported (p<0.05).

For bed dust, the relative abundance and absolute read counts of *Planomicrobium* were enriched in subjects who had been staying in the same house for at least 5 years compared to those who had not (p<0.05). Being of non-Indian ethnicity was associated with a higher relative abundance and absolute read counts of *Propionibacterium* than other ethnic groups (p<0.05). Being primiparous was associated with a higher relative abundance, but not absolute read counts of *Propionibacterium* compared to being multiparous (p<0.05). In contrast, factors such as being of Indian ethnicity and multiparous were associated with higher relative abundance and absolute read counts of *Prevotella* than non-Indian ethnicity and being primiparous (p<0.05). Living near the expressway and being of male gender were associated with lower relative abundance and absolute read counts of *Anaerococcus* (p<0.05).

For living room floor dust, higher frequency of living floor cleaning (cleaning daily or at least once a week) was associated with a higher relative abundance and absolute read counts of *Planomicrobium* (p<0.05). Being multiparous and living away from expressway were associated with higher relative abundance of *Finegoldia* and *Prevotella* (p<0.05). Of note, the same significance was only observed with analysis on absolute read counts of *Finegoldia*, but not *Prevotella*. Being of non-Chinese ethnicity and living in condominium/private property were associated with higher relative abundance and absolute read counts of *Acinetobacter* (p<0.05). Subjects living in a same house for less than 5 years had a higher relative abundance and absolute read counts of *Micrococcus* in the living room floor dust than those living in a same house for more than 5 years (p<0.05).

**DISCUSSION**

The early life period has been proposed to be a critical window during which environmental exposures can significantly affect the health outcomes of the child (Calatayud et al., 2019). In this longitudinal study, serial sampling of household dust samples over critical time periods i.e. preconception, pregnancy and postnatally provides important evidence on the putative influence of the constant exposure to environmental microbiota in preventing or predisposing to childhood eczema.

**Distinct bacterial profiles between living room floor and bed dust**

Two distinct dust microbiota profiles were observed in the bed and living room floor dust. Higher microbial diversity was found in the living room floor dust samples. Täubel et al. also observed higher microbial diversity in floor dust than mattress dust that possibly could be attributed to walk-in exposures or deposition from air ventilation (Gusareva et al., 2019; Taubel et al., 2009). The continuous exchange of microbiota between outdoors and indoor household microbiota, tracked indoors from contact via the shoes and feet can explain the increased diversity of living room floor compared to bed.

Bacteria families driving the bacterial composition from bed dust originated mainly from human sources, while those influencing living room floor dust composition originated from human and environmental sources. Our results were corroborated by findings from the Inner City Asthma Consortium that human associated microbiota are significantly more abundant in bedroom floor dust (Fujimura et al., 2012). Another study by Dunn et al. reported significant intra-site differences in bacterial composition. Human-associated bacteria were abundant on most interior surfaces including pillowcases, toilet seats, and door handles, whereas environment-associated bacteria were more likely to be found on exterior door trim samples (Dunn et al., 2013). The study also found significant positive correlation between the bacteria communities that were found inside the house and those found on surfaces outside the house, implicating that the outdoor microbial composition can directly affect the indoor microbial composition (Dunn et al., 2013). These results further highlight the strong influence of outdoor environmental microbiota in shaping indoor environmental microbiota.

**Distinct house dust bacterial profiles between the eczema and control groups**

Atopic eczema is an inflammatory skin disease usually associated with elevated levels of total and allergen-specific IgE in blood and skin (Wuthrich, 1978). The subgroup of patients with normal IgE levels and negative sensitization to typical food allergens are known to have a condition termed non-atopic eczema, also known as intrinsic type eczema (Akdis and Akdis, 2003). It has been postulated that intrinsic type eczema could be a transitional form of eczema (Cork et al., 2006), hence we also analysed the influence of the environmental microbiota on the development of non-atopic and atopic eczema.

1. **Association of environmental and human-associated bacteria with development of eczema**

In the eczema group, we observed an increased relative abundance of human-associated bacteria *Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium*, suggesting their possible role in shaping atopy risk. Although *Micrococcus*, *Actinomyces* and *Propionibacterium* have been reported as commensal skin bacteria (Hetem et al., 2017), the proinflammatory role of these bacteria has also been suggested. Turturice et al. reported that for the subjects who produced IL-13 in response to aeroallergens, they had corresponding increased levels of *Micrococcaceae*, suggesting a relationship between exposure to environmental bacteria during the perinatal period and subsequent immune responses to aeroallergens (Turturice et al., 2017). *Actinomyces* has also been reported to induce inflammatory cytokines, via activation of the host’s immune system, thereof potentially initiating the pathogenesis of inflammatory diseases (Moreillon and Majcherczyk, 2003; Sato et al., 2012; Wolf and Underhill, 2018). *Propionibacterium* can induce *Staphylococcus aureus* biofilm formation through secretion of coproporphyrin III molecules, hence increasing opportunities for *S. aureus* colonization (Wollenberg et al., 2014). Higher levels of *Propionibacterium* in houses of eczema subjects might result in eczema development by increasing infection by *S. aureus.* Certain strains including *Propionibacterium acnes* are capable of producing molecules such as porphyrins which may promote inflammation (Brüggemann et al., 2004).

Literature on *Prevotella* has been conflicting, while there are studies that have reported on the protective role of *Prevotella* in asthma (Hilty et al., 2010; Zhang et al., 2016), Larsen et al. provided evidence that linked *Prevotella* to inflammatory diseases by the activation of Th17 pathway (Grice and Segre, 2011; Larsen, 2017).

In our eczema subgroup analysis, the increased relative abundance of *Acinetobacter* (*Moraxellaceae* family); *Finegoldia* (*Tissierellaceae* family); *Micrococcus* (*Micrococcaceae* family) and *Prevotella* (*Prevotellaceae* family) in the living room floor dust were further exaggerated in eczema subjects with allergen sensitization compared to non-sensitized NAE subjects. These findings further highlight the possible proinflammatory role of these bacteria in the development of atopy.

1. **Environmental bacterium *Planomicrobium* potentially protective against eczema development**

Environmental bacteria *Planomicrobium* (*Planococcaceae* family) was significantly enriched in the houses of control compared to eczema subjects. *Planomicrobium* usually originates from environmental sources, including aquatic and land habitats such as soil and lakes (Shivaji et al., 2014). *Planococcaceae* has also been detected in mattress dust from farming environment (Birzele et al., 2017). *Planomicrobium* belongs to the phylum Firmicutes, which has been previously reported to be present in higher relative abundance in bedroom and living room dust from houses of healthy controls versus subjects with atopy or wheeze (Lynch et al., 2014). In this study, we observed that members of *Planococcaceae* conferred protection against the development of eczema due to their higher abundance in controls as compared to eczema subjects. It is possible that increased contact with nature such as going to the gardens and parks brings home beneficial environmental microbiota which not only influences living room dust composition but is also transferred to the bed. A study by Steglinska and colleagues analyzing the microbiome on human feet similarly provided evidence of *Planomicrobium* being an inhabitant on the foot microbiome of subjects, suggesting the influence of environmental microbiome on the skin microbiome (Steglińska et al., 2019). The possible role of *Planococcaceae* in regulating host immunity and atopy development was further strengthened by our findings of a lower relative abundance of *Planococcaceae* in living room floor and bed dust of AE subjects compared to NAE subjects.

**Environmental microbiota exposure in household dust regulates the potentially proinflammatory shed human microbiota**

Bacteria are likely to function as networks within a consortium, forming clusters of exposures (von Mutius, 2014). Hence, we investigated the network correlation analysis between different bacterial genera to understand the inter-bacteria interaction in the environment, in relation to atopic eczema.

Network correlation analysis suggested an inverse relationship between beneficial *Planomicrobium* and human associated bacteria (*Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium*) to be protective against atopy and eczema. Absence of these interactions in eczema groups may indicate the lack of inhibitive effects by beneficial environmental bacteria *Planomicrobium* on the human-associated bacteria. Presence of only human associated microbiota indoor might be detrimental and predispose us to develop allergy in the absence of regulation by natural environmental microbiota. We postulate that we need a diverse range of human associated and natural environmental bacteria to train our immune system during early life to minimize development of allergic diseases.

In the PASTURE birth cohort, Karvonen et al. identified 7 protective outdoor-associated microbiota present in indoor dust, that are commonly found in sources such as soil, water and compost which are linked to lower asthma risk. The study also postulated that composition of environmental bacterial communities from outdoor sources could potentially be more useful as compared to bacterial richness in the prevention of asthma (Karvonen et al., 2019). Other supporting evidence stems from a study by Kirjavainen et al., where mimicking a farm-like home dust microbiota in the houses of non-farm children reduced the probability of later asthmatic outcomes at six years (Kirjavainen et al., 2019). A farming environment has been proposed to be more diverse and enriched in environment-associated microbiota with lower relative abundance of human-associated bacteria, which confers protective effect against allergy-related diseases (Bendiks and Kopp, 2013; Douwes et al., 2008; Heederik and von Mutius, 2012; Ruokolainen et al., 2015). Besides this, an intervention study involving enrichment of environmental biodiversity in urban daycare centers showed increase in skin microbial diversity of children in the intervention group with a corresponding increase in the proportion of regulatory T cells and cytokines (Roslund et al., 2020). This highlights the importance of environmental biodiversity in regulating immune responses and preventing immune-mediated diseases.

In support, the network analysis strengthens our findings on the protective role of natural environmental bacteria against eczema. This is mediated possibly by the inhibition of certain human-associated bacteria which increase the risk of developing eczema. Shed human associated bacteria may affect host immunity via modulation of skin and gut microbiota and predisposes to development of atopic eczema.

**Factors affecting house dust bacterial profiles**

We observed a higher relative abundance of *Planomicrobium* in houses with longer period of occupancy as well as higher cleaning frequency, suggesting that *Planomicrobium* accumulates with time and is resistant to cleaning. Supporting evidence is provided by studies showing that *Planomicrobium* is able to form biofilms (Liu et al., 2012; Rafi et al., 2019; Siddik and Satheesh, 2019), which may enable it to be more resistant to cleaning and survive longer in houses (Bridier et al., 2011). Conversely, we found lower abundance of human-associated *Micrococcus* even with longer period of occupancy, suggesting that human-associated *Micrococcus* is not resistant to cleaning. Królasik et al showed that *Micrococcus luteus* biofilms had lower resistance to disinfectants as compared to other bacteria (Królasik et al., 2010). Taken together, environmental microbiota might be more resistant to cleaning compared to human associated bacteria.

Higher parity was associated with higher relative abundance of *Finegoldia* and *Prevotella.* Higher human occupancy may lead to more shedding of these human-associated bacteria. Guo et al. found the abundance of *Alloprevotella*, another human-associated bacteria from the *Prevotellaceae* family*,* to be positively correlated with occupancy density (Guo et al., 2020).

Living near the expressway was linked to lower abundance of human skin-associated *Anaerococcus* and *Finegoldia*. Close proximity to expressway may indicate higher traffic air pollution. He et al. reported that exposure to ground-level ozone resulted in oxidative damage and reduced the skin microbiome population by half (He et al., 2006).

We also observed that gender and ethnicity influenced the composition of human-associated dust microbiome. Male gender was linked to lower *Anaerococcus* abundance. Males and females were found to have highly different bacteria on their hand surfaces, where female skin had a higher microbial diversity (Fierer et al., 2008). These variations may be attributed to differences in skin pH and thickness, production of hormones and sweat and also use of topical products. We postulate that lifestyle differences due to ethnic and cultural diversities may influence house dust microbiome composition. For instance, a higher proportion of Singaporean Malays are pet owners (including cats, small mammals and birds) as compared to other races (Goh et al., 2020).

**Strengths and limitations of study**

The strengths of our study include longitudinal assessment of environmental microbiota during critical early life periods with dust collection at multiple timepoints, accompanied with objective diagnosis of eczema performed by trained clinicians. The prospective design of this study also allows us to determine the possible contribution of environmental microbiota before the onset of disease. In addition, we used dust samples as an objective assessment of environmental microbiota exposure with high-throughput sequencing to generate the microbiome data instead of a questionnaire-based approach. We also analyzed dust from the living room besides the bed which had been commonly studied, this allows us to have a more comprehensive understanding of the indoor house microbiota. However, the main limitation of the study is the lack of skin and gut microbiome data hence we are unable to determine the interactions and effect of environmental microbiome on skin and gut microbiome and establish causality. Another limitation is the modest sample size; however, we have conducted rigorous statistical analysis such as adjusting for multiple comparison to increase the reliability of the results. Further studies are also needed to assess the role of the fungal community in developing eczema. Data on immune profiling is not available currently and it should be examined in future research.

**CONCLUSIONS**

In this study, we report the observation that constant exposure to household environmental microbiota during critical periods in early life may be a risk factor for eczema development in early childhood. We postulate that natural environmental microbiota exposure is needed to regulate the shed human associated microbiota that surrounds our aura microbiota. Future studies will focus on evaluating the effect of environmental microbiota on skin and gut microbiota to develop strategies and guidelines to modify household environmental microbiota exposure. Future urban design should also focus on incorporation of environmental microbiota in construction projects so as increase functionality of ecosystem for public health (Watkins et al., 2020).

**Experimental Procedures**

**S-PRESTO study population**

The S-PRESTO study design has been reported previously (Loo et al., 2020). Briefly, S-PRESTO study recruited Chinese, Malay or Indian (or any combinations thereof) women aged 18 to 45 years and who intended to get pregnant and deliver in Singapore. Written informed consent was provided by the participants. Ethical approval was obtained from the SingHealth Centralised Institutional Review Board (reference 2014/692/D). This study has been registered at ClinicalTrials.gov (NCT 03531658).

**Assessment of allergic outcomes and selection of subjects**

The offspring were followed up at birth, 1, 3 and 6 weeks, 3, 6, 12 and 18 months. Allergy status was determined through questionnaires during the follow-ups. Additionally, at the 6-month and 18-month visit, clinical evaluation by Hanifin and Rajka criteria (Hanifin and Rajka, 1980) and severity scoring (SCORAD) for eczema (European Task Force on Atopic Dermatitis, 1993) was performed by clinicians. Skin prick testing to common allergens was also carried at 6-month and 18-month visits (detailed in Supplementary Appendix).

In this study, 36 subjects with eczema and 25 non-eczema and non-allergen-sensitized controls were included. Controls were matched with eczema subjects based on date of birth. These 36 subjects with physician-diagnosed eczema were further classified into non-allergen sensitized eczema (NAE) (n=22) and allergen-sensitized atopic eczema (AE) (n=14) for sub-group analysis. These maternal-offspring pairs also stayed at the same house from preconception to postnatal phase with no major renovations being carried out during this time period.

**Sample collection and processing**

At preconception (approximately 14 weeks before pregnancy) and pregnancy (at 34-36weeks) timepoints, dust was collected from the subject’s bed and the floor in the living room (a 2 m by 1 m area furthest from the main door). In the postnatal phase (when the child was 3 months old), dust was collected from the bed where the infant slept on (either the parent’s bed if they were co-sleeping or the infant’s cot) and the living room floor. An interviewer administered questionnaire collected information such as pet ownership, eating habits and cleaning practices at every dust collection visit. Dust was collected using vacuum cleaners (Dyson DC63; Dyson, UK) with 40 μm nylon mesh DUSTREAM® filters (Indoor Biotechnologies, India). Each area was vacuumed for 4 min. Debris were manually removed from the dust samples and all samples were then stored at − 80 °C until further processing.

The details for skin prick testing, DNA extraction, bacterial 16S rRNA gene sequencing and statistical analysis are included in the Supplementary Appendix.

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**Availability of data and material**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request with approval from the S-PRESTO scientific review board. All raw microbiome data are available at NCBI (https://www.ncbi.nlm.nih.gov) (Accession Number PRJNA668050).

**Conflict of Interest**

Godfrey KM has received reimbursement for speaking at conferences sponsored by Nestle and Shek LP has received reimbursement for speaking at conferences sponsored by Danone and Nestle and consulting for Mead Johnson and Nestle. Shek LP has received research funding from Danone. Godfrey KM and Chong YS are part of an academic consortium that has received research funding from Abbot Nutrition, Nestle and Danone. Chong YS is a co-inventor on patent filings by Nestlé S.A. Christophe Lay and Jan Knol are employees of Danone Nutricia Research.

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**Table Legend**

**Table 1**: **Demographics and lifestyle factors of the subjects**

**Figure Legends**

**Graphical abstract.** The study findings of S-PRESTO are depicted in the infographic. Dust microbiota composition of bed dust and living room floor dust was compared between houses of subjects with and without eczema. Increased abundance of human-associated bacteria *Actinomyces*, *Anaerococcus, Finegoldia, Micrococcus, Prevotella* and *Propionibacterium* was associated with eczema development, suggesting the possible regulation of host immunity resulting in atopy. Conversely, *Planomicrobium*, an environmental bacterium, was found to possibly prevent the development of eczema by inhibiting proinflammatory human associated bacteria. Exposure to natural environmental microbiota may be needed to modulate shed human associated microbiota in an indoor environment.

**Figure 1: Distribution of top 30 bacteria families in bed and living room floor dust.** Data presented as mean of relative abundance (%), only the top 30 bacterial families with relative abundance of at least 0.1% are shown, regardless of clinical outcome. At the postnatal timepoint, dust was collected from the bed that the baby slept on.

**Figure 2: PCoA based on Bray–Curtis dissimilarity between dust microbiota profiles (family level) for the 2 locations (bed and living room floor) at various timepoints:** all timepoints combined, preconception, pregnancy and post-natal. Arrow shows directions from the origin for which clusters have significant abundances for the bacterial groups (PERMANOVA test, p<0.01, 999 permutations), and its length is proportional to the correlation between ordination and bacterial groups.

**Figure 3: Abundance plots of bacteria genera from (A) bed and (B) living room floor dust of clinical groups.** Data presented as geometric mean of relative abundance ± geometric SD range (**Supplementary Table S2, S3**). # denote significant longitudinal difference of bacterial relative abundance between groups (p<0.05; linear mixed model analysis). \* denote significant difference at p<0.05 between groups at specific timepoint (general linear model analysis).

**Figure 4: Bacterial correlation networks of environment dust between controls and eczema.** Two sets of bacterial correlation networks, consisting of bacteria that were longitudinally different between control and eczema group, in living room floor and bed dust were analyzed. For bed and living room floor dust, seven and five genera were included respectively. Green and red lines indicate the significant positive and negative interaction respectively between two genera (p<0.05).