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Rhinitis in the first 18 months of life: Exploring the role of respiratory viruses

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Abstract

Background: Rhinitis is common in early childhood, but allergic rhinitis is considered a later manifestation of the atopic march. This study aimed to evaluate rhinitis (allergic and non-allergic) in the first 18 months of life, its link with other atopic manifestations and the role of respiratory viruses.

Methods: Subjects (n = 1237) of the Singapore GUSTO birth cohort were followed up quarterly until 18 months of age with questionnaires to screen for rhinitis symptoms lasting at least 2 wk and with monthly calls to positive subjects to detect prolonged/recurrent rhinitis symptoms (total duration ≥4 wk). Anterior nasal swabbing for molecular-based virus detection was conducted during these visits and near (within a month) rhinitis episodes. Skin prick testing to common environmental and food allergens was conducted at the 18 month visit.

Results: Prolonged/recurrent rhinitis was significantly associated with history of parental atopy (mother: aOR = 2.17; father: aOR = 1.82) and atopic comorbidities of eczema (aOR = 2.53) and wheeze (aOR = 4.63) ($p < 0.05$), though not with allergen sensitization. Although the frequency of nasal respiratory virus detection during scheduled quarterly visits did not differ between prolonged/recurrent rhinitis and matched controls ($p > 0.05$), virus detection was higher in swabs obtained within a month following rhinitis episodes in prolonged/recurrent rhinitis subjects compared with scheduled visits (adjusted $p = 0.04$).

Conclusions: Based on the duration of rhinitis symptoms, this study defined a subset of early childhood rhinitis which was associated with atopic predisposition and comorbidities. Persistent respiratory viral shedding may contribute to the symptomatology. Whether this entity is a precursor of subsequent childhood allergic rhinitis will require longer follow-up.

Abbreviations
GEE, Generalized Estimating Equations; TNSS, Total Nasal Symptom Score; SPT, Skin Prick Test; HDM, House Dust Mites; ARIA, Allergic Rhinitis and its Impact on Asthma; ISAAC, International Study on Asthma and Allergies in Childhood; GUSTO, Growing Up in Singapore Towards healthy Outcomes; HRV, Human Rhinovirus; RSV, Respiratory Syncytial Virus; PIV, Parainfluenza Virus.

Unlike asthma, rhinitis does not cause mortality but has been acknowledged to be a global problem as it imposes considerable cost and burden to the quality of life (1). In addition, there is strong evidence for an association with asthma (2).

Rhinitis has not been as extensively studied as its counterparts, eczema and asthma, in very young children. This may stem from the notion that allergic rhinitis is one of the later manifestations of the atopic march and therefore not significant in early life (2, 3). A cross-sectional survey of Singaporean toddlers in 2005 showed that rhinitis had a cumulative prevalence of as high as 42.7% in the first 2 yr of life (4). This suggests that, contrary to the allergic march model which described rhinitis to occur later in life, allergic and non-allergic rhinitis might, in at least a subset of atopic individuals, have their onset as early as infancy.

Another reason for the gaps in our knowledge of the early-onset rhinitis might be the difficulty in recognizing allergic and non-allergic rhinitis at this early age. They are difficult to differentiate from recurrent infectious rhinitis (i.e. back-to-back infections of the upper airways), which is a common cause of rhinitis in young children (1). The current prospective birth cohort study thus aimed to evaluate rhinitis from birth to 18 months of age and its link to atopy in a general population birth cohort. This study also assessed the role of respiratory viruses in the inception of rhinitis using molecular-based virological studies on nasal swabs collected during scheduled quarterly visits and near (within a month) rhinitis episodes.

Methods

Subject assessment

This study involved Singaporean newborns enrolled in the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort (n = 1237), which is a general population cohort. The methodology of the GUSTO study has been described elsewhere (5). Briefly, mothers were recruited antenatally and their offspring followed from birth by scheduled visits at 3 wk, and 3, 6, 9, 12, 15 and 18 months. 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.

During the visits, caretakers were interviewed for rhinitis and concomitant symptoms (Fig. 1). Subjects were screened with the question 'Has your child ever had sneezing, running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has lasted for two or more weeks duration?' Subjects positive for this question were followed up by monthly phone calls to track rhinitis progression until remission for 3 months was reached. Data on demographics, family history and environmental conditions were also obtained using a structured questionnaire.

Definitions

Rhinitis was defined as symptoms of sneezing, runny and/or blocked nose for more than 2 wk duration based on questionnaire and subsequent phone interview. We based our 2-wk definition from the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline, which states that symptoms lasting for more than 2 wk should be investigated for a cause aside from infection (1). Rhinitis symptoms were considered 'prolonged/recurrent' if the symptoms lasted for at least 4 wk in single or multiple episodes (each lasting at least 2 wk) anytime within the first 18 months of life. Eczema was defined as physician-diagnosed eczema. Wheezing was defined as the presence of wheezing symptoms (noisy breathing with a high-pitched, whistling sound heard from the chest, not the mouth) associated with inhalational therapy as a more stringent definition and to differentiate wheezing from snoring.

Scoring symptom severity

A four item Total Nasal Symptom Score (TNSS) (6) question was used to assess the severity of four symptom items: blocked nose, runny nose, sneezing and itchy nose symptoms. Each item was subjectively scored by caretakers during monthly calls from 0 (none) to 3 (severe) giving a maximum total score of 12. For subjects with more than one scoring between each quarterly survey, the highest scores for each item were selected for analysis. The degree of disturbance of sleep and feeding activities was also scored in a similar way.

Skin prick test for allergen sensitization

At the 18 month visit, subjects were assessed for the presence of allergic sensitization to a panel of food (whole egg, cow's

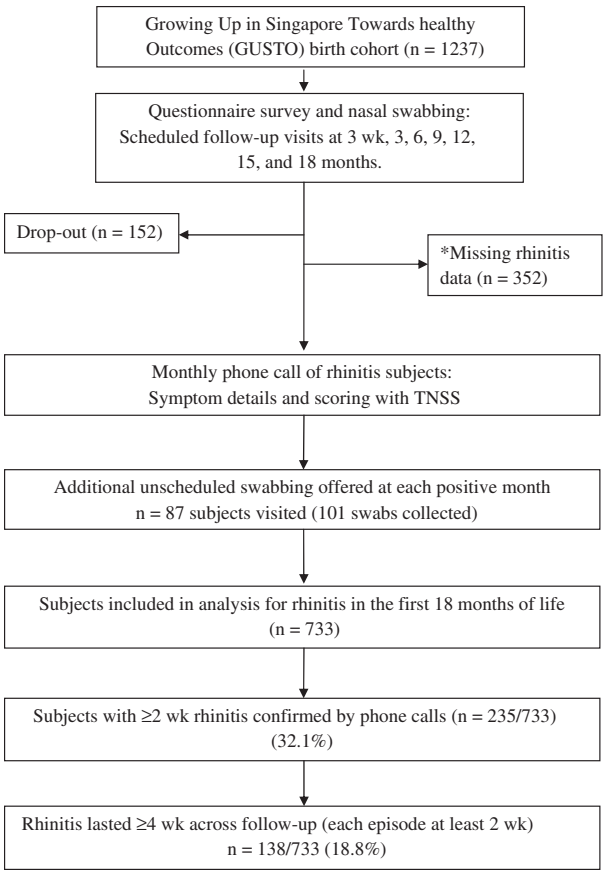

Figure 1 Flowchart of subject assessment and rhinitis outcomes.

milk and peanut) and house dust mites allergens (HDM: *Blomia tropicalis*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*) by skin prick testing (SPT). Skin prick test reagents were obtained from Greer Laboratories (Lenoir, NC, USA), except for *B. tropicalis* which was obtained from our in-house laboratory. A positive reaction was defined as a wheal size of 3 mm larger than the negative control (saline).

Molecular detection of respiratory virus

Anterior nasal swabs for detection of upper respiratory viruses were obtained from every consenting subject at each scheduled quarterly visit until the 18 month visit. A dry flocked swab (COPAN, USA) was inserted to the nostril of the subjects until a resistance was felt and then twisted. Swabs were transferred to a tube of 3 ml universal transport media (COPAN, USA) and stored at −80°C until analysis.

Samples were aliquoted, and total nucleic acid was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Multiplex real-time PCR was conducted using AB7500 fast machine (Thermo Fisher Scientific, Waltham, MA, USA) for testing of 16 respiratory viruses across four multiplex panels as described by Tan et al. (7). The multiplex panels were as follows: panel 1 (human rhinovirus [HRV], influenza A & B, respiratory syncytial virus [RSV] A&B and human metapneumovirus), panel 2 (adenovirus and bocavirus), panel 3 (parainfluenza virus 1–4) and panel 4 (coronavirus: OC43, NL63, HKU1, and 229E). Positive controls, purchased from Vircell (Spain) and also generously gifted by TBH's laboratory and by Dr Lynette Oon Lin Ean from Singapore General Hospital Department of Pathology, were included in every run.

Human Rhinovirus subtyping was conducted by reverse transcription of extracted RNA using Roche cDNA Transcriptor Kit according to the manufacturer's instructions (Roche Diagnostics, Basel, Switzerland). Resulting cDNA underwent nested PCR using Invitrogen Platinum Taq (Invitrogen, Waltham, Massachusetts, USA) and primers targeting the VP4-VP2 adapted from Wisdom et al. (8) according to the kit's instruction, but with 0.5 μl of Taq polymerase instead. PCR products were gel-purified and sequenced (1st Base, Singapore). Rhinovirus subtype was determined by comparing the nucleotide sequences (NCBI nucleotide blast) to published subtypes deposited in GenBank.

Scheduled visit nasal swabs were analysed from a subgroup of 32 subjects with prolonged/recurrent rhinitis in the first 18 months of life and 32 control subjects without rhinitis or wheeze matched for known confounders (9): order of birth and day care attendance during the first year of life. Subjects were also matched for their month of birth. A sample size of 32 pairs was estimated to be able to detect a 30% difference in the prevalence at a single time point. Rhinitis subjects were randomly selected from those with the most number of nasal swabs (minimum of four swabs from seven scheduled visits). Subjects presenting with rhinitis during monthly phone calls were offered additional visit for nasal swabbing within 1 month of the call. Additional nasal swabs obtained (n = 101 from 87 prolonged/recurrent rhinitis subjects) were also analysed.

Statistical analysis

Analysis was conducted on IBM SPSS Statistics version 19 (IBM Corporation, Armonk, New York, USA). Univariate analysis was conducted by chi-square test. Significant factors and household income were included into logistic regression model for multivariate analysis. Linear regression was used to calculate the trend of nasal symptom severity across time and to examine the effect of total symptom score on disturbances scores. Generalized Estimating Equations (GEE) was performed with the matching variables as the random factors to compare respiratory virus detection between case and control groups. A p value <0.05 was considered statistically significant.

Results

Subject characteristics and prevalence of rhinitis

Of the cohort of 1237 subjects, there were 733 (59.3%) evaluable subjects. The reasons for exclusion were as follows: drop outs (n = 152), and subjects with missing rhinitis data at any time point and with negative response for rhinitis at all other time points (n = 352) (Fig. 1).

Demographics and clinical characteristics of the 733 analysed subjects are presented in Table 1. Based on our definition of rhinitis (see methods), the 18-month cumulative prevalence of rhinitis and prolonged/recurrent rhinitis were 32.1% (95% CI: 28.8–35.5%) and 18.8% (95% CI: 16.2–21.8%), respectively. The median cumulative symptom duration in subjects with prolonged/recurrent rhinitis was three (interquartile range 2–4; range 1–12) months. Fig. 2 depicts the cumulative prevalence of rhinitis and prolonged/recurrent rhinitis, which increased by 4.75% (95% CI: 4.34–5.16%) and 2.92% (95% CI: 2.46–3.38%) at each time point, respectively. Only 20.7% (15.7% only to HDM) of prolonged/recurrent rhinitis subjects were sensitized to at least one of the allergens tested (Table 1).

Risk factors associated with rhinitis

The two subsets of rhinitis based on duration (2–4 wk and ≥4 wk) were compared against subjects without rhinitis using multinomial regression analysis (Table 2). Except for usage of antibiotics in the first year of life (adjp = 0.008) and eczema (adjp = 0.042) which were associated with both subsets of rhinitis, only prolonged/recurrent rhinitis (≥4 wk) was significantly associated with maternal (adjp = 0.011) and paternal (adjp = 0.031) histories of atopic symptoms, wheezing (adjp < 0.001), male gender (adjp = 0.003) and low household income (adjp = 0.042). To reinforce these findings, a binary logistic regression was conducted to compare subjects with prolonged/recurrent rhinitis against subjects without prolonged/frequent rhinitis (no rhinitis or 2–4 wk rhinitis), and similar results were observed.

In lieu of the large proportion of missing rhinitis data (32.4%) in this cohort, a sensitivity analysis was conducted to test the stability of these associations. The binary

Table 1 Demographics and clinical characteristics of rhinitis in the GUSTO birth cohort

		All Rhinitis (≥2 wk)†		
	Healthy† (n = 498) (67.9%)	(n = 235) (32.1%)	2–4 wk† (n = 97) (13.2%)	≥4 wk† (n = 138) (18.8%)
Male gender	242 (48.6)	138 (58.7)**	46 (47.4)	92 (66.7)**
Have siblings	277 (55.6)	129 (54.9)	48 (49.5)	81 (58.7)
Caesarean delivery	147 (29.5)	74 (31.5)	39 (40.2)*	35 (25.4)
<37 wk gestational	40 (8.0)	19 (8.1)	10 (10.3)	9 (6.5)
Prenatal tobacco exposure	157 (33.2)	88 (39.1)	32 (34.8)	56 (42.1)
Antibiotics usage during labour	132 (26.7)	68 (29.1)	25 (25.8)	43 (31.4)
Household income (Singapore $)				
0–1999	63 (13.8)	31 (14.0)	10 (10.9)	21 (16.2)
2000–3999	133 (29.0)	66 (29.7)	29 (31.5)	37 (28.5)
4000–5999	111 (24.2)	61 (27.5)	23 (25.0)	38 (29.2)
≥6000	151 (33.0)	64 (28.8)	30 (32.6)	34 (26.2)
Ethnicity				
Chinese	320 (64.3)	125 (53.2)**	53 (54.6)	72 (52.2)**
Malay	98 (19.7)	72 (30.6)**	30 (30.9)*	42 (30.4)**
Indian	80 (16.1)	38 (16.2)	14 (14.4)	24 (17.4)
Maternal history				
Rhinitis	49 (10.2)	38 (18.0)**	11 (13.1)	27 (21.3)**
Eczema	34 (7.1)	30 (14.2)**	9 (10.7)	21 (16.5)**
Asthma	37 (7.7)	28 (13.3)*	5 (6.0)	23 (18.1)**
Paternal history				
Rhinitis	55 (11.5)	40 (19.0)**	13 (15.5)	27 (21.3) **
Eczema	20 (4.2)	18 (8.5)*	6 (7.1)	12 (9.4)*
Asthma	42 (8.8)	23 (10.9)	5 (6.0)	18 (14.2)
Childcare attendance within 1st yr of life	27 (6.2)	25 (12.6)**	8 (10.7)	17 (13.8)*
Atopic symptoms				
Eczema‡	81 (16.7)	66 (36.3)**	22 (31.4)**	44 (39.3) **
Wheeze§	30 (6.1)	47 (25.3)**	8 (11.6)	39 (33.3)**
Allergen sensitization¶				
Any of six tested	53 (11.8)	41 (20.9)**	16 (21.3)*	25 (20.7)*
Egg	10 (2.2)	14 (7.1)*	4 (5.3)	10 (8.2)*
Milk	3 (0.7)	4 (2.0)	1 (1.3)	3 (2.5)
Peanut	3 (0.7)	7 (3.6)*	3 (4.0)*	4 (3.3)*
Dermatophagoides pteronyssinus	36 (8.0)	23 (11.6)	6 (7.9)	17 (13.9)*
Dermatophagoides farina	28 (6.2)	18 (9.1)	7 (9.2)	11 (9.1)
Blomia tropicalis	3 (0.7)	3 (1.5)	0	3 (2.5)
Antibiotics usage in the first 12 months	166 (33.7)	123 (60.3)**	43 (56.6)**	80 (62.5)**

†Some variables had subjects with missing data (range from 0 to 13%).
‡Physician-diagnosed eczema.
§Wheezing symptoms accompanied with nebulizer usage.
¶Tested at 18 months.
*Significant at ≤0.05 compared to healthy group in univariate analysis.
**Significant at ≤0.01 compared to healthy group in univariate analysis.

multivariate regression analysis was repeated. Subjects with missing data in 1–6 of the seven time points (n = 338 of 504 excluded subjects) were classified as 'rhinitis negative' subjects and in another separate analysis with these subjects classified as positive for prolonged/recurrent rhinitis. Similar results were obtained in both extreme scenarios, suggesting consistency of the associated factors (data not shown).

Severity of prolonged/recurrent rhinitis

There was an increasing trend in the TNSS with increasing age, with mean scores increasing by 0.52 (95% CI: 0.35–0.68, $p < 0.001$) at each quarterly survey. Sleeping and feeding disturbances were reported in 71.7% and 58.0% of subjects with prolonged/recurrent rhinitis, respectively. Severe impairment of sleeping (barely able to sleep, awake most of the

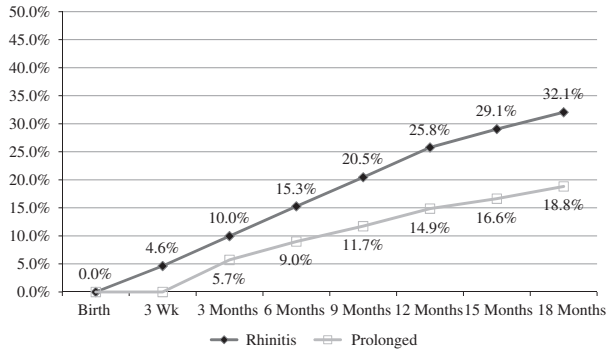

Figure 2 Cumulative prevalence of subjects with history of rhinitis symptoms (sneezing, runny/blocked nose for ≥2 wk) (♦ and black lines) or prolonged rhinitis (≥4 wk symptoms throughout follow-up, each episode lasting ≥2 wk) (□ and grey lines) at each time points till 18 months.

night on most nights) and feeding (feeds very slowly most of the time due to interruptions or irritability) was reported by 17.4% and 8.7% of the subjects, respectively. The disturbance of sleeping and feeding scores correlated positively with TNSS ($R = 0.593$, $p < 0.001$ and $R = 0.496$, $p < 0.001$, respectively).

Detection of specific respiratory viruses in anterior nasal swabs

Analysis of nasal swabs collected at scheduled visits was conducted to examine the role of the persistence of respiratory virus colonization in early-onset prolonged/recurrent rhinitis (≥4 wk). A total of 205 anterior nasal swab samples from 32 subjects with prolonged/recurrent rhinitis in the first 18 months of life and 215 from 32 matched control subjects were analysed. Respiratory virus detection rates showed a peak detection rate at 15 months of age in the case group (34.5%) and at 18 months in the controls (26.7%) (Fig. 3). Fig. 4a depicts the detection rate of each respiratory virus in scheduled nasal swab samples. The most commonly detected virus was HRV, found in 29/205 (14.1%) of swabs from cases (19/32 subjects, 59.3%) and 27/215 (12.6%) from controls (16/32 subjects, 50%). Generalized estimating equations (GEE) analysis result showed no significant difference in virus detection rate between the case and the control ($p = 0.840$). Of interest, we observed that among infants with prolonged/recurrent rhinitis, HRV-positive infants had a higher rate of wheeze (6/19, 31.6%) compared with HRV-negative infants (2/12, 17%). However, this difference was not statistically significant ($p = 0.166$), likely due to the relatively small sample size in the current analysis.

Analysis of nasal swabs collected near (within a month) rhinitis episodes, however, showed a higher detection rate in the nasal swabs of prolonged/recurrent rhinitis subjects obtained at visits within a month of rhinitis episodes (29/101, 28.7%), compared with scheduled visits regardless of case or control ($p = 0.027$; adjp = 0.041, adjusted for age of nasal swabbing, Fig. 4b).

Discussion

Current guidelines such as the ARIA (1) have very little information on rhinitis in infancy and early childhood due to a paucity of studies in this age group. This study thus aimed to provide a better understanding of rhinitis in the first 18 months of life.

We observed a high prevalence rate of rhinitis of 32.1%, and even after taking into account missing rhinitis data (32.4%), the lowest possible prevalence rate (assuming every subject with missing data were rhinitis negative) would have been 21.7%. This prevalence is higher than those reported from the PARIS (9.1% in first 18 months) (10) and the Isle of Wight (15.8% cumulative over first 2 yr of life) (11) cohorts. Our higher prevalence is likely due to more frequent assessments (seven time points in 18 months) and to focussing on the duration instead of using the International Study of Asthma and Allergies in Childhood (ISAAC) definition of symptoms outside cold/flu (12). The only other published study which utilized a duration cut-off is the BAMSE cohort study (13), which reported a lower prevalence rate of 3.5% in the first year and 3.8% in the second year of age. This lower prevalence maybe attributed to their stricter cut-off duration of at least 2 months in the past 12 months.

In the current study, the 2-wk definition for rhinitis based on the ARIA guideline which states that symptoms lasting for more than 2 wk should be investigated for a cause aside from infection (1). This recommendation was substantiated in a recent study which observed that upper respiratory infection in children rarely lasted beyond 14 days (14). In addition, it can be difficult for parents to exclude symptoms due to cold or the flu (ISAAC definition) in this age group (15) as seen in the high rate of positive responses (42.7%) in a cross-sectional study performed on children <2 yr of age in our population (4), most likely an overestimation of the true prevalence of rhinitis.

Prolonged/recurrent rhinitis (≥4 wk in the first 18 months) was found to be significantly associated with parental history of atopic symptoms, and atopic comorbidities (eczema and wheezing). Associations with parental history (13, 16, 17), eczema (17) and wheezing (16–18) have been observed across studies of rhinitis in preschoolers. This result suggests that using a duration cut-off of ≥4 wk in the first 18 months may be a useful approach in defining allergic rhinitis in this age group and therefore deserves further clinical validation.

House dust mites are the most important aeroallergens in Singaporean children (19) and sensitization exclusively to non-HDM aeroallergens is rare (20). HDM allergen sensitization among our rhinitis subjects was uncommon (15.2%, Table 1), implying that the majority of the rhinitis was non-atopic. Our observations support the proposal by the European population-based cohort study MeDALL (Mechanisms of the Development of ALLergy study) that eczema, wheeze and rhinitis may coexist even in the absence of IgE sensitization, and that IgE sensitization may not be a dominant causal mechanism involved in comorbidity of these disorders (21). Another possible explanation would be that there was localized IgE production which was still undetectable by SPT (22) or that the clinical rhinitis phenotypes precede the development of atopic

Table 2 Multivariate analyses for risk factors and comorbidities associated with rhinitis in the first 18 months of life

	Multinomial logistic		Binary logistic
	Rhinitis lasting 2–4 wk* adj OR (95% C.I.)‡	Prolonged/Recurrent rhinitis (≥4 wk)* adj OR (95% C.I.)‡	Prolonged/Recurrent rhinitis (≥4 wk)† adj OR (95% C.I.)‡
Risk factors§			
Gender			
Male	0.61 (0.31–1.21)	**2.36 (1.32–4.22)**	**2.46 (1.40–4.34)**
Female *(ref)*	1.00	1.00	1.00
Delivery			
Caesarean	1.45 (0.74–2.85)	0.95 (0.52–1.74)	0.87 (0.48–1.56)
Vaginal *(ref)*	1.00	1.00	1.00
Ethnicity			
Malay	2.14 (0.92–4.97)	1.21 (0.62–2.38)	1.18 (0.61–2.23)
Indian	1.17 (0.44–3.16)	0.93 (0.39–2.21)	1.00 (0.43–2.33)
Chinese *(ref)*	1.00	1.00	1.00
Household income			
0–$1999	0.43 (0.13–1.46)	**2.51 (1.04–6.05)**	**3.03 (1.27–7.22)**
$2000–$3999	0.63 (0.25–1.56)	1.01 (0.44–2.32)	1.14 (0.51–2.55)
$4000–$5999	0.67 (0.27–1.67)	1.58 (0.73–3.31)	1.82 (0.87–3.79)
≥$6000 *(ref)*	1.00	1.00	1.00
Parental history of atopy symptoms			
Mother			
Yes	1.01 (0.45–2.27)	**2.23 (1.23–4.03)**	**2.17 (1.23–3.83)**
No *(ref)*	1.00	1.00	1.00
Father			
Yes	0.97 (0.43–2.17)	**1.97 (1.08–3.61)**	**1.82 (1.01–3.27)**
No *(ref)*	1.00	1.00	1.00
Post-natal environment			
Childcare attendance in 1st year of life			
Yes	2.17 (0.75–6.31**)**	1.68 (0.64–4.43)	1.72 (0.70–4.23)
No *(ref)*	1.00	1.00	1.00
Antibiotic usage in 1st year of life			
Yes	**3.43 (1.74–6.76)**	**2.24 (1.28–3.91)**	**1.90 (1.10–3.29)**
No *(ref)*	1.00	1.00	1.00
Comorbid			
Eczema¶			
Yes	**2.27 (1.03–5.01)**	**2.71 (1.45–5.07)**	**2.53 (1.38–4.65)**
No *(ref)*	1.00	1.00	1.00
Wheeze**			
Yes	0.96 (0.29–3.20)	**4.99 (2.23–10.86)**	**4.63 (2.22–9.66)**
No *(ref)*	1.00	1.00	1.00
Sensitization††			
Yes	1.90 (0.82–4.38)	1.29 (0.60–2.78)	1.08 (0.52–2.26)
No *(ref)*	1.00	1.00	1.00

*Comparison group was subjects without rhinitis.
†Comparison group was subjects without rhinitis or rhinitis lasted only 2–4 wk.
‡Factors adjusted with each other in a logistic regression model.
§Each variable subset compared with reference subset for odds of being in outcome group compared to being in comparison group.
¶Physician-diagnosed eczema.
**Wheezing symptoms accompanied with nebulizer usage.
††IgE sensitization to egg, milk, peanut and house dust mite allergens at 18 months.
Significant ($p<0.05$) variables are highlighted in bold.

sensitization (23). Thus, a longer follow-up is warranted to monitor the progression of rhinitis within this cohort.

We observed that a substantial proportion of subjects with prolonged/recurrent rhinitis suffered from disturbances to sleep and feeding, highlighting the importance of studying rhinitis in infancy. These findings mirror studies that have shown a significant impact of rhinitis on the quality of life of preschool and school aged children (24).

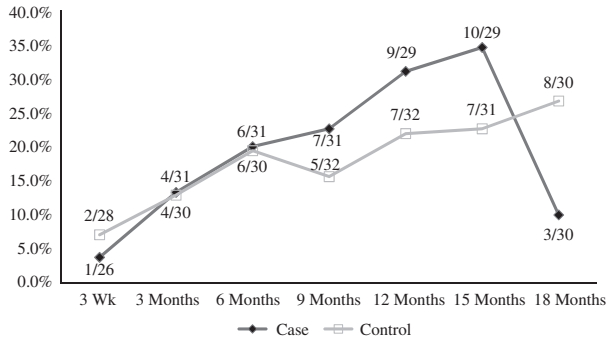

Figure 3 Proportion of samples at each time points positive for real-time PCR detection of any of the 16 targeted respiratory viruses: human rhinovirus (HRV), human respiratory syncytial virus (RSV) A and B, human metapneumovirus (HMPV), human influenza virus (Flu) A and B, human adenovirus (AdV) and human bocavirus (BoV), human parainfluenza virus (PIV) 1–4, and human coronavirus: OC43, HKU1, 229E and NL63. Results are stratified based on cases (♦) or controls (□).

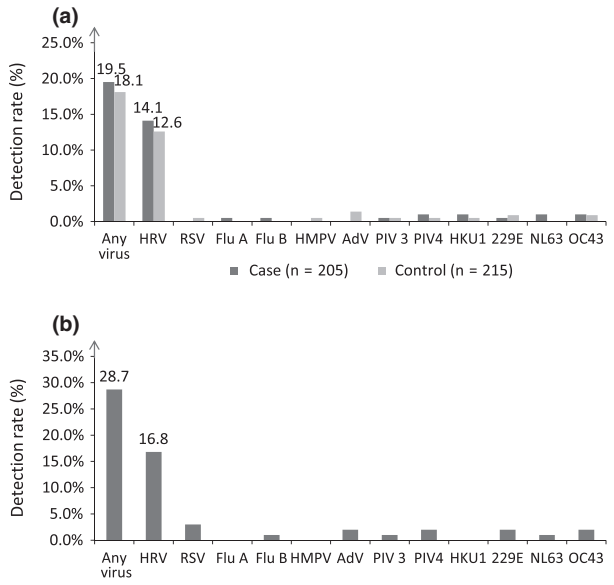

Figure 4 Respiratory virus detection (a) In scheduled visit nasal swab samples collected from cases (n = 205) and controls (n = 215) subjects, (b) In samples collected during additional visits (87 prolonged/recurrent subjects, 101 swabs). Respiratory viruses detected by multiplex PCR across 4 time points. HRV: human rhinovirus, RSV: respiratory syncytial virus, Flu A: human influenza A, Flu B: human influenza B, HMPV: human metapneumovirus, AdV: human adenovirus, PIV: human parainfluenza.

Respiratory virus detection rate of our study was lower than other cohorts such as the COAST (90% overall detection) (25) and the COPSAC cohort (64% overall detection) (26). Our study utilized nasal swabbing instead of nasopharyngeal aspirate which is less invasive considering the young age of our subjects. Regardless, both methods have been shown to produce comparable results (27). Our low rate of detection may be in part due to the collection of nasal swabs at scheduled intervals rather than during acute respiratory symptoms. Furthermore, the additional clinic visit swabs were collected only when the symptoms had been ongoing for at least 2 wk since the onset, as per our definition of rhinitis.

The subcohort virus analysis did not reveal an association between respiratory virus detected at quarterly scheduled visits and prolonged/recurrent rhinitis in the first 18 months of life. Analysis of larger sample size was not pursued as the difference in respiratory virus detection rate between the case and the matched control group was small. Statistically, it is unlikely that a difference would be discerned even if the entire cohort was analysed.

In contrast, analysis of nasal swabs collected from prolonged/recurrent rhinitis subjects within a month following rhinitis episodes showed a higher rate of virus detection compared with those of scheduled swabs (adjp = 0.041). This suggests that whilst these subjects did not persistently shed respiratory virus more than controls during symptom free intervals, an extended period of respiratory viruses shedding within a month since the onset of symptom episodes (of at least 2 wk) was observed. These findings may provide clues to the mechanisms of prolonged rhinitis in early life. Whilst it has been shown that respiratory viruses shedding may persist beyond 2 wk of onset of symptoms in 25% of children aged 2–9 yr old (28), respiratory virus shedding rarely persists beyond 2 wk following the start of respiratory infections in infants (29). This persistence of virus shedding observed in our prolonged/recurrent rhinitis subjects may be an inherent inability of the subjects in clearing respiratory virus infection, or that prolonged/recurrent rhinitis subjects were more susceptible to new infections following each rhinitis episode. On the other hand, it is also possible that respiratory viruses play a role in the symptomatology of prolonged/recurrent rhinitis in this age group.

Our observations mirror studies on the role of respiratory viruses in early childhood wheezing, where HRV was also reported as the most frequently detected virus in the Perth (30), WHISTLER (31) and the COAST (25) cohorts. These studies showed that rhinovirus-associated wheeze was a risk factor of subsequent asthma. While not statistically significant, our study observed numerically higher rate of wheezing in subjects with positive respiratory viruses detection among wheezing subjects. The notion that HRV-related prolonged/recurrent rhinitis might also predispose to wheezing deserves further evaluation.

We also looked into the seasonality of nasal swab HRV detection rates. While RSV and influenza was previously reported to have some seasonal pattern in tropical Singapore (32), we did not observe any seasonal pattern of respiratory virus detection in our study (data not shown).

A recently discovered subtype C of HRV had been reported to result in severe respiratory virus illnesses (33, 34). HRV subtyping was also conducted in our subjects but no statistical difference between case and control was found (data not shown). Further follow-up of this cohort would be required to determine whether a subset of the prolonged/recurrent rhinitis subjects would progress to allergic rhinitis in later childhood as this would suggest that respiratory viruses may play a role in the development of subsequent allergic rhinitis.

In conclusion, our data suggest that epidemiological studies on rhinitis in this age group should focus not just on the presence of symptoms but also on its duration, as rhinitis which lasted for at least 4 wk in duration was associated with eczema, wheezing and parental histories of atopy. Respiratory viruses may also play a role in the development of early-onset prolonged/recurrent rhinitis. Whether this entity is a precursor of later childhood allergic rhinitis will require longer follow-up. This study substantiates the need for further studies in the management of rhinitis in young children, which is hitherto an unmet need.

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Conflict of interests

All authors declare that they do not have any relevant conflict of interest pertaining to this study.

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