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Co-existence of wheezing and constipation in childhood: explanations from an epidemiological study

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Abbreviations: IBS, irritable bowel syndrome; AD, atopic dermatitis; ISAAC, international study of asthma and allergies in childhood; BMI, body mass index; NOSIK, nijmeegse ouderlijke stress index—kort (parenting stress index—short form); OR, odds ratio; CI, confidence interval; HPA, hypothalamic pituitary-adrenal

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CONTRIBUTOR'S STATEMENT:

Jessica Kiefte-de Jong has analyzed the data, written the first draft of the paper, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. She fulfils the criteria as described by the ICMJE as follows:

1. Substantial contributions to conception and design, acquisition of data, and analysis and interpretation of the data.
2. Drafting the article .
3. Final approval of the version to be published.

Ankie Lebon has participated in the data analysis and had access to all the data in the study. She fulfils the criteria as described by the ICMJE as follows:

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2. Critical revision of the paper.
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Johan de Jongste, Vincent Jaddoe, and Albert Hofman fulfil the criteria as described by the ICMJE as follows:

1. Substantial contribution to conception and design, acquisition of data, and interpretation of the data.
2. Critical revision of the paper.
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Henriëtte Moll supervised the study. She fulfils the criteria as described by the ICMJE as follows:

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ABSTRACT

Objective: To assess whether wheezing and atopic dermatitis were associated with constipation in pre-school children and to what extent shared risk-factors contribute to this relationship.

Methods: A population-based sample of 4651 pre-school children was used. At the age of 24, 36 and 48 months, parental report of functional constipation was available according to the ROME II criteria and data on atopic dermatitis and wheezing was available using age-adapted questionnaires from the International Study of Asthma and Allergies in Childhood. Stepwise multivariate analyses were performed to assess whether body mass index, inflection exposure, food allergy and infant nutrition, and parental stress explained the association between wheezing, atopic dermatitis and constipation.

Results: Out of 4651 children, 12-17% had functional constipation between 24 and 48 months. Symptoms of wheezing and atopic dermatitis decreased from 20% and 30% at 24 months to 10% and 18% at 48 months. Between the age of 24 and 48 months, wheezing symptoms were significantly associated with functional constipation (aOR: 1.16; 95%CI: 1.01, 1.33) but these results were mainly explained by the child's infections exposure (aOR: 1.10; 95%CI: 0.96 – 1.25). No significant association was found between symptoms of atopic dermatitis and functional constipation (aOR: 1.04; 95%CI: 0.91, 1.18).

Conclusions: These findings suggest that functional constipation frequently co-exists with wheezing in childhood but is mainly explained by the child's infection exposure. Therefore, an independent association between respiratory symptoms and functional bowel disorders as suggested in previous studies may be discussed.

ARTICLE SUMMARY

Article focus

- Both constipation, wheezing and atopic dermatitis are common symptoms in the paediatric population.
- Functional bowel disorders is linked with asthma and atopy in previous studies in adults but in children these results are equivocal.
- Functional bowel disorders, asthma and atopic disease may share common risk-factors which include overweight, stress, infection exposure, food allergy and nutritional practice during infancy. However, most studies did not take into account for these potential confounders.

Key messages

- Wheezing but not atopic dermatitis is associated with functional constipation in pre-school children but this is mainly explained by a history of infections of the child whereas other potential shared-risk factors as BMI, food allergy, timing of introduction of food allergens, breast-feeding duration and parental stress do not have a major role in explaining the association between wheezing and constipation..
- This study suggests that the association between wheezing and functional constipation is not independent in childhood. Further research is needed to explore whether this result also apply to the outcome of asthma.

Strengths

- Population-based study population. This study includes a large study population who were not selected according to medical care and, therefore, a high external validity applies to this study.
- This study addresses a topical area that has not had sufficient study and can have a contribution on the discussion how asthma or atopy may be associated with functional bowel disorders. This study took multiple shared risk-factors of wheezing, atopic dermatitis and constipation into account that sheds light on the suggested association in literature.

Limitations

- Symptoms were available only from parental-reported questionnaires. This could have led to misclassification of wheezing, atopic dermatitis and constipation since doctor diagnosis provides a more accurate diagnosis.
- Early wheezing in infancy is not a sufficient predictor of childhood asthma. Hence, our study precludes conclusions with respect to functional bowel disorders and its link with asthma.
- No data was available regarding parental concerns of the child's health status. Bias may occur when parents with high concerns are more likely to report symptoms in their child as wheezing, constipation and infectious disease.

INTRODUCTION

Functional bowel disease accounts for the majority of gastrointestinal symptoms worldwide (1). The prevalence of atopic diseases such as asthma and atopic dermatitis is also increasing in Western Countries(2). Several studies linked asthma and atopy in adults with the irritable bowel syndrome (IBS)(3-6), but in children different results have been described(7-8).

Some pathophysiological overlaps may explain the correlation between IBS and atopy. For example, atopic disease, which affects approximately 30% of the population, include food allergies that may cause various organ dysfunction implying that the link between IBS and atopy may be explained by food allergy(9). Likewise, a shared risk-factor for both asthma and functional bowel disorders is obesity. As reported in several studies, asthma is highly prevalent in children with obesity(10) and obesity has been found to be more common in subjects with functional constipation(11).

Since both atopy and functional bowel disorders are particularly prevalent in developed countries, a role for the 'hygiene hypothesis' in these two disorders is also proposed. The hygiene hypotheses concedes that current hygiene practices lead to reduced or altered exposure of bacteria and other micro-organisms that may result in an imbalance of the immune system and microflora early in life (12). Early microbial colonization of the infant's gastro-intestinal tract might reduce the risk of atopic disorders and altered intestinal flora has been an area of interest for years in both IBS and atopy (13-14). Finally, studies have addressed the role of stress in the aetiology both of atopic disorders(15) and functional bowel disorders(16). Prenatal and childhood stress have been suggested to disturb the regulation of immune and autonomic functions(17) that makes children vulnerable to develop both atopy and altered bowel habits.

To better understand the mechanisms playing a role in these disorders, it is warranted to examine which common risk-factors explain the association between atopy and functional bowel disorders as described in the literature. In addition, most of the exposures associated with disease start early in life and the majority of IBS subjects experience symptoms of constipation that are already present during childhood(18). The aim of our study, therefore, is to examine whether wheezing and atopic dermatitis are associated with functional constipation in childhood. A second aim is to assess if and to what extent cow's milk allergy, overweight, indices of infection exposure and parental stress contribute to the association between wheezing, atopic dermatitis and functional constipation in young children.

PATIENTS AND METHODS

Participants and study design

This study was embedded in the Generation R study, a population-based prospective cohort study from foetal life until young adulthood and has been described in detail previously (19-20). In total, 7893 mothers with a delivery date between April 2002 and January 2006 provided consent for follow-up. The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, The Netherlands.

Functional constipation

At the age of 24, 36 and 48 months, stool pattern of the child was assessed by using a questionnaire (response rates: 70%, 64% and 63% respectively). Accordingly, the outcome of functional constipation was defined in this study if at least one of the following symptoms of ROME II(21)were reported: 1) defecation frequency <3 times a week for at least 2 weeks or 2) predominantly hard faeces for the majority of stools for at least 2 weeks.

To avoid the influence of metabolic disorders and clustering, children were excluded in the analyses in case of the following: 1) twinborn (*n*=238), 2) siblings within the Generation R cohort (*n* =343) 3) presence of a congenital heart condition (*n* =47), 4) history of anaemia (*n* =58) or 5) growth retardation defined as height for age < -2SD based on the Netherlands growth curves (*n* =163)(22).

Wheezing and atopic dermatitis

Information regarding atopic dermatitis and wheezing was obtained using core questions from an age-adapted version of the validated "International Study of Asthma and Allergies in Childhood" (ISAAC) and doctor-attendance of eczema or episodes of itchy rash at the age of 24, 36 and 48 months(23). These outcomes were defined dichotomously: presence or absence of eczema and presence or absence of wheezing at 24, 36 and 48 months of age.

Covariates

Weight and height at 24, 36 and 48 months were available from the child health centres. Body Mass index (BMI) was then calculated and overweight was defined according to age- and gender-adapted BMI-thresholds for young children from the International Obesity Task Force(24).

At the age of 6 and 12 months, questionnaire data were available about a history of doctor-attended food allergy in the first year of life and the introduction of food allergens and breast-feeding duration that we described in detail in a previous study (25).

At the age of 24, 36 and 48 months, questionnaire data was available for the following indicators which were used as proxy for infection exposure of the child: attendance of daycare and doctor-attendance for fever (> 38°C) accompanied with symptoms of cough, runny nose, earache, diarrhoea or vomiting in the past year.

The level of parental stress was assessed at the child's age of 24 months by using the Nijmeegse Ouderlijke Stress Index—Kort (NOSIK(26)), the Dutch version of the Parenting Stress Index—Short Form that has been shown to be reliable and valid(27). The NOSIK comprises two domains consisting of 25 items; parenting stress due to parent factors and parenting stress due to child factors. Only the items on the parent domain were available in this study (n=15). Items were assessed on a 4-point scale and the scores were summed and divided by the number of fulfilled items. Higher scores indicate greater levels of parental stress. In addition, these scores were demonstrated to be an important predictor of somatic complaints in this birth cohort (28).

Other variables possibly related to functional constipation, wheezing and atopic dermatitis were obtained from prenatal and postnatal questionnaires filled in by the parents (ethnicity, mother's educational level, maternal and paternal history of atopic dermatitis, asthma, hay fever, allergy to house dust and prenatal smoking). From obstetric records assessed in mid-wife practices and from hospital registries data on gender, birth weight and gestational age were available as described previously(19-20).

Statistical analysis

Firstly, univariate analyses were performed by using Chi-square tests for categorical variables and the Mann-Whitney U test for continuous variables. Secondly, hierarchical logistic regression analyses (29) by using generalized estimating equations (GEE) were performed. GEE analysis assesses the

association by correction for the within subject's dependence as a result of the repeated observations on wheezing, eczema and functional constipation (30). Since the within-subject correlation coefficient for wheezing, atopic dermatitis and functional constipation at the three time points were comparable (r=0.3-0.4), an exchangeable working correlation structure was used in the GEE analyses.

Generalized Estimation equations were performed with functional constipation compared to no constipation as dependent variables and the presence of any wheezing or atopic dermatitis in the past year as independent variable. In addition, model 1 was adjusted for time, and major child characteristics as gender, mother's educational level, ethnicity (western versus non-western), birth weight, gestational age, maternal smoking and family history of atopic disorders (ie. asthma or atopic dermatitis). Subsequently to assess whether the association between functional constipation and atopy was dependent on BMI, infection exposure, food allergy and infant nutrition, or parental stress; the following variables were added separately to model 1: BMI (model 2), daycare attendance and history of respiratory or gastrointestinal infections in the previous year (model 3), history of food allergy, breast-feeding duration, and introduction of food allergens ≤ 6 months (model 4) and NOSIK score (model 5). To test whether the associations between wheezing, atopic dermatitis and functional constipation were different at the age of 24, 36 and 48 months, effect modification by time was evaluated by adding the product-term of wheezing/atopic dermatitis and time (e.g. wheezing*time) as independent variable to the crude GEE model.

Non-response analysis at 48 months showed that non-responders slightly reported more often wheezing at 36 months (17.1% vs. 11.9%, $P<0.01$), functional constipation at 24 and 36 months (18% vs. 11.3% and 23.2 and 16.2% respectively, $P<0.01$), had more often mothers with a lower social economic background (16.6% vs. 5.8% $P<0.01$) and a non-western ethnicity (54.5% vs. 25.2%, $P<0.01$) and smoked more often during pregnancy (77.4% vs. 65.5%$P<0.01$). To reduce potential bias associated with missing data, missing values (approximately 0.1% - 40%) were multiple imputed (n=5 imputed datasets). The multiple imputation was based on the correlation between each variable with missing values with the other subject characteristics as described previously by Sterne et al(31). Data were analyzed in each data set separately to obtain desired effect sizes and standard errors. Finally, the results of the 5 imputed analyses were pooled and reported in this paper as odds ratios (OR) and 95% confidence interval (95%CI). A P-value <0.05 was considered as statistically

significant. Statistical analyses were carried out by using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL).

RESULTS

Characteristics of the study population are presented in table 1. Prevalence of functional constipation remained relatively stable between 12% and 14% at the age of 24, 36 and 48 months. The highest prevalence of wheezing symptoms during childhood was at the age of 24 months (20%) and this decreased to a prevalence of 12% at 36 and 48 months. The prevalence of atopic dermatitis decreased from 30% at the age of 24 months to 20% and 18% at the age of 36 and 48 months respectively. Out of 4651 children, 3-4% had persistent symptoms of functional constipation, atopic dermatitis or wheezing at all time points (i.e. at 24, 36 and 48 months).

At the age of 48 months, functional constipation was more prevalent in girls (16% vs 13%, $P<0.01$), children of non-Western origin (23% vs. 12%, $P<0.01$), children with a history of respiratory tract and gastrointestinal infections in the previous year (12% vs 20% and 14% vs 18%, $P<0.01$) and in children whose mothers who smoked during pregnancy (14% vs 16%, $P<0.01$). Children with functional constipation at 48 months had a lower birth weight (3373 vs 3488 grams, $P<0.01$) and had a higher parental stress score (0.37 vs 0.29, $P<0.01$) than children with no functional constipation.

The distribution of wheezing and atopic dermatitis in children with and without functional constipation between 24 and 48 months is shown in figure 1. Wheezing symptoms were significantly associated with symptoms of functional constipation in childhood but no significant association was found between atopic dermatitis and functional constipation after adjustment for gender, birth weight, gestational age, maternal smoking, maternal educational background, ethnicity and parental history of atopy (Table 2; model 1). The greatest alteration in the effect estimate in the analyses of wheezing and functional constipation was found after adjustment for infection exposure of the child, which mainly explained the association between wheezing and constipation (Table 2; model 3). Adjustment in addition to model 1 for other potential shared risk-factors did not explain the association between wheezing and functional constipation (Table 2).

No effect modification by the different time points (ie. 24, 36 an 48 months) was found for the analyses between wheezing, atopic dermatitis and functional constipation ($P=0.57$ for statistical interaction between wheezing and time and $P=0.43$ for statistical interaction between eczema and time).

DISCUSSION

This study shows that wheezing symptoms and functional constipation frequently coexist in pre-school children but this relationship was markedly influenced by infection exposure of the child. Other potential shared-risk factors as BMI, history of food allergy, infant nutrition, and parental stress did not explain the association between wheezing and constipation.

Several studies in adults showed a link between IBS and asthma (3-6), but results in children are inconsistent so far. In addition, Caffarelli et al. demonstrated no difference in constipation in allergic children compared to controls(8). Also, Simeone et al. showed no association between constipation and atopy in young children(7) whereas a small increase in IBS prevalence over time was observed in subjects with asthma aged 5-65 years(5).

The strength of our study lays in the large study population who were not selected according to medical care, thereby diminishing selection bias. Earlier studies have been carried out in selected populations of patients seeking medical care. This could lead to selection towards for instance children at high risk for atopic constitution that may have a higher proportion of IBS than in the general population. Also, most studies did not take account for potential shared risk-factors that may improperly suggest that the relationship is independent. The broad range of available data of potential confounders in our study elucidates the contribution of shared risk-factors in the association between atopy and functional bowel disorders.

Our study confirmed that the association between wheezing and functional constipation was influenced by infection exposure. Respiratory infections are risk-factors for asthma(32) but gastro-intestinal infections are also associated with IBS(33). It is, therefore, more likely that infection exposure of the child increases the risk of wheezing and constipation rather than that it acts protectively as suggested by other studies. In addition, from animal studies it is known that early exposure to micro-organisms is required to have a proper maturation of the immune system in and outside the gut that may have a protective effect on allergic disease (34). Also, it is speculated that childhood exposure to infection is responsible for low prevalence of IBS in developing countries and that early colonization may enable the intestinal epithelium to respond efficiently to antigenic challenge that may result in a pronounced amelioration of gastro-intestinal symptoms after

infections(35). Further in-depth research exploring the process of colonization may elucidate the contribution of infections in the association between wheezing and constipation.

Underlying mechanisms as altered contractility of smooth muscles cells and dysfunction of the autonomic nervous system that can be triggered by emotional stress is suggested to play a common role in asthma and bowel disorders (15). Since parental stress was demonstrated to be an important predictor of somatic symptoms in children in a previous study (28) we expected a certain explanatory effect of parental stress on the association between wheezing and constipation. However, adjustment for parental stress only slightly weakened the association between wheezing and functional constipation. Nevertheless, it may well be that stress operates at the spectrum of infection exposure of the child since prenatal and postnatal stress can be associated with an increased risk of infectious disease in the offspring (36-37) which itself was thus of major importance in explaining the association between wheezing and constipation.

To appreciate the results some limitations of this study should be considered. We had data on functional constipation by the use of parental derived questionnaires and no additional information from medical records or physical examinations was available. Therefore, some subjects may be misclassified concerning the diagnosis of functional constipation. However, only if this misclassification is also related to wheezing, this misclassification has overestimated our results.

It has been suggested that food allergy in early infancy may be a risk factor for wheezing attacks(38). The limited clarifying effect of food allergy on our study results might be explained by the fact that not all food-sensitized children have manifest food allergy(39). Consequently, sensitization to food allergens, as demonstrated by tests of specific serum IgE, might be a better explanation rather than report of food allergy or timing of introduction of food allergens in the association between atopic disorders and functional constipation. We did not have other evidence of food allergy in the first year available than the parental report of doctor-diagnosed food allergy. It is known that self-report of food allergy overestimates the true prevalence of food allergy. On the other hand, we expect that this information bias may have led to over-adjusted odds ratios and thereby an underestimation of the final results because parents who believe that their child had food allergy may also have reported symptoms related to atopic diseases or constipation more frequently. Nevertheless, bias may occur when parents with high concerns are more likely to report symptoms in their child as wheezing, constipation and infectious disease. Adjustment for NOSIK score may slightly reduce this effect.

However, it only consisted of information on parental stress due to parent factors but not stress due to the child's health status.

At last, previous studies on functional bowel disorders focused on the link with asthma. Early wheezing in infancy is not a major predictor of childhood asthma(32). Hence, our results do not allow for conclusions regarding functional constipation and childhood asthma. Although we took several shared-risk factors into account when analyzing the association between wheezing and constipation, other determinants may also explain the association between asthma and functional bowel disorders in other studies. Recently it has been demonstrated that consumption of fruit and vegetables is associated with a lower prevalence of asthma (40) but also with constipation in children(41). Another symptom that may overlap wheezing with constipation is gastro-oesophageal reflux disease. Gastro-oesophageal reflux disease is a relatively common disorder in infants and children which has been demonstrated to be associated with respiratory symptoms(42) and functional bowel disorders(43). Therefore, explaining the association between other respiratory symptoms and asthma, and functional constipation in both children and adults needs further clarification.

Conclusion

In a population-based setting, functional constipation and wheezing symptoms may coexist in pre-school children. However, this association is not independent and can be mainly explained by infection exposure of the child. Other potential shared risk-factors as BMI, food allergy, infant nutrition and parental stress do not explain this association. Further research should elucidate whether colonization plays a role in the explanatory effect of infectious disease in the association between wheezing and constipation and if these results do also apply to the adult population and to secondary care patients.

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	X
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	X
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	X
Objectives	3	State specific objectives, including any prespecified hypotheses	X
Methods			
Study design	4	Present key elements of study design early in the paper	X
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	X
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	X
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	X
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*X*
Bias	9	Describe any efforts to address potential sources of bias	X
Study size	10	Explain how the study size was arrived at	X
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	X
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	X
		(*b*) Describe any methods used to examine subgroups and interactions	X
		(*c*) Explain how missing data were addressed	X
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	X
		(b) Give reasons for non-participation at each stage	X (non-response Analyse described)
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	X
		(b) Indicate number of participants with missing data for each variable of interest	

		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	Report numbers of outcome events or summary measures over time	X
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	X
		(*b*) Report category boundaries when continuous variables were categorized	X
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	X
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	X
Discussion			
Key results	18	Summarise key results with reference to study objectives	X
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	X
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	X
Generalisability	21	Discuss the generalisability (external validity) of the study results	X
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	X

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Table 1. Maternal and Child Characteristics of the Study Population (N=4651)

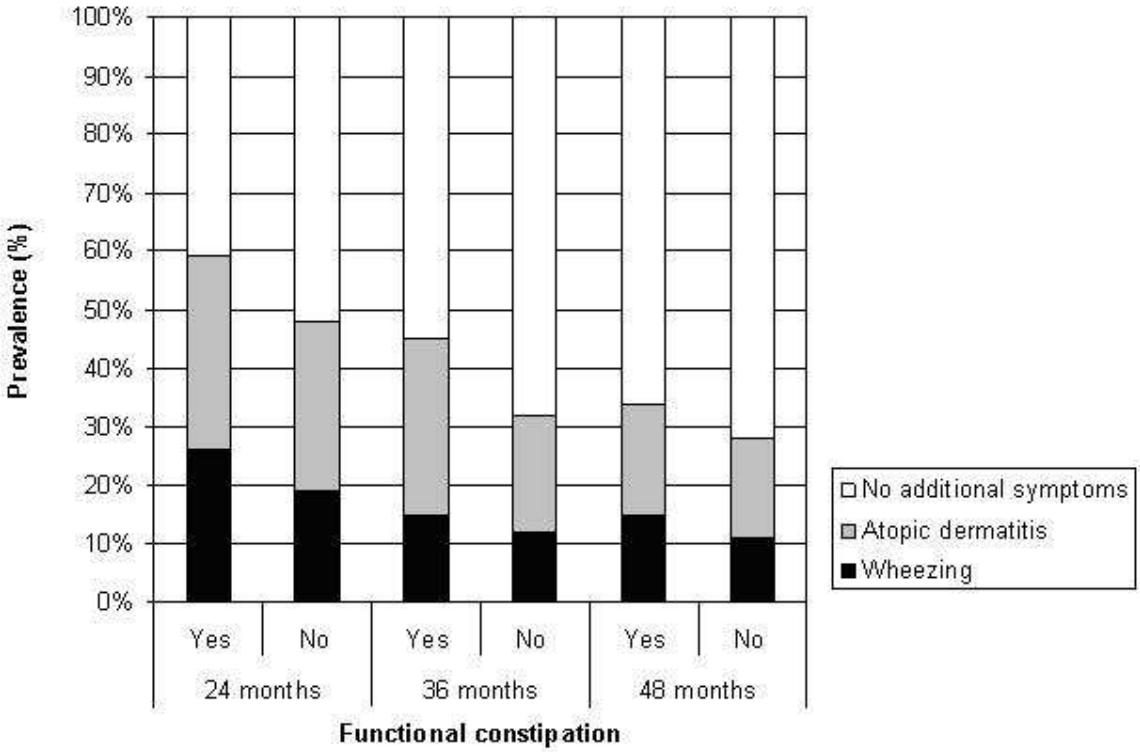
		N (%)
Mother		
Educational level of mother		
	Low	774 (16%)
	Midlow	1365 (29%)
	Midhigh	1148 (25%)
	High	1363 (29%)
Maternal smoking		1154 (25%)
Maternal alcohol consumption		1869 (40%)
Parental stress score mean (SD)		0.36 (0.30)
History of atopy		2238 (48%)
Child		
Male N (%)		2333 (50%)
Ethnicity N (%)		
	Dutch/other Western	3421 (74%)
	Non-Western	1230 (26%)
Birth weight mean (SD)		3431 (540)
Gestational age at delivery mean (SD)		39.9 (1.6)
History of food allergy in first year of life		265 (6%)
Introduction of food allergens ≤ 6 months		3472 (75%)
Duration of breast-feeding in months mean (SD)		4.8 (3.8)
Crèche attendance in first two years of life		3579 (77%)
Overweight in second year of life		915 (20%)

Table 2. Hierarchical logistic models on the association between wheezing, eczema and functional constipation between 24 and 48 months of age (N=4651)

	No constipation	Functional Constipation
Wheezing		**OR 95%CI**
Crude	Reference	1.11 (0.98 – 1.27)
Model 1	Reference	1.16* (1.01 – 1.31)
Model 2 (body mass index)	Reference	1.16* (1.01 – 1.32)
Model 3 (infection exposure)	Reference	1.10 (0.96 – 1.25)
Model 4 (food allergy & infant nutrition)	Reference	1.15* (1.01 – 1.32)
Model 5 (parental stress)	Reference	1.14* (1.00 – 1.30)
Eczema		**OR 95%CI**
Crude	Reference	1.03 (0.91 – 1.16)
Model 1	Reference	1.04 (0.91 – 1.18)
Model 2 (body mass index)	Reference	1.04 (0.91 – 1.18)
Model 3 (infection exposure)	Reference	1.04 (0.91 – 1.19)
Model 4 (food allergy & infant nutrition)	Reference	1.03 (0.60 – 1.18)
Model 5 (parental stress)	Reference	1.03 (0.90 – 1.18)

OR: odds ratio; 95%CI: 95% confidence interval; * $P<0.05$; Model 1: baseline model adjusted for time, gender, mothers educational level, ethnicity, birth weight, gestational age, maternal smoking and family history of atopy; Model 2: model 1+ adjustment for body mass index; Model 3: model 1+ adjustment for history of respiratory tract or gastrointestinal infections in past year and daycare attendance in first two years of life; Model 4: model 1+ adjustment for history of food allergy, breast-feeding duration, and introduction of food allergens ≤6 months; Model 5: model 1+ parental stress.

Figure 1: Distribution of wheezing and atopic dermatitis symptoms in children with and without functional constipation (N=4651)



Association between wheezing and childhood in pre-school children: explanations from a longitudinal birth cohort.

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Is there an association between wheezing and constipation in pre-school children? Explanations from a longitudinal birth-cohort

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Keywords: functional constipation, wheezing, eczema

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Financial disclosure and conflict of interest: none

Abbreviations: IBS, irritable bowel syndrome; AD, atopic dermatitis; ISAAC, international study of asthma and allergies in childhood; BMI, body mass index; NOSIK, nijmeegse ouderlijke stress index—kort (parenting stress index—short form); OR, odds ratio; CI, confidence interval; HPA, hypothalamic pituitary-adrenal

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CONTRIBUTOR'S STATEMENT:

Involvement in the design, planning, conducting the study and data collection: H.A. Moll, V.W.V. Jaddoe, A. Hofman, J.C. de Jongste, J.C. Kiefte-de Jong. Statistical analyses, interpreting data: J.C. Kiefte-de Jong, H.A. Moll, A. Lebon. Drafting the final manuscript: J.C. Kiefte-de Jong, H.A. Moll. All authors critically reviewed the manuscript and approved the final version of the manuscript to be published.

ABSTRACT

Objective: To assess whether wheezing and atopic dermatitis were associated with constipation in pre-school children and to what extent shared risk-factors contribute to this relationship.

Methods: A population-based sample of 4651 pre-school children was used. At the age of 24, 36 and 48 months, parental report of functional constipation was available according to the ROME II criteria and data on atopic dermatitis and wheezing was available using age-adapted questionnaires from the International Study of Asthma and Allergies in Childhood. Stepwise multivariate analyses were performed to assess whether body mass index, infection exposure, food allergy and infant nutrition, and parental stress explained the association between wheezing, atopic dermatitis and constipation.

Results: Out of 4651 children, 12-17% had functional constipation between 24 and 48 months. Symptoms of wheezing and atopic dermatitis decreased from 20% and 30% at 24 months to 10% and 18% at 48 months. Between the age of 24 and 48 months, wheezing symptoms were significantly associated with functional constipation (OR: 1.17; 1.02 – 1.34) but these results were mainly explained by the child's infections exposure and use of antibiotics (aOR: 1.08; 95%CI: 0.95 – 1.24). No significant association was found between symptoms of atopic dermatitis and functional constipation (OR: 1.08; 95%CI: 0.94, 1.23).

Conclusions: These findings suggest that functional constipation co-exists with wheezing in childhood but is mainly explained by the child's infection exposure and use of antibiotics. Therefore, an independent association between respiratory symptoms and functional bowel disorders as suggested in previous studies is questionable.

ARTICLE SUMMARY

Article focus

- Constipation, wheezing and atopic dermatitis are common symptoms in children.
- Functional bowel disorders are linked with asthma and atopy in adults.
- Functional bowel disorders, asthma and atopic disease may share common risk-factors that may explain co-existence of these symptoms.

Key messages

- Wheezing but not atopic dermatitis is associated with functional constipation in pre-school children. The association is mainly explained by a history of infection exposure.
- Hence the association between wheezing and functional constipation is not independent. Further research is needed to explore whether this result also apply to the outcome of asthma.

Strengths

- Population-based study population. The study group were not selected according to medical care.
- This study addresses a topical area that has not had sufficient study and can have a contribution on the discussion how asthma or atopy may be associated with functional bowel disorders.
- This study took multiple shared risk-factors of wheezing and constipation into account that sheds light on the suggested association in literature.

Limitations

- Symptoms were available only from parental-reported questionnaires. This may led to misclassification of the symptoms.
- Early wheezing in infancy is not a sufficient predictor of childhood asthma.

- No data was available regarding parental concerns of the child's health status. Bias may occur when parents with high concerns are more likely to report symptoms in their child as wheezing, constipation and infectious disease.
- No data was available on IgE sensitization, thus the study preclude conclusions on the association between allergic disease and constipation.

INTRODUCTION

Functional constipation and other functional bowel diseases account for the majority of gastrointestinal symptoms worldwide and is increasing in the Western world (1-2). The prevalence of atopic diseases such as asthma and atopic dermatitis is also increasing in Western Countries(3). In adults, several studies have suggested that asthma or atopy may be linked with constipation(4) or other functional gastro-intestinal disorders (5-8). In children, different results with regard to constipation have been described (9-10). Some pathophysiological overlaps may explain the proposed correlation between constipation and atopy. For example, atopic disease, which affects approximately 30% of the population, include food allergies that may cause various organ dysfunction implying that the link between constipation and atopy may be explained by food allergy(11). Likewise, a shared risk-factor for both asthma and constipation is obesity. As reported in several studies, asthma is highly prevalent in children with obesity(12) and obesity has been found to be more common in subjects with functional constipation(13).

Since both atopy and functional bowel disorders are particularly prevalent in developed countries, a role for the 'hygiene hypothesis' in these two disorders is also proposed. The hygiene hypotheses concedes that current hygiene practices lead to reduced or altered exposure of bacteria and other micro-organisms that may result in an imbalance of the immune system and microflora early in life (14). Early microbial colonization of the infant's gastro-intestinal tract might reduce the risk of atopic disorders and altered intestinal flora has been an area of interest for years in both constipation and atopy (15-16). Finally, studies have addressed the role of stress in the aetiology both of atopic disorders(17) and constipation (18). Prenatal and childhood stress have been suggested to disturb the regulation of immune and autonomic functions(19) that makes children vulnerable to develop both atopy and altered bowel habits.

To better understand the mechanisms playing a role in these disorders, it is warranted to examine which common risk-factors explain the association between atopy and constipation as described in the literature. In addition, most of the exposures associated with disease start early in life and the majority of adults with other functional bowel disorders experience symptoms of functional constipation that are already present during childhood (20). The aim of our study, therefore, is to examine whether wheezing and atopic dermatitis are associated with functional constipation in

childhood. A second aim is to assess if and to what extent cow's milk allergy, overweight, indices of infection exposure and parental stress contribute to the association between wheezing, atopic dermatitis and functional constipation in young children.

PATIENTS AND METHODS

Participants and study design

This study was embedded in the Generation R study, a population-based prospective cohort study from foetal life until young adulthood and has been described in detail previously (21-22). In total, 7893 mothers with a delivery date between April 2002 and January 2006 provided consent for follow-up. The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, The Netherlands.

Functional constipation

At the age of 24, 36 and 48 months, stool pattern of the child was assessed by using a questionnaire (response rates: 70%, 64% and 63% respectively). Accordingly, the outcome of functional constipation was defined in this study if at least one of the following symptoms of ROME II(23)were reported: 1) defecation frequency <3 times a week for at least 2 weeks or 2) predominantly hard faeces for the majority of stools for at least 2 weeks.

To avoid the influence of metabolic disorders and clustering, children were excluded in the analyses in case of the following: 1) twinborn (*n*=238), 2) siblings within the Generation R cohort (*n* =343) 3) presence of a congenital heart condition (*n* =47), 4) any history of doctor diagnosis of anaemia, collected by questionnaire (*n* =58) or 5) growth retardation defined as height for age < -2SD based on the Netherlands growth curves (*n* =163)(24).

Wheezing and atopic dermatitis

Information regarding atopic dermatitis and wheezing was obtained on the basis of core questions from "International Study of Asthma and Allergies in Childhood" (ISAAC) and doctor-attendance of eczema or episodes of itchy rash at the age of 24, 36 and 48 months(25) which has been used succesfully in a previous study to predict asthma (26). In addition, the following questions were asked at the age of 24, 36 and 48 months: “Has your child had problems with a wheezing chest during the last year?” and “Has your child had an itchy rash that came and went during the past year?” These outcomes were analyzed dichotomously: presence or absence of eczema and presence or absence of wheezing in the previous year at 24, 36 and 48 months of age.

Covariates

Weight and height at 24, 36 and 48 months were available from the child health centres. Body Mass index (BMI) was then calculated and overweight was defined according to age- and gender-adapted BMI-thresholds for young children from the International Obesity Task Force(27).

At the age of 6 and 12 months, questionnaire data were available about a history of doctor-attended food allergy in the first year of life and the introduction of food allergens and breast-feeding duration that we described in detail in a previous study (28). Briefly, parents were asked at what age they had introduced the following products in the infant's diet for the first time: milk, yoghurt, porridge, egg, bread or biscuits, peanuts, nuts and soy products. The reported introduction of these food products were cross-checked with a short food-frequency questionnaire filled in at the children's age of 6 and 12 months consisting of food-products frequently consumed according to a Dutch food consumption survey in infants. For example, if the parent indicated at the age of 12 months that they had never introduced gluten in their infant's diet but at the infant's age of 6 months the parent filled in that the infant consumed porridge based on wheat for at least once, then the introduction of this gluten was considered to be before or equal to 6 months of age. Breast-feeding duration was assessed according to five variables: ever breast-feeding, cessation of breast-feeding and receiving any breast-feeding at the age of 2, 6 and 12 months.

At the age of 24, 36 and 48 months, questionnaire data was available for the following indicators which were used as proxy for infection exposure of the child: attendance of daycare and doctor-attendance for fever (> 38°C) accompanied with symptoms of cough, runny nose, earache, diarrhoea or vomiting in the past year, and any use of antibiotics in the previous year (yes vs no).

The level of parental stress was assessed at the child's age of 24 months by using the Nijmeegse Ouderlijke Stress Index—Kort (NOSIK(29)), the Dutch version of the Parenting Stress Index—Short Form that has been shown to be reliable and valid(30). The NOSIK comprises two domains consisting of 25 items; parenting stress due to parent factors and parenting stress due to child factors. Only the items on the parent domain were available in this study (n=15 items on partenal stress due to parent factors). Items were assessed on a 4-point scale and the scores were summed and divided by the number of fulfilled items. Higher scores indicate greater levels of parental stress. In addition, these scores were demonstrated to be an important predictor of somatic complaints in this birth cohort (31).

Other variables possibly related to functional constipation, wheezing and atopic dermatitis were obtained from prenatal and postnatal questionnaires filled in by the parents (ethnicity, mother's educational level, maternal and paternal history of atopic dermatitis, asthma, hay fever, allergy to house dust and prenatal smoking). From obstetric records assessed in mid-wife practices and from hospital registries data on gender, birth weight and gestational age were available as described previously(21-22).

Statistical analysis

Firstly, univariate analyses were performed by using Chi-square tests for categorical variables and the Mann-Whitney U test for continuous variables. Secondly, logistic regression analyses by using generalized estimating equations (GEE) were performed. Briefly, regression analysis by GEE assesses the association between two variables by correction for the within subject's dependence as a result of the repeated observations on wheezing, eczema and functional constipation since repeated measurements within one individual are frequently correlated (32). The within-subject correlation coefficient for wheezing, atopic dermatitis and functional constipation at the three time points were comparable (r=0.3-0.4). Therefore, an exchangeable working correlation structure was used in the GEE analyses as adjustment for the dependency between the repeated measurements . Logistic regression analysis with GEE was performed with functional constipation compared to no constipation as dependent variables and the presence of any wheezing or atopic dermatitis in the past year as independent variable. In addition, model 1 was adjusted for time (i.e. age), and major child characteristics as gender, mother's educational level, ethnicity (western versus non-western), birth weight, gestational age, maternal smoking and family history of atopic disorders on the basis factors associated with asthma-like symptoms and constipation in literature(28, 33) . Subsequently, to assess whether the association between functional constipation and atopy was dependent on BMI, infection exposure, food allergy and infant nutrition, or parental stress; the following variables were added separately to model 1: BMI (model 2), daycare attendance, use of antibiotics in previous year, and history of respiratory or gastrointestinal infections in the previous year (model 3), history of food allergy, breast-feeding duration, and introduction of food allergens ≤ 6 months (model 4), and parental stress score (NOSIK) (model 5). To test whether the associations between wheezing, atopic dermatitis and functional constipation were different at the age of 24, 36 and 48 months, effect

modification by time was evaluated by adding the product-term of wheezing/atopic dermatitis and time (e.g. wheezing*time) as independent variable to the crude GEE model.

To reduce potential bias associated with missing data, missing values (approximately 0.1% - 40%) were multiple imputed (*n*=5 imputed datasets). The multiple imputation was based on the correlation between each variable with missing values with the other subject characteristics as described previously by Sterne et al(34). Data were analyzed in each data set separately to obtain desired effect sizes and standard errors. Finally, the results of the 5 imputed analyses were pooled and reported in this paper. In addition, the pooled odds ratio (OR) was calculated by taking the average of the OR's of the 5 imputed datasets. The pooled standard error to assess the 95% confience intervals (95%CI) was then calculated by using Ruben's rule(35): $\sqrt{[W+(1+1/m)*B]}$ with W= mean variance of the effect size within the imputed datasets; B=variance of the effect sizes between the imputed datasets; m= number of imputed datasets (n=5).A *P*-value <0.05 was considered as statistically significant. Statistical analyses were carried out by using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL).

RESULTS

Characteristics of the study population are presented in table 1.

Non-response analysis at 48 months showed that non-responders slightly reported more often wheezing at 36 months (17.1% vs. 11.9%, *P*<0.01), functional constipation at 24 and 36 months (18% vs. 11.3% and 23.2 and 16.2% respectively, *P*<0.01), had more often mothers with a lower social economic background (16.6% vs. 5.8% *P*<0.01) and a non-western ethnicity (54.5% vs. 25.2%, *P*<0.01) and smoked more often during pregnancy (77.4% vs. 65.5%, *P*<0.01).

Prevalence of functional constipation remained relatively stable between 12% and 14% at the age of 24, 36 and 48 months. The highest prevalence of wheezing symptoms during childhood was at the age of 24 months (20%) and this decreased to a prevalence of 12% at 36 and 48 months. The prevalence of atopic dermatitis decreased from 30% at the age of 24 months to 20% and 18% at the age of 36 and 48 months respectively. Out of 4651 children, 3-4% had persistent symptoms of functional constipation, atopic dermatitis or wheezing at all time points (i.e. at 24, 36 and 48 months).

At the age of 48 months, functional constipation was more prevalent in girls (16% vs 13%, *P*<0.01), children of non-Western origin (23% vs. 12%, *P*<0.01), children with a history of respiratory tract and gastrointestinal infections in the previous year (12% vs 20% and 14% vs 18%, *P*<0.01) and in children whose mothers who smoked during pregnancy (14% vs 16%, *P*<0.01). Children with functional constipation at 48 months had a lower birth weight (3373 vs 3488 grams, *P*<0.01) and had a higher parental stress score (0.37 vs 0.29, *P*<0.01) than children with no functional constipation.

The distribution of wheezing and atopic dermatitis in children with and without functional constipation between 24 and 48 months is shown in figure 1. Wheezing symptoms were significantly associated with symptoms of functional constipation in childhood but no significant association was found between atopic dermatitis and functional constipation in univariate analyses (Table 2). After adjustment for age, gender, birth weight, gestational age, maternal smoking, maternal educational background, ethnicity and parental history of atopy, wheezing remained significantly associated with functional constipation (Table 2; model 1), which was also the case after stepwise adjustment for BMI, history of food allergy and infant nutrition and parental stress (Table 2; model 2, 4 and 5). The greatest alteration in the effect estimate in the analyses of wheezing and functional constipation was found after adjustment for infection exposure and use of antibiotics of the child, which mainly

explained the association between wheezing and constipation (Table 2; model 3). Adjustment in addition to model 1 for other potential shared risk-factors did not explain the association between wheezing and functional constipation (Table 2).

The results between wheezing and constiaption did not significantly differ by the different time points of 24, 36 an 48 months (P=0.57 for statistical interaction between wheezing and age and P=0.43 for statistical interaction between eczema and age).

DISCUSSION

This study shows that wheezing symptoms and functional constipation frequently coexist in pre-school children but this relationship was markedly influenced by infection exposure of the child. Other potential shared-risk factors as BMI, history of food allergy, infant nutrition, and parental stress did not explain the association between wheezing and constipation.

Several studies in adults showed a link between functional bowel disorders and asthma (5-8), but different results in children are described so far. In addition, Caffarelli et al. demonstrated no difference in constipation in allergic children compared to controls(36) but reported that other gastrointestinal symptoms as diarrhoea and vomiting are more prevalent in children with atopic eczema than in controls(10). Also, Simeone et al. showed no association between constipation and atopy in young children(9) whereas a small increase in the prevalence irritable bowel syndrome over time was observed in subjects with asthma aged 5-65 years(7). Yet, Leander et al(4) showed recently that constipation was associated with asthma independently of age (9)

The strength of our study lays in the large study population who were not selected according to medical care, thereby diminishing selection bias. Earlier studies have been carried out in selected populations of patients seeking medical care. This could lead to selection towards for instance children at high risk for atopic constitution that may have a higher proportion of functional bowel disorders than in the general population. Also, most studies did not take account for potential shared risk-factors that may improperly suggest that the relationship is independent. The broad range of available data of potential confounders in our study elucidates the contribution of shared risk-factors in the association between atopy and functional bowel disorders.

Our study confirmed that the association between wheezing and functional constipation was influenced by infection exposure. This finding can be explained in several ways. First, respiratory infections are risk-factors for wheezing and asthma (26) but gastro-intestinal infections are also associated with constipation (37). During for example an influenza-like ilnesses, both respiratory and gastrointestinal symptoms may co-exist (38-39) and may clarify that our association wheezing and constipation is explained by history of infections as defined by fever acompanied with diarrhoea or respiratory symptoms. Second, from animal studies it is known that very early exposure to micro-organisms, for example by crowding or infections earlier in life, is required to have a proper maturation

of the immune system in and outside the gut which may have a protective effect on allergic disease (40). Also, it is speculated that frequent exposure to childhood infections is responsible for the low prevalence of functional bowel disorders in developing countries and that early colonization may enable the intestinal epithelium to respond efficiently to antigenic challenge that may result in a pronounced amelioration of gastro-intestinal symptoms after infections (41). However, if the latter was the case in our study, we would expect that adjustment for infection exposure would make the association between wheezing and constipation even stronger instead of weaker. Further in-depth research exploring the process of colonization may elucidate the contribution of infections and exposure to microorganisms in the association between wheezing and constipation.

Underlying mechanisms as altered contractility of smooth muscles cells and dysfunction of the autonomic nervous system that can be triggered by emotional stress is suggested to play a common role in asthma and constipation (17-18). Since parental stress was demonstrated to be an important predictor of somatic symptoms in children in a previous study (31) we expected a certain explanatory effect of parental stress on the association between wheezing and constipation. However, adjustment for parental stress only slightly weakened the association between wheezing and functional constipation. Nevertheless, it may well be that stress operates at the spectrum of infection exposure of the child since prenatal and postnatal stress can be associated with an increased risk of infectious disease in the offspring (42-43) which itself was thus of major importance in explaining the association between wheezing and constipation.

To appreciate the results some limitations of this study should be considered. We had data on functional constipation by the use of parental derived questionnaires and no additional information from medical records or physical examinations was available. Therefore, some subjects may be misclassified concerning the diagnosis of functional constipation. However, only if this misclassification is also related to wheezing, this misclassification has overestimated our results.

Loss to follow-up is a common phenomenon in large birth-cohort studies. Relative to other birth cohort studies, the particpation rate of our study was high (60-70% in our study group versus 30-40% in other studies)(44). As in most other studies, the non-response analyses showed that the participation in our study was selective. Nevertheless, this would only have influenced our results when the association between wheezing and constipation would be completely different among participants of this study relative to those who did not participate(44). It has been suggested that food allergy in early

infancy may be a risk factor for wheezing attacks(45). The limited clarifying effect of food allergy on our study results might be explained by the fact that not all food-sensitized children have manifest food allergy(46). Consequently, sensitization to food allergens, as demonstrated by tests of specific serum IgE, might be a better explanation rather than report of food allergy or timing of introduction of food allergens in the association between atopic disorders and functional constipation. We did not have other evidence of food allergy in the first year available than the parental report of doctor-diagnosed food allergy. It is known that self-report of food allergy overestimates the true prevalence of food allergy. On the other hand, we expect that this information bias may have led to over-adjusted odds ratios and thereby an underestimation of the final results because parents who believe that their child had food allergy may also have reported symptoms related to atopic diseases or constipation more frequently. Nevertheless, bias may occur when parents with high concerns are more likely to report symptoms in their child as wheezing, constipation and infectious disease. Adjustment for NOSIK score may slightly reduce this effect. However, it only consisted of information on parental stress due to parent factors but not stress due to the child's health status.

At last, previous studies on functional bowel disorders focused on the link with asthma and allergic disease. Early wheezing in infancy is not a major predictor of childhood asthma (26) and we did not have data on allergic sensitization as measured by IgE. Hence, our results do not allow for conclusions regarding functional constipation and childhood asthma and allergic disease. Although we took several shared-risk factors into account when analyzing the association between wheezing and constipation, other determinants may also explain the association between asthma and functional bowel disorders in other studies. Recently it has been demonstrated that consumption of fruit and vegetables is associated with a lower prevalence of asthma (47) but also with constipation in children(48). Another symptom that may overlap wheezing with constipation is gastro-oesophageal reflux disease. Gastro-oesophageal reflux disease is a relatively common disorder in infants and children which has been demonstrated to be associated with respiratory symptoms(49) and functional bowel disorders(50). Therefore, explaining the association between other respiratory symptoms and asthma, and functional constipation in both children and adults needs further clarification.

Conclusion

In a population-based setting, functional constipation and wheezing symptoms may coexist in pre-school children. However, this association is not independent and can be mainly explained by infection exposure of the child and antibiotic use. Other potential shared risk-factors as BMI, food allergy, infant nutrition and parental stress do not explain this association. Further research should elucidate whether colonization plays a role in the explanatory effect of infectious disease in the association between wheezing and constipation and if these results do also apply to the adult population and to secondary care patients.

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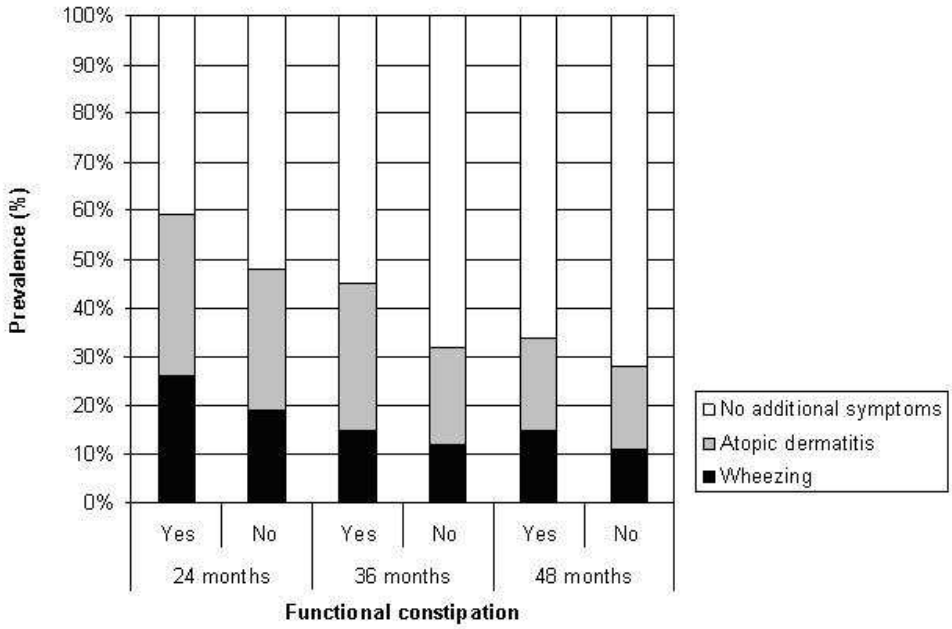
Figure 1: Distribution of wheezing and atopic dermatitis symptoms in children with and without functional constipation (N=4651)



54x43mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	X
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	X
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	X
Objectives	3	State specific objectives, including any prespecified hypotheses	X
Methods			
Study design	4	Present key elements of study design early in the paper	X
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	X
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	X
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	X
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*X*
Bias	9	Describe any efforts to address potential sources of bias	X
Study size	10	Explain how the study size was arrived at	X
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	X
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	X
		(*b*) Describe any methods used to examine subgroups and interactions	X
		(*c*) Explain how missing data were addressed	X
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	X
		(b) Give reasons for non-participation at each stage	X (non-response Analyse described)
		(c) Consider use of a flow diagram	X
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	X
		(b) Indicate number of participants with missing data for each variable of interest	X

		(c) Summarise follow-up time (eg, average and total amount)	X
Outcome data	15*	Report numbers of outcome events or summary measures over time	X
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	X
		(*b*) Report category boundaries when continuous variables were categorized	X
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	X
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	X
Discussion			
Key results	18	Summarise key results with reference to study objectives	X
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	X
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	X
Generalisability	21	Discuss the generalisability (external validity) of the study results	X
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	X

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.