Establishment of the Nasal Microbiota in the first 18 Months of Life – Correlation with Early Onset Rhinitis and Wheezing

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Abstract

Background—The dynamic establishment of the nasal microbiota in early life influences local mucosal immune responses and the susceptibility to childhood respiratory disorders.

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Objective—The aim of this case-control study was to monitor, evaluate and compare the development of the nasal microbiota of infants who developed rhinitis and wheeze in the first 18 months of life with those of healthy controls.

Methods—Anterior nasal swabs of 122 subjects belonging to the GUSTO birth cohort were collected longitudinally over seven time points in the first 18 months of life. The nasal microbiota signatures were analyzed using 16S rRNA multiplexed pair-end sequencing from three clinical groups (1) rhinitis alone (n=28), (2) rhinitis with concomitant wheeze (n=34) and (3) healthy controls (n=60).

Results—The maturation of the nasal microbiome followed distinctive patterns in infants from both rhinitis groups compared to controls. Bacterial diversity increased over the period of 18 months of life in control infants, whilst infants with rhinitis showed a decreasing trend (p<0.05). An increase in abundance of *Oxalobacteraceae* family (*Proteobacteria* phylum) and *Aerococcaceae* family (*Firmicutes* phylum) was associated with rhinitis and concomitant wheeze (adj p<0.01) whilst *Corynebacteriaceae* family (*Actinobacteria* phylum) and early colonization with *Staphylococcaceae* family (*Firmicutes* phylum) (3 weeks till 9 months) was associated with controls (adj p<0.05). The only difference between the rhinitis group and controls was a reduced abundance of *Corynebacteriaceae* family (adj p<0.05). Determinants of nasal microbiota succession included gender, mode of delivery, presence of siblings and infant care attendance.

Conclusion—Our results support the hypothesis that nasal microbiome is involved in the development of early onset rhinitis and wheeze in infants.

Introduction

Rhinitis and wheezing disorders in infancy and childhood are common [1, 2]. Birth cohort studies, including our "Growing Up in Singapore Towards healthy Outcomes" (GUSTO) birth cohort, have described infants and toddlers with persistent and recurrent rhinitis. Although the majority do not have allergen sensitization in infancy, early onset rhinitis has been associated with eczema, wheeze and parental history of allergic disorders, suggesting that atopy may play a role in the manifestation of rhinitis. [3–5]

Considerable current evidence supports bacteria-virus interactions as an important factor in lower respiratory tract infections. Respiratory syncytial virus and rhinovirus have been implicated as triggers of wheezing disorders in infants and young children, and as predictors of subsequent asthma at school age [6–10]. It has also been recognized that the resident microbiota of the respiratory tract plays a role as gate keeper that either facilitates or provides resistance to colonization by respiratory virus [10, 11]. The presence of several potentially pathogenic bacteria such as *Moraxella catarrhalis* in the airways has been associated with asthma and wheeze development [12, 13]. Moreover, farming studies support the concept that environmental microbial exposure has a protective effect on the

development of allergic disease [14, 15]. This brings up the concept of "exposome" in which the health status of an individual is determined by his environmental and lifestyle exposure.

Recently, Bosch et al. identified significant differences in the nasal microbiome profiles in infants predisposed to upper respiratory infections in a longitudinal study [16]. Despite these findings, a detailed description of the nasal microbiota in infants with respect to allergic diseases such as rhinitis and wheezing has not been studied longitudinally. In this study, we monitored and evaluated the dynamics of the nasal microbiota in a subcohort of infants belonging to the GUSTO birth cohort over seven time points. The subjects of this subcohort had early onset childhood rhinitis which was associated with parental atopy and atopic comorbidities of atopic dermatitis and wheeze [4]. Given the influential role of the microbiome in the pathogenesis of respiratory tract infections and atopic diseases, the microbiome of the infant's airways may provide insights into the contributory or inhibitory role of bacteria on the manifestations of atopy.

Methods

Study Population

This case-control study recruited a subset of infants from the GUSTO birth cohort of 1,237 Singaporean newborns [17]. GUSTO is a general population birth cohort and not at risk for atopy cohort. Primary objective of GUSTO study is to identify and evaluate the role of risk factors and determinants influencing body composition and metabolism during early development, and their influence on infants' health in later childhood. Rhinitis and other atopic outcomes is included as a sub-study of this cohort. Briefly, mothers were recruited antenatally and their children were followed up with scheduled home visits at 3 weeks, 3, 6, 9, 12, 15 months and a clinic visit at 18 months. During these seven visits, caretakers of the subjects were administered a questionnaire on demographic, lifestyle and symptoms of rhinitis and wheeze. Positive cases of rhinitis were then followed up by monthly phone calls to monitor rhinitis progression until a 3-month remission period was over to minimize false-positive cases. This study was approved by the relevant institutions' ethical review boards (DSRB:B/2009/584 and CIRB:2009/1024/E). Written informed consent was obtained from parents or legal guardians of the subjects.

There were 733/1,237 (59.3%) evaluable subjects (excluding drop-outs and those with missing clinical data) who completed follow up till 18 months of age (Fig 1). There were 138 subjects who fulfilled our criteria of rhinitis by 18 months at age. Selection of rhinitis subjects was based on the highest number of available nasal swabs. A total of 62/138 (44.9%) subjects that provided at least four out of the seven nasal swab samples, were then categorized into two groups: rhinitis without wheeze (n=28) and rhinitis with wheeze (n=34). Sixty controls without rhinitis or wheeze were selected with similar characteristics (mode of delivery, age, birth order and day-care attendance) to minimize potential selection bias. Controls had at least four nasal swabs and had negative responses for rhinitis and wheeze (as defined in section below) in the first 18 months of life.

Case Definitions

Rhinitis was defined as having symptoms of sneezing, runny and/or blocked nose that lasted for at least four weeks in single or multiple episodes (each episode lasting at least two weeks) This definition was based on the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines [4]. Wheeze was defined as presence of wheeze symptoms (noisy breathing with a high-pitched, whistling sound heard from the chest, not the mouth). Eczema was defined as physician-diagnosed eczema in the first 18 months. At the 18 month visit, subjects were assessed via skin prick test (SPT) for the presence of allergic sensitization to a panel of food (milk, peanut, egg) and house dust mites allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*) from Greer Laboratories (Lenoir, NC, USA) and *Blomia tropicalis* (extract obtained from in-house laboratory) [18]. Wheal size of at least 3mm larger than the negative control (saline) was inferred as a positive reaction.

Sample Collection

Anterior nasal swabbing was carried out on infants during the visits at seven time points (3 weeks, 3, 6, 9, 12, 15 and 18 months) using dry flocked swabs and stored in Universal Transport Medium (Copan Diagnostics, USA) tubes at -80°C on the same day of collection until analysis.

Nucleic Acid Extraction, 16S rRNA Gene Amplification and Sequencing

DNA was extracted using QIAGEN EZ1 DNA Extraction kit and QIAGEN EZ1 Advanced XL automated purification machine (Qiagen, Germany) according to the manufacturer's instruction. Bacterial 16S rRNA gene sequences (V3-V6) were amplified and purified as described in Ong et al. [19]. Purified amplicons were fragmented using E220 Focused-ultrasonicator (Covaris, USA) to yield product of an average length of 180 bases. QIAGEN GeneRead DNA Library Prep I Kit (QIAGEN, Germany) was used for library preparation. Multiplexing Sample Preparation Oligonucleotide Kit (Illumina, USA) was used to label DNA sequencing libraries. Multiplexed pair-end sequencing (2 x 76bp reads) was performed using Illumina HISEQ RAPID platform.

16S rRNA Sequencing Analysis

Samples with less than 100,000 reads were removed. Reconstruction of the original amplicon (V3-V6) sequences and classification of amplicon sequences followed the workflow described in Ong et al. [19], which is based on "Expectation Maximization Iterative Reconstruction of Genes from the Environment" (EMIRGE) [20]. EMIRGE-reconstructed sequences were trimmed to the primer-amplified section and searched using BLAST against the complete Greengenes database (dated May, 2013; greengenes/13_5/99_otus.fasta). Hits below predefined percent identity (97% at the species level, 94.5% at the genus level, 86.5% at the family level and 75% at the phylum level) were not considered for classification and removed. EMIRGE assigns abundance estimates to reconstructed sequences. Those quality-passed sequences were clustered into operational taxonomy units (OTUs). The relative abundance of OTUs and Shannon diversity index (SDI) were calculated for each sample at each taxonomic level.

Bioinformatics Analysis

All bioinformatics analyses were performed in the R version 3.4.1 within R studio version 1.0.136. Package "vegan" was used to plot rarefaction curves and Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance to visualize the separation of microbiota profiles between groups. "Envfit" function was used to fit bacterial abundance onto ordination space (p<0.001, 999 permutations) to determine the microbial determinants that shape the bacterial community composition [21]. The Sankey plots was constructed by a custom C++ script (https://github.com/BetaCollins/Sankey). Spearman correlation analysis was done using CoNet plugin in Cytoscape as described by Hannigan et al. [22, 23]. Correlation heatmap and network were visualized in Graphpad Prism 7.0 and Cytoscape 3.4.0 respectively.

Statistical Analysis

All statistical analyses were carried out using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 22 (IBM Cooperation, New York). Univariate analysis (chi-square test) was used to study the demographic, lifestyle and clinical factors between groups. Linear mixed model and linear regression were used to evaluate the bacterial abundance longitudinally and at specific time points respectively, as well as to explore the effect of demographic factors on abundance of main bacterial groups, while adjusting for potential confounders. All statistical significance tests and confidence intervals were two-sided and set at p<0.05. Geometric means were used to represent bacterial abundance and SDI due to non-parametric data distribution.

Results

Demographic, Lifestyle and Clinical Characteristics

The demographic, lifestyle and clinical characteristics of the population were summarized in Table 1. Eczema symptoms and allergen sensitization to any of the 6 tested allergens were more common in rhinitis groups than controls (p<0.05). Maternal history of eczema, and paternal history of rhinitis and eczema was also higher in rhinitis without wheeze group than controls (p<0.05). The use of postnatal antibiotics, Chinese/Malay ethnicity, maternal history of rhinitis, infant-care attendance in the first year were significantly associated with rhinitis with concomitant wheeze compared to control infants (p<0.05). Other demographic and clinical factors were not significantly different between groups.

In the clinical groups with rhinitis, the mean (± SD) onset of rhinitis symptoms was 7.1 ± 4.8 months (range 3 weeks to 18 months) and wheeze symptoms onset was 8.2 ± 4.6 months (range 3 to 18 months).

16S rRNA Sequencing Summary

There was a total of 670 nasal swab samples collected from 122 subjects (62 cases and 60 controls). Excluding samples with low yield of DNA concentration and low reads count, this yielded 620 nasal swab samples. The sequencing summary is tabulated in Table E1. The rarefaction curve for number of observed OTUs at family level were generated (Fig E1).

Dynamics of Bacterial Diversity

Overall SDI was significantly lower in both rhinitis groups (without wheeze: geometric mean SDI=0.85; with wheeze: geometric mean SDI=0.83) compared to controls (geometric mean SDI=0.97, p=0.03 and 0.01 respectively) (Fig 2A). Diversity in controls increased (p=0.019) while diversity in infants from both rhinitis groups declined over time (without wheeze, p=0.028; with wheeze, p=0.025) (Fig 2B).

Maturation of the Nasal Microbiota In the First 18 Months of Life

The overall distribution of nasal microbiota down to species level is shown using Sankey plots (Fig 3). The Sankey plots for each clinical group are shown in Figure E2. The abundance of predominant phyla, family and genus are summarized in Figure 4 and Table E2. Abundance data down to species level were not analyzed further due to low bacterial identification rate.

Phyla and Family—For all the three clinical groups, the predominant phyla were *Actinobacteria*, *Proteobacteria* and *Firmicutes*, which accounted for more than 97% of the total nasal microbiota. Other minor phyla made up less than 2% of the whole bacterial community.

Higher abundance of *Actinobacteria* was associated with control group compared to both rhinitis groups till the age of 18 months (without wheeze: adj p=0.034; with wheeze: adj p<0.01). *Corynebacteriaceae* family comprised 95.1% of this phylum. Correspondingly, the abundance of *Corynebacteriaceae* was higher in controls than both rhinitis groups (without wheeze: adj p=0.037; with wheeze: adj p<0.01).

In contrast, higher abundance of *Proteobacteria* phylum was observed for the rhinitis groups compared to controls, but statistical significance was seen only for rhinitis with wheeze (adj p <0.01). At family level, *Oxalobacteraceae* and *Moraxellaceae* comprised 36.9% and 39.3% of this phylum respectively. However, only *Oxalobacteraceae* was significantly higher in rhinitis with wheeze than controls over time (adj p<0.01).

Firmicutes phylum did not show distinct trends longitudinally for all clinical groups. However, *Aerococcaceae* family, which comprised 67.6% of this phylum, was higher in rhinitis with wheeze group compared to controls (adj p <0.01). *Staphylococcaceae* made up 14.8% of the *Firmicute* phylum and was significantly decreased in both rhinitis groups till 9 month (adj p<0.05).

Genera—The *Corynebacteriaceae*, *Aerococcaceae*, *Moraxellaceae* and *Staphylococcaceae* families were made up mainly of *Corynebacterium spp.* (82.0%), *Alloiococcus spp.* (94.3%), *Moraxella spp.* (43.7%) and *Staphylococcus spp.* (91.1%) respectively. The trends of these genera in the first 18 months were similar to their corresponding families (Fig 4C). *Janthinobacterium spp.* was the only genus of *Oxalobacteraceae* family that was identified with the sequencing data processing method employed. However, the average relative abundance of this genus was only 0.3%.

In summary, the relative abundance of bacteria between the three clinical groups showed distinct trends throughout 18 months of life. Although differences in bacteria composition were observed for both groups of rhinitis (with and without wheeze) compared to controls, the differences were more marked in the rhinitis with concomitant wheeze group. Furthermore, correlation analysis showed that *Oxalobacteraceae* family (*Proteobacteria* phylum) had direct negative interactions with *Aerococcaceae* family (*Firmicutes* phylum) and *Corynebacteriaceae* family (*Actinobacteria* phylum) in all three clinical groups throughout the first 18 months. This reinforces the results of our longitudinal analysis (Fig 4) that a distinct pattern of microbial abundance of the major bacterial groups was associated with the clinical outcomes; where high abundance of *Corynebacteriaceae* and *Staphylococcaceae*, and low abundance of *Oxalobacteraceae* and *Aerococcaceae* were associated with controls and the reverse pattern of abundance for rhinitis and wheeze (Fig E3). There was lower abundance of the *Corynebacteriaceae* and showed little fluctuation throughout the 18 month period in both rhinitis groups (with and without wheeze) compared to controls.

Multivariate Analysis of Nasal Microbial Community Composition

To investigate the differences in community composition between clinical groups, PCoA was plotted at seven time points (Fig 5). The five bacterial groups (*Corynebacteriaceae, Oxalobacteraceae, Moraxellaceae, Aerococcaceae* and *Staphylococcaceae* families) together explained an average of 54.6% (range: 49.87%-57.69%) of the overall variability in the microbiota composition. Distinct bacterial community composition was seen between two groups of rhinitis and controls at 3 weeks and 3 months. At these time points, *Corynebacteriaceae* and *Staphylococcaceae* significantly influenced controls whilst *Oxalobacteraceae* and *Aerococcaceae* shaped the rhinitis groups ($p<0.001$). The clusters between groups became less distinct from 6 months onwards. *Moraxellaceae* drove bacterial composition of rhinitis groups at this time point ($p<0.001$). The microbial communities after 9 months showed an obvious shift and disappearing of distinct clusters between clinical groups. Of note, *Corynebacteriaceae* remained the key bacterial drivers of bacterial community in controls till 12 months ($p<0.001$).

Influence of Demographic and Clinical Determinants on Establishment of the Main Nasal Bacterial Groups

Linear regression was employed to evaluate the effect of demographic and clinical factors on the overall dynamic establishment of the five main bacterial families (Fig E4). Caesarean-delivery and male gender were associated with higher abundance of *Aerococcaceae* and lower abundance of *Staphylococcaceae* (adj $p<0.05$). Presence of siblings and infant care attendance were associated with lower colonization of *Corynebacteriaceae*, and higher carriage of *Oxalobacteraceae* and *Moraxellaceae* (adj $p<0.05$). Other factors (pre-term, use of intrapartum antibiotic prophylaxis and postnatal antibiotics, family history of atopic diseases and exclusive breastfeeding pattern) did not show significant associations.

Discussion

The longitudinal design of this study with serial sampling over seven time points describes the dynamic changes and provides valuable snapshots of the succession of the nasal microbiota in early childhood. We observed distinct differences in the profiles of microbiota of infants who developed rhinitis, especially in those who also had concomitant wheeze in the first 18 months of life compared to healthy controls. As these differences were present from the earliest time points (3 weeks and 3 months of age), and before the onset of clinical symptoms in the majority of subjects, our findings strongly suggest a role of the nasal microbiome in the development of respiratory disease.

Nasal Microbial Diversity

The nasal microbial diversity increased with age in the controls, whilst the rhinitis groups (with or without concomitant wheeze) was reversed with decreasing trends with age. Other cross-sectional studies in infants and young children with respiratory disorders such as asthma, allergic rhinitis and acute respiratory infection, have described similar findings [24–26].

Distinct Bacterial Profiles Between Disease Groups and Healthy

The three dominating phyla of the infant nasal microbiome were *Actinobacteria*, *Proteobacteria* and *Firmicutes* which is consistent with other longitudinal studies in children [13, 16].

Both the rhinitis (without and with wheeze) groups had decreased abundance of *Corynebacteriaceae* family (*Actinobacteria* phylum) compared to controls. Other studies have also shown reduced abundance of *Corynebacteriaceae* with other respiratory conditions such as acute otitis media and wheezing [13, 27]. Of interest is the finding that *Corynebacterium accolens* from the human nasal mucosa releases antibacterial free fatty acid from human triacylglycerols [28]. Taken together, these findings suggest that a high abundance of *Corynebacteriaceae* confers protection by inhibiting other potentially pathogenic bacteria against respiratory diseases in infants.

In contrast to *Corynebacteriaceae*, there was higher abundance of *Proteobacteria* in those with rhinitis and concomitant wheeze compared to controls. This observation was consistent with other studies that reported its association with wheezing respiratory disorders [29]. Although there have not been other reports of *Oxalobacteriaceae* family in association with wheeze in infants specifically, its relation with asthma in adults has been reported [30]. Our current study in infants strengthens the possibility that *Oxalobacteraceae* may contribute to wheezing disorders.

The evidence that *Moraxella spp.* (*Moraxellaceae* family) influences subsequent respiratory illness appears to vary between studies. Several studies have shown that *Moraxella spp.* was associated with an increased risk of pneumonia in children in their first 3 years of life [31]. On the other hand, it has also been shown to be a part of a commensal bacterial community in the first 2 years of life and protects against subsequent respiratory infection [32]. In our study, there was no difference in abundance of *Moraxella spp.* between clinical groups

longitudinally but it was significantly increased in both rhinitis groups at the 6 month time point.

Similar to *Oxalobacteraceae* family, the abundance of *Aerococcaceae* family (*Firmicutes* phylum) was significantly higher with the rhinitis and concomitant wheeze group compared to controls. As in all clinical groups, *Aerococcaceae* colonization appears later in age (6 months and beyond) compared to *Oxalobacteriaceae* and *Corynebacteriaceae*. This observation is consistent with previous reports in children studied longitudinally from 1 till 24 months [32, 33]. The microbials belonging to the *Aerococcaceae* were further identified to belong to the *Alloiococcus spp.* Although this genus appeared to be strongly associated with rhinitis and wheeze in our study, other studies have shown the contrary, in that lower abundance of *Alloiococcus spp.* during infancy was associated with higher numbers of acute respiratory infections [13]. Little is known of its role in early nasal microbial colonization. *Alloiococcus* is a Gram-positive genus with only one known species, *Alloiococcus otitidis* (*A. otitidis*) [34]. This species is frequently detected in the outer/middle ear canal and nasopharyngeal of children with acute otitis media. Studies from Japan found that nasopharyngeal colonization of *A. otitidis* is increased in otitis-prone children compared to that in non-otitis-prone children [35, 36]. This organism is also shown to induce local immune response in the middle ear as a pathogen [37, 38].

Staphylococcaceae family (*Firmicutes* phylum) differ between clinical groups longitudinally till 9 months, and its abundance was significantly higher at early time points (3 week and 3 month) in controls compared to the rhinitis groups. This was strengthened by PCoA at 3 week and 3 month, *Staphylococcaceae* is, therefore, a feature of early colonization in healthy individuals before its declines in later infancy, and this is supported by other studies in healthy children aged below 18 months of age [13, 33]. Although cutaneous colonization of *Staphylococcus spp.*, particularly *Staphylococcus aureus* is associated with atopic eczema [39], our longitudinal analysis did not show any association between eczema and colonization of nasal *Staphylococcus spp.* (data not shown).

Our data indicate that the succession of nasal bacterial over the first 18 months of life influence the susceptibility to wheeze. We observed that high carriage of *Oxalobacteraceae* and low abundance of *Corynebacterium spp.* (*Corynebacteriacecae* family) and *Staphylococcaceae* in the early time points (till 9 months) appeared to encourage the early colonization and growth of *Alloiococcus spp.* (*Aerococcaceae* family) at an accelerated rate in subjects with wheeze compared to controls. Thus, the initial establishment of *Oxalobacteraceae* rather than *Corynebacterium spp.* might be a predictive marker of transition to the subsequent increase in abundance of *Alloiococcus spp.* Of note is the 'graded' degree of dysbiosis between the rhinitis alone and rhinitis with concomitant wheeze groups, with the rhinitis with wheeze group showing the more extreme difference from controls. It is tempting to speculate that larger deviation from 'normal' microbial establishment in the airway is required to predispose wheeze in infancy. Our findings are supported by Bosch et al. who reported an accelerated maturation of microbiota in children with more than two respiratory tract infection episodes per year compared to controls with less than two episodes [16].

Determinants of Establishment of Nasal Microbiota

This study found that male gender, presence of siblings, Caesarean delivery and infant-care attendance influenced the establishment and maturation of the nasal microbiome. These factors could be the determinants for dysbiotic nasal microbiota that could predispose to rhinitis and wheeze later in life.

The presence of siblings and daycare attendance was associated with lower abundance of *Corynebacteriaceae* and higher abundance of *Oxalobacteraceae* and *Moraxellaceae*. This interaction with other young children has also been shown to be related to frequent wheezing at the age of 2 years compared to children with little or no exposure [40]. An Australian study by Teo et al., showed that significantly higher relative abundances of *Moraxella spp.* and lower relative abundances of *Corynebacterium spp.* in infants attending day care compared to those at home [13]. A cross-sectional American study showed that infants with siblings were more likely be dominated by *Moraxella* than a *Corynebacterium* while opposite pattern was observed in those without siblings [41]. Whether the increased exposure to respiratory viruses or its influence on the establishment of the nasal microbiome through the interaction with other young children plays a more important role in the development of wheezing illness in early life will require further study.

Our results also showed that male gender and delivery by Caesarean section was associated with a more 'dysbiotic' nasal microbial profile with higher abundance of *Aerococcaceae* and lower abundance of *Staphylococcaceae.* Although not observed in other studies, the mode of delivery did impact on the succession of nasal microbiome in early life [16]. A birth cohort study up to 6 months led by Bosch et al. showed that children born by Caesarean section had a higher abundance of *Staphylococcus aureus* than vaginally born children, even though this trend was most prominent at the early time points. In relation to gender, male gender has been shown to be a universal risk factor for respiratory disorders such as rhinitis, wheeze and asthma in early childhood in most populations published and not confined to this cohort [42, 43]. This may arise from gender differences in lung airway anatomy and maturation [44]. The airways in males have been found to be structurally narrower than females and this anatomical difference may contribute to the higher prevalence of wheezing disorders in the male [45, 46]. However, there are other gender related differences that may explain the gender differences in nasal bacterial colonization that was observed in our cohort. Firstly, the delay in pulmonary surfactant in the male fetal lung compared to females [47, 48] may contribute to the pattern of early microbial colonization. Surfactant proteins have the ability to aggregate and control the clearance of bacteria through binding and neutralization by acting as opsonin [49–51]. Secondly, the innate and adaptive immune responses of male infants are also weaker than females, and this difference may influence differential colonization of the nasal mucosa [52–54].

Our study did not show a significant impact of intrapartum antibiotic prophylaxis or post-natal antibiotic on the nasal microbiome. Other studies in infants with respiratory tract infection and acute otitis media have shown a negative association between antibiotic usage and *Corynebacteriaceae* carriage [16, 27]. These differences between studies may in part be explained by the different collection sites of our study (anterior nares) and other studies (nasopharyngeal).

Limitations and Conclusion

The limitation in this study is that the sampling area is the anterior nares. Several studies have highlighted the variation in bacterial composition between different sites of respiratory tract [55–58]. Moreover, the impact of virus was not analyzed in our study which deserves further investigation. We were also unable to analyze all the bacteria groups down to species level. Future studies involving metagenomics sequencing may provide more precise identification of bacteria and may also provide information on viruses.

Despite these limitations, our longitudinal birth cohort with dense serial sampling support the hypothesis that distinct profiles of the nasal microbiota in the first 18 months of life impact on the development of rhinitis and wheezing in later infancy. Our GUSTO follow up data showed that 20% of infants with rhinitis persisted to have rhinitis at the age 5 years, whilst controls remained rhinitis free (data not shown), indicating that rhinitis status at 18 months is a predictor for rhinitis status at 5 years. Further work to verify these findings and its impact on subsequent allergic rhinitis and asthma in later childhood will provide important insights into the role of the nasal microbiome in these disorders. These are important steps towards developing novel strategies for the treatment and management of these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations Used

ARIA   Allergic Rhinitis and its Impact on Asthma

DNA   Deoxyribonucleic Acid

EMIRGE	Expectation Maximization Iterative Reconstruction of Genes from the Environment
GERMS	Genome Institute of Singapore Efficient Rapid Microbial Sequencing
GUSTO	Growing Up in Singapore Towards healthy Outcomes
OTUs	Operational Taxonomic Units
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
SDI	Shannon Diversity Index
SPT	Skin Prick Test
spp.	species
16S rRNA	16S Ribosomal Ribonucleic Acid

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Capsule Summary

The nasal microbiome in the first 18 months of life is involved in the development of rhinitis and wheeze in early life. A decrease in *Corynebacteriaceae* and *Staphylococcaceae,* and increase in *Oxalobacteraceae* and *Aerococcaceae* of the anterior nares are associated with early onset rhinitis and wheeze.

Clinical Implications

Research into the mechanisms behind longitudinal variation in the respiratory microbiome may pave the way for the prevention and treatment of early onset rhinitis and wheezing disorders.
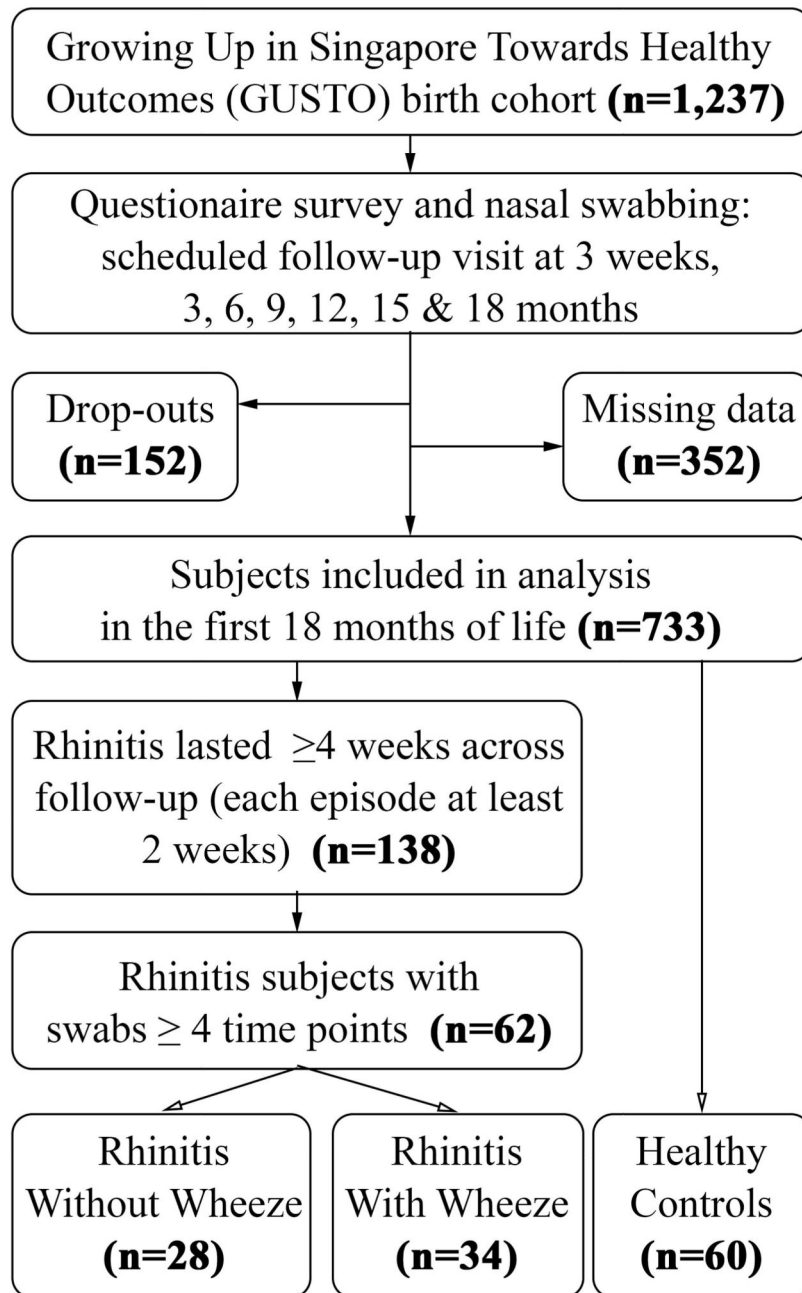
Figure 1.
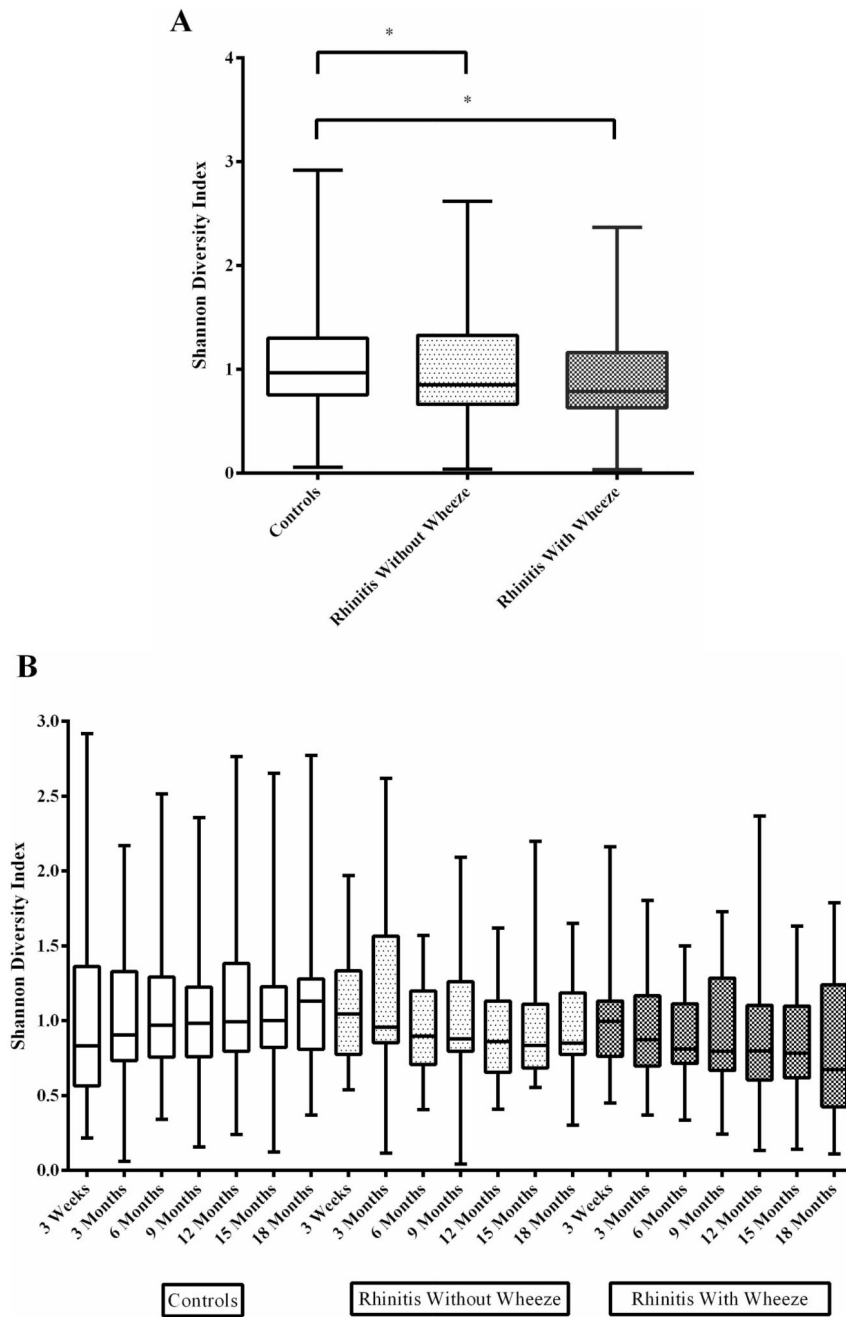Flowchart of Subject Selection.

Figure 2.

Microbial diversity of the three clinical groups. (A) Overall Shannon Diversity combining all seven time-points from 3 weeks till 18 months; (B) Shannon diversity of the three clinical groups by time points. Max, min, 25th percentile, 75th percentile and geometric mean of relative abundance were used for box and whisker plot.

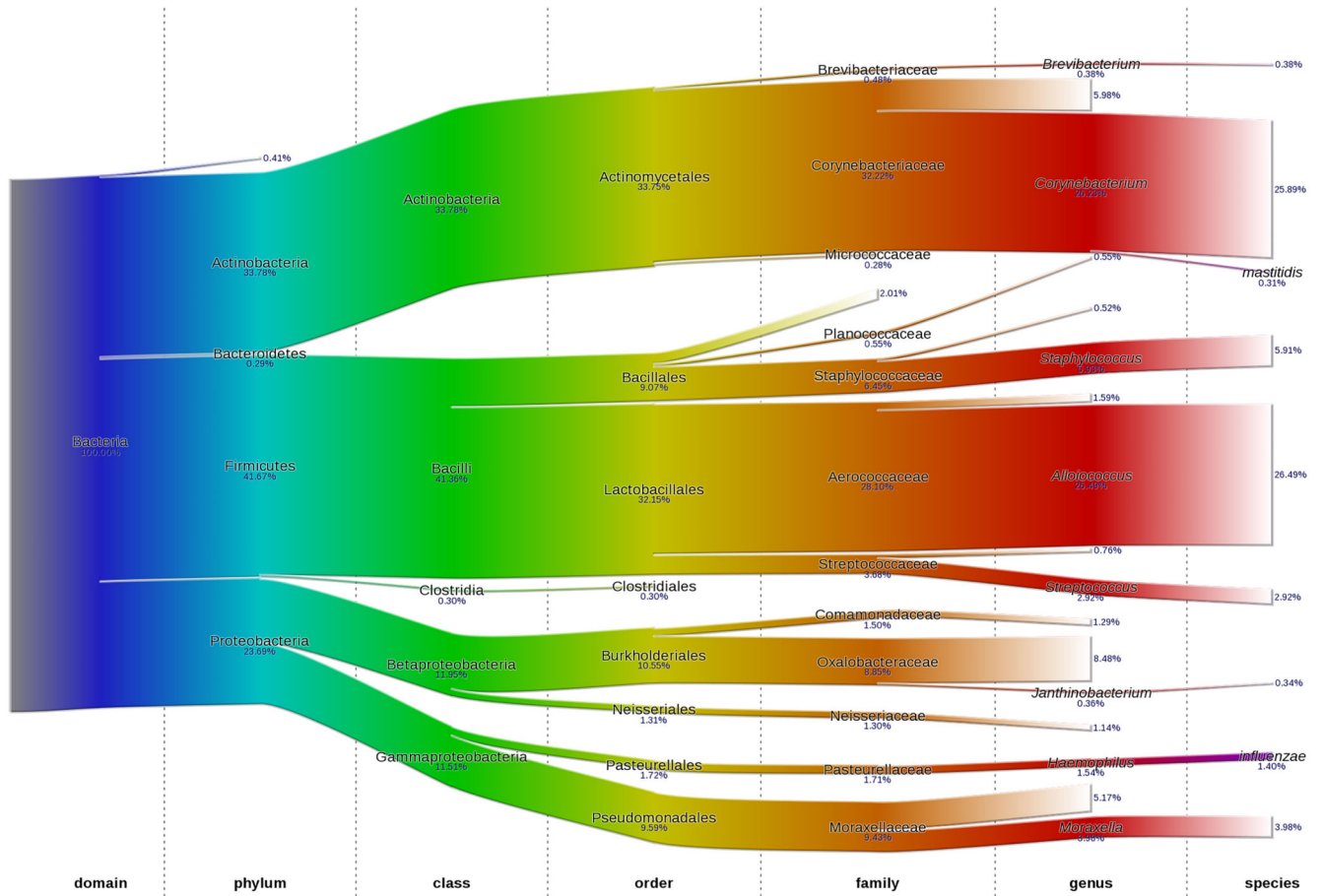* denotes significance at p<0.05 between groups.

Figure 3.
Sankey plot representing the overall nasal bacterial composition and their corresponding average abundance for three clinical groups. Each colour represents different taxonomy classification levels. The arithmetic mean of relative abundance is inserted below each microbial group.
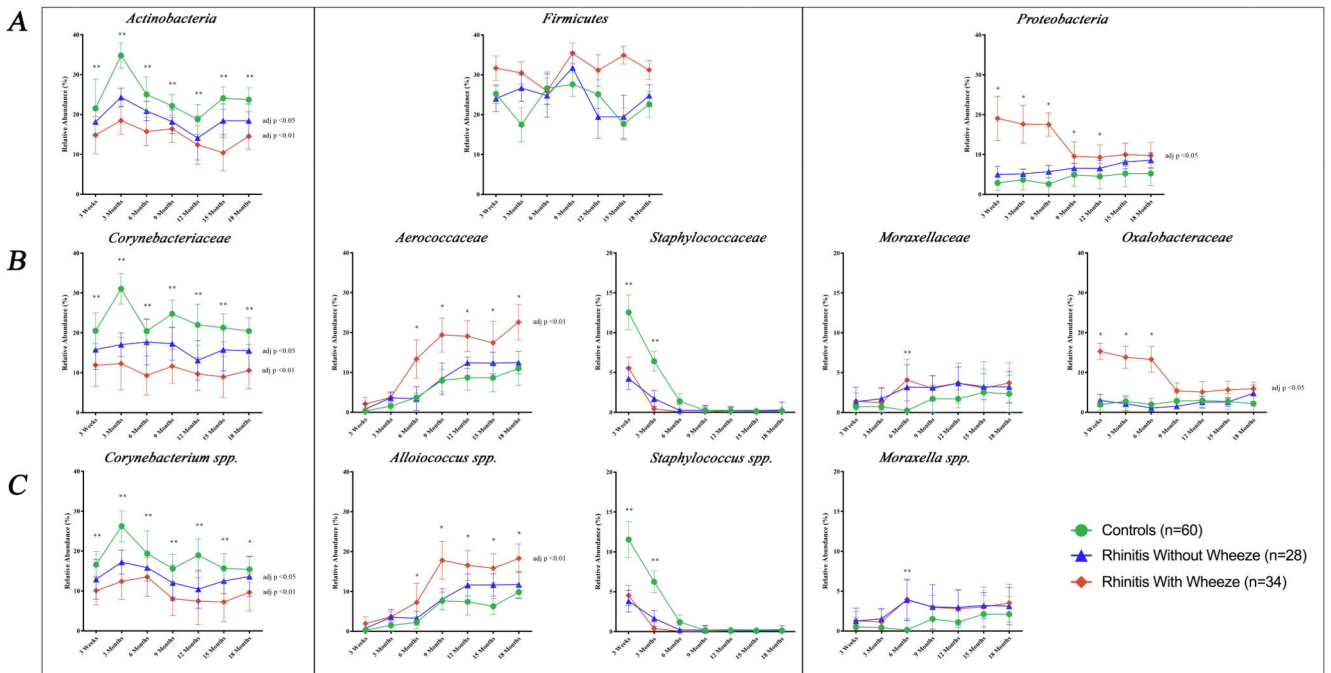
Figure 4.
Bacterial abundance of major (A) Phyla, (B) Family and (C) Genus of the three clinical groups by time points. Data presented as geometric mean of relative abundance. Adjusted p values denotes significant difference of bacterial relative abundance between rhinitis and control groups using linear mixed model analysis and linear regression after adjusting for confounders (gender, family history of respiratory disease, presence of siblings, mode of delivery, use of intrapartum antibiotic prophylaxis and postnatal antibiotics and breastfeeding pattern).
* denotes significant difference at p<0.05 between rhinitis with wheeze and control group at specific time point.
** denotes significant difference p<0.05 between both rhinitis groups and control group at specific time point.
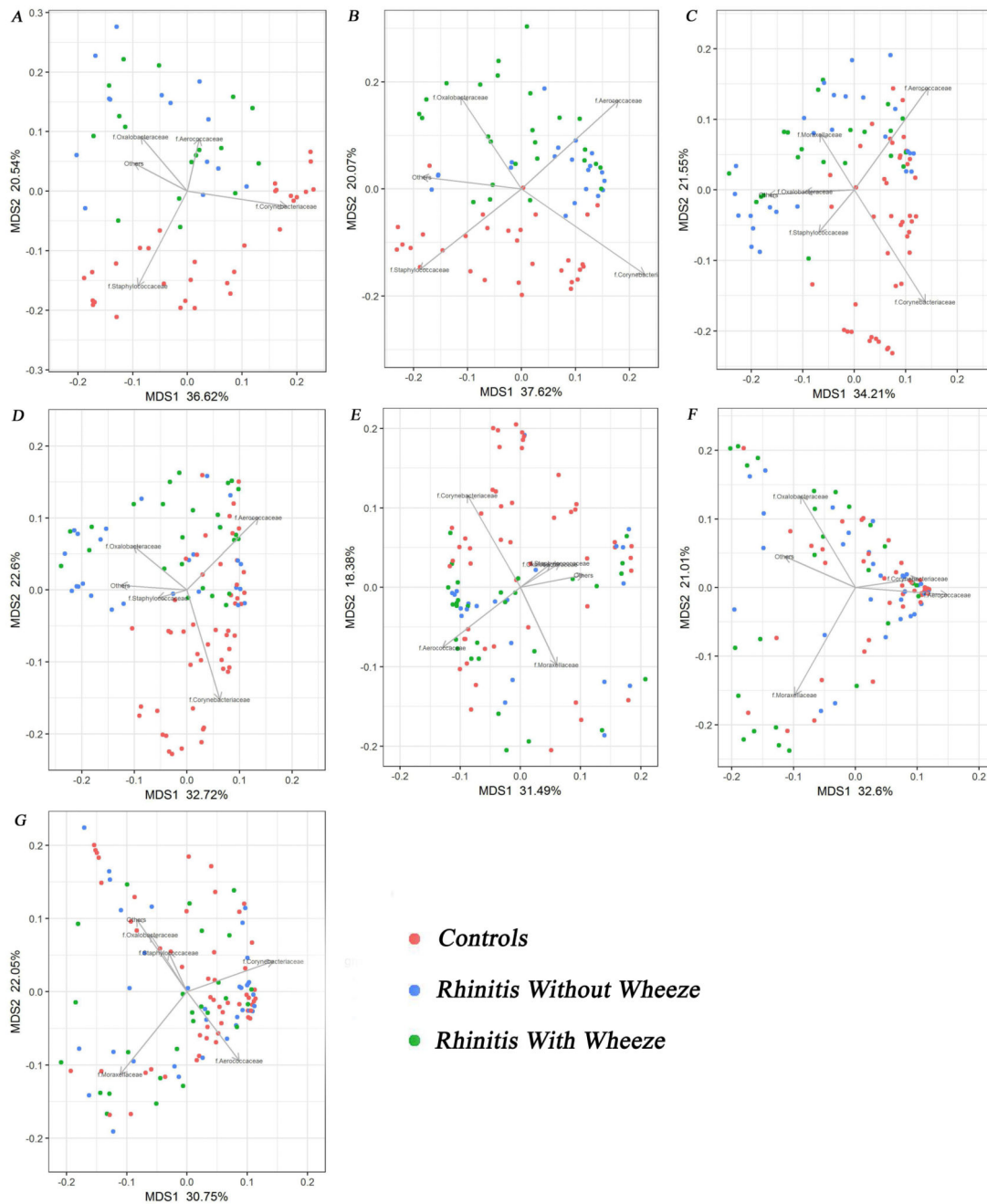
Figure 5.
Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity between nasal microbiome profiles (Family level) for the three clinical groups at various time-points. (A) 3 week; (B) 3 month; (C) 6 month; (D) 9 month; (E) 12 month; (F) 15 month and (G) 18 month. Arrow shows the directions from the origin for which clusters have significant abundances for the bacterial groups (p<0.001, 999 permutations) and its length is proportional to the correlation between ordination and the bacterial groups.

Table 1

Demographic, Lifestyle and Clinical Characteristics of the Three Clinical Groups.

	Control†	All Rhinitis†	Rhinitis Without Wheeze†	Rhinitis With Wheeze†
	n = 60	n = 62	n = 28	n = 34
Male gender	31 (51.67)	42 (67.74)	19 (67.86)	23 (67.65)
Presence of siblings	37 (61.67)	38 (61.29)	17 (60.71)	21 (61.76)
Caesarean delivery	10 (16.67)	14 (22.58)	5 (17.86)	9 (26.47)
Pre-term <37 weeks gestation	4 (6.67)	4 (6.45)	2 (7.14)	2 (5.88)
Intrapartum antibiotic prophylaxis	18 (30.51)	22 (35.48)	5 (17.86)	17 (50.00)
Postnatal antibiotics within first year	19 (31.67)	35 (58.33)**	14 (51.85)	21 (63.64)**
Ethnicity				
Chinese	36 (60.00)	30 (48.39)*	17 (60.71)	13 (38.24)**
Malay	10 (16.67)	21 (33.87)*	8 (28.57)	13 (38.24)*
Indian	8 (13.33)	11 (17.74)	3 (10.71)	8 (23.53)
Maternal history				
Any of three atopic diseases	12 (20.34)	23 (39.66)*	11 (40.74)	12 (38.71)*
Rhinitis	6 (10.17)	13 (22.41)	4 (14.81)	9 (29.03)*
Eczema	3 (5.08)	11 (18.97)*	7 (25.93)*	4 (12.90)
Asthma	7 (11.86)	9 (15.52)	2 (7.41)	7 (22.58)
Paternal History				
Any of three atopic diseases	18 (30.51)	25 (43.10)	16 (59.26)*	9 (29.03)
Rhinitis	11 (18.64)	13 (22.41)	10 (37.04)*	3 (9.68)
Eczema	3 (5.08)	7 (12.07)	5 (18.52)*	2 (6.45)
Asthma	8 (13.56)	10 (17.24)	4 (14.81)	6 (19.35)
Infant care attendance within first year	7 (15.56)	6 (17.65)	2 (10.53)	4 (26.67)*
Exclusive breastfeeding till month 6	10 (16.67)	8 (12.90)	7 (25.00)	1 (2.94)
Eczema symptoms	6 (10.00)	19 (30.65)**	9 (32.14)**	10 (29.41)*

	All Rhinitis[†]		Rhinitis Without Wheeze[†]	Rhinitis With Wheeze[†]
	Control[†]			
	n = 60	n = 62	n = 28	n = 34
Allergen sensitization				
Any of six tested allergens	4 (7.02)	13 (23.21)[*]	7 (28.00)[*]	6 (19.35)
Egg	1 (1.75)	4 (7.14)	3 (12.00)	1 (3.23)
Milk	0 (0.00)	3 (5.36)	1 (4.00)	2 (6.45)
Peanut	0 (0.00)	4 (7.14)	3 (12.00)[*]	1 (3.23)
Dermatophagoides pteronyssinus	3 (5.26)	10 (17.86)	6 (24.00)[*]	4 (12.90)
Dermatophagoides farina	3 (5.26)	5 (8.93)	3 (12.00)	2 (6.45)
Blomia tropicalis	0 (0.00)	2 (3.57)	2 (8.00)	0 (0.00)

[*]
significant at p<0.05 compared to healthy group in univariate analysis.

[**]
significant at p<0.01 compared to healthy group in univariate analysis.

[†]
Some variables had subjects with missing data.