Risk Factors and Treatment of Paediatric Chronic Diseases

Type 1 diabetes, Asthma and Allergy

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Risicofactoren en Behandeling van Pediatrische Chronische Ziekten
Type 1 diabetes, Astma en Allergie

(met een samenvatting in het Nederlands)

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"The sun will shine tomorrow"

TABLE OF CONTENTS

Chapter 1 General introduction

A chronic disease is defined as any physical, emotional, or mental condition that prolongs for at least three months, affects a child's normal activities and requires frequent hospitalizations[1,2]. One out of four children in the United States suffers from a chronic disease which accounts for 15-18 million children younger than 17 years of age[3,4]. The impact of chronic diseases on patients, their families, and societies is tremendous. They adversely affect quality of life and increase the rate of comorbidities and mortality[5-7]. In the last decades major progress in diagnosis and treatment of pediatric chronic disorders has been realized. Improved screening and diagnostic tests leading to early detection, proper diagnosis based on updated guidelines, and improved drug therapy have led to that chronic illnesses can now be effectively treated. High rate of children with chronic diseases that survive childhood is a consequence of progress in clinical care[1,2].

TYPE 1 DIABETES (T1DM) IN CHILDREN

Trend in epidemiology of T1DM

T1DM is one of the most common autoimmune disorders, with a worldwide estimated prevalence rate of 497,000/1.9 billion children aged 1-14 years in 2013[8] . The incidence rate of T1DM in children is increasing especially in those younger than 15 years of age. Although there are geographic differences in reported trends (**Table 1**), the increasing trend is estimated as being around 3% annually since the 1980s[8,9].

Table 1. Top ten countries for number of children with T1DM (<15 years) in 2015; ***Source: IDF Diabetes Atlas. 2015***

Rank	Country/territory	Number of children with T1DM
1	United States	84,100
2	India	70,200
3	Brazil	30,900
4	China	30,500
5	United Kingdom	19,800
6	Russian Federation	18,500
7	Saudi Arabia	16,200
8	Germany	15,800
9	Nigeria	14,400
10	Mexico	13,500

Abbreviation: T1DM: type 1 diabetes mellitus

Risk factors related to T1DM

Susceptibility of T1DM results from interactions between genetic disease susceptibility and environmental factors as diabetogenic triggers or potentiators of beta-cell destruction[10,11]. HLA class II genes on chromosome 6 are the most important genes for T1DM; accounting for nearly 50% of the genetic contribution to the disease[12,13].

It has been suggested that the disease process leading to overt T1DM is triggered by an infectious agent in which the strongest candidate is a diabetogenic enterovirus[11]. Lack of vitamin D supplementation in infancy[14] and infant's age at introduction to certain complementary foods have also been associated with the risk of T1DM[15]. Prospective studies in patients with T1DM have shown that there are distinct identifiable stages prior to the onset of disease; including stage I defined as the presence of islet autoantibodies, stage II as the presence of b-cell autoimmunity with dysglycemia and stage III as onset of symptomatic disease[16]. Observational studies are needed to better understand the stages prior to the onset of diabetes and the risk factors involved.

Glycaemic control in T1DM

Diabetes is a complex disease that requires continuous medical care as well as multifactorial risk reduction strategies. Poor glycemic control is defined as glycosylated hemoglobin (HbA1c) >7.5% (58 mmol/mol) which is a determinant for CVD risk factors[17]. The Diabetes Control and Complications Trial (DCCT) clearly showed the benefits of good glycemic control to prevent micro and macrovascular complications in patients with T1DM[18,19]. Observational studies have also shown that only a minority of individuals with diabetes achieves good glycemic control[20,21]. The average HbA1c in the pediatric population is 8.3 ± 1.6 %[22], while it is strongly recommended for diabetic patients to keep HbA1c<7.5%[23].

Comorbidities and medication utilization related to T1DM

Children with T1DM are typically at increased risk of serious health problems and complications e.g. psychiatric comorbidity[24] and cardiovascular disease (CVD) risk factors[25] (e.g. hypertension and hypercholesterolemia). CVD is a long-term complication of T1DM however, manifestation of CVD risk factors including hypertension and dyslipidemia normally starts in early life. Duration of diabetes, child's age, gender, and race/ethnicity are shown to be potential effect modifiers in the association of T1DM and higher rate of CVD[26,27]. Patients with childhood-onset T1DM have a higher mortality rate compared with the general population where the leading cause of death is acute diabetic complications; corresponding to 26.1% of all deaths. Death caused by CVD also is the major cause of death after the age of 30 years (33.6%)[28].

Higher prevalence rates of CVD risk factors in children with T1DM have been shown by previous studies, however the use of CV medication to treat these risk factors is low in this population[29-33]. The available guidelines advice to start treatment in diabetic patients with CVD risk factors, however longitudinal data on efficacy of CV drugs in this population is limited[34,35]. Information on the utilization pattern of CV medication use in children with T1DM is limited. Therefore, it is important to quantify the use of these medications to evaluate whether and what improvements are necessary in daily clinical practice.

It has also been reported that children with T1DM have an abnormal lung function e.g. lower forced vital capacity (FVC) and forced expired volume in 1s (FEV1C) compared with an age and gender matched general population[36,37]. However, the link between T1DM and asthma in children has been studied with controversial results. Some studies showed a

significant lower risk of asthma in children with T1DM[38,39], or higher risk of asthma[40] or no association[41]. So far, no study has quantified asthma medication use in children with T1DM before and after the onset of diabetes. Quantification of asthma medication use can provide further insight into the relation between T1DM and impaired lung function or asthma in this population.

ASTHMA AND ALLERGY IN CHILDREN

Trend in epidemiology of asthma/allergy

Worldwide prevalence rates of allergic diseases e.g. asthma, allergic rhinitis, eczema and food allergy are increasing[42]. Respiratory allergic diseases including asthma and allergic rhinitis affect almost 700 million subjects throughout the world. Asthma is the 14th most important disorder in terms of the duration of disability in the world with 334 million people affected. Almost 1–18% of the population in different countries suffers from asthma[43]. Fourteen percent of the global children population experience asthma symptoms and the burden of asthma is highest for children aged 10-14 compared to other age categories including 1-4, 5-9 and 15-19 years[42].

The International Study of Asthma and Allergies in Childhood (ISAAC) showed geographic variations in the prevalence rate of asthma symptoms even within genetically similar groups which represents the strong effect of environmental factors. In some countries, the prevalence rate of childhood asthma is close to 35%–40%, whereas in other countries it is reported as less than 5%[44,45]. Lai CK et al. has studied the global variation in the prevalence and severity of asthma symptoms in phase III of the ISAAC which is a cross-sectional questionnaire survey including 388,811 children aged 6–7 years from 144 centers in 61 countries between 2000 and 2003, and 798,685 children aged 13–14 years from 233 centers in 97 countries[44]. Prevalence rates of asthma symptoms in children aged 6–7 year by region has been shown in **Table 2**. As shown, the global asthma prevalence rate in children aged 6-7 years is 9.4% where the rate of asthma exacerbations is 4.9% in this population. These rates are 12.6% and 6.9% in children aged 13-14 years, respectively.

Table 2. Prevalence rate of asthma symptoms in children aged 6-7 years by regions; ***Source: C K W Lai, 2009***

Regions	Children, n	Year	Asthma, n (%)	Severe asthma, n (%)
Africa	5,876	2003	202 (3.4)	532 (9.1)
Asia	60,052	2000	6,572 (10.9)	1,893 (3.2)
Eastern Mediterranean	40,573	2000	2,823 (7.0)	1,920 (4.7)
Indian sub-countries	50,106	2002	2,271 (4.5)	1,766 (3.5)
Latin America	93,851	2002	10,495 (11.2)	7,289 (7.8)
North America	4,014	2002	803 (20.0)	283 (7.1)
Northern and Eastern Europe	42,583	2001	1,719 (4.0)	1,350 (3.2)
Oceania	13,888	2002	4,053 (29.2)	1,318 (9.5)
Western Europe	77,868	2002	7,536 (9.7)	2,826 (3.6)

Risk factors related to asthma and allergy

Asthma and allergy/atopy are common conditions with complex etiologies involving both genetic and environmental factors. Associations with variation in genes such as IL1RL1 gene encoding the IL-33 receptor and interleukin-33 (IL33) highlight the important roles for innate immune response pathways that promote the activation of T-helper 2 (Th2) cells in the pathogenesis of both asthma and allergic diseases[46].

Although, it is clear that genetics has an effect on the development of allergic disease the effect is small and only a small proportion of the prevalence rate in asthma can be explained by genetic factors[47]. It is also obvious that environmental factors play a significant role in this association. Several host risk factors have been related to the incidence of asthma and wheeze in children e.g. gender, parental history of asthma and eczema[48]. Additionally, epidemiological studies in adults have shown a modest association between overweight or obesity and the risk of asthma/exacerbations[49,50] but investigations in children have led to conflicting results[51-53].

Heterogeneity of asthma prevalence rate exists at multiple levels including clinical and inflammatory heterogeneity and genetic factors that contribute to asthma risk as previously discussed by Huang YJ. et al.. We should now move on to the gut microbiome role in the development of asthma/allergy[54].

Novel insight into gut microbiome and the association with asthma and allergy

Microbial gut colonization starts usually at the time of birth. It has been suggested that gut microbiome is influenced by environmental factors such as maternal microbiome which is changed based on the feeding practices (formula feeding vs. breast-feeding), and antibiotics consumption[55-57]. Gut microbiome diversity during the first month of life, which is the critical period of microbiome development[58], influences immune function either in maintaining a very well-balanced immune response between T-helper (Th)-1 and Th2 cells or in terms of its function. The composition of healthy gut microbiome is diverse and it can be altered by environmental factors e.g. bacterial infections, antibiotic therapy and lifestyle in early infancy [58-61]. It is known that Th2 cells contribute to the development of and maintenance of allergic responses, while Th1 cells contribute in the modulation of cell-mediated immunity[54,58]. Therefore, there might be a causal pathway linking early life exposure to environmental factors to the development of asthma and allergy: firstly, environmental exposures shape the composition of gut microbiome, secondly gut microbiome shape the pattern of immune function, and finally differences in immune function shape the nature of responsiveness to allergens encountered[54].

The impact of factors influencing the gut microbiome and immune system has been studied in many studies. The associations of early life environmental exposures and increased risk of asthma and allergies is controversial[62].

Complications related to asthma and allergy

Severe asthma is defined as a condition in which children with exacerbations do not adequately respond to the medication and continue to experience respiratory distress despite treatment[63]. Severe asthma is the most common medical emergency in children

with asthma in which is responsible for annually half a million admissions to the pediatric intensive care in the US[64].
Allergic and immunologic response associated with immunoglobulin E (IgE) mediated hypersensitivity is not always a stand-alone problem. Allergy might increase the risk of other medical problems, e.g. anaphylaxis, asthma, sinusitis, otitis and fungal complications such as allergic fungal sinusitis[65].

THESIS OBJECTIVE

The main aims of this thesis were to study:

1. *Comorbidities and co-medication use in childhood T1DM including CVD risk factors, and asthma*
2. *Risk factors (genetic and environmental) associated with the occurrence of childhood asthma/asthma exacerbations/allergy*

THESIS OUTLINE

This thesis explored childhood chronic comorbidities including T1DM, asthma and allergy, and focused on risk factors and treatment according to most recent guidelines.
In ***Chapter 2***, we studied the trends in prevalence and incidence rates of CVD risk factors (hypertension and hypercholesterolemia), CVD and CV medications use in children with T1DM using two different databases; PHARMO in the Netherlands (***Chapter 2.1)*** and CPRD in the UK (***Chapter 2.2***). We studied asthma medication use and asthma exacerbations among children with T1DM to study the link between asthma and T1DM (***Chapter 2.3***).
In ***Chapter 3***, we assessed the role of genetic factors (***Chapter 3.1***) and environmental factors on asthma and allergy; the role of breast-feeding and early life antibiotic exposure (as markers for influence on the gut microbiome) on later asthma onset/asthma exacerbations and allergy were assessed (***Chapters 3.2 & 3.3 & 3.4***). In ***Chapter 3.5***, we explored childhood obesity and the association with asthma severity in children with asthma.
In ***Chapter 4***, the general discussion, we discussed our key findings in comparison with literature. Furthermore, the strengths and limitations of the studies in this thesis were described. And finally, we discussed implications for clinical practice and for future research.

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Chapter 2

Trends in comorbidities related to type 1 diabetes in children

Chapter 2.1

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Cardiovascular medication use and cardiovascular disease in children and adolescents with type 1 diabetes: a population-based cohort study

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ABSTRACT

Objectives: To investigate the 5-years prevalence and incidence rates of cardiovascular medication and cardiovascular disease before and after onset of type 1 diabetes mellitus (T1DM) in children and adolescents.

Methods: Children and adolescents (<19 years) with T1DM (n=925), defined as at least two insulin prescriptions, and a four times larger reference cohort (n=3,591) with the same age and gender in the Dutch PHARMO record linkage system were studied in a retrospective cohort study between 1999 and 2009. The date of first insulin dispensing was selected as the index date.

Results: The overall prevalence rate of cardiovascular medication use was substantially higher in the T1DM cohort before (2.2% vs. 1.0%, p<0.001) and after (9.2% vs. 3.2%, p<0.001) the index date. After the index date angiotensin converting enzyme inhibitors (2.0%) and statins (1.5%) were the most prevalent cardiovascular medications in the T1DM cohort. The highest incidence rate of cardiovascular medication use was observed in the first year after the index date (28.1 per 1000 person years)**.** Furthermore, three type 1 diabetic patients were hospitalized due to cardiomyopathy (n=2) and heart failure (n=1) and one child from the reference group was hospitalized due to cardiomyopathy in the five years after the index date.

Conclusions: Children with T1DM were more likely to use cardiovascular medications in the years before and after the onset of diabetes. Our study emphasizes the importance of routine screening tests and timely treatment of CVD risk factors in the pediatric population with diabetes.

INTRODUCTION

It is well established that type 1 diabetes mellitus (T1DM) is associated with an increased risk of cardiovascular disease (CVD) that already starts in childhood[1]. CVD risk factors such as hyperlipidemia, hypertension and diabetes cause damage to arterial vessels due to atherosclerosis. This is a process that develops over many years, starting in childhood and manifesting as coronary artery disease and stroke in adulthood[2]. Children and adolescents with T1DM have a higher prevalence of CVD risk factors such as hypertension and hyperlipidemia compared with children without diabetes [3-7]. In line with this, they have a higher risk of cardiovascular (CV) abnormalities such as vascular endothelial and smooth muscle dysfunction, arterial stiffness and increased carotid intima media thickness [8-11]. Furthermore, they have a six-fold higher risk of prolonged heart rate corrected QT interval (≥450 milliseconds) [12-15].

The available guidelines [16,17] advice to start treatment in diabetic patients with CVD risk factors, however there is a lack of longitudinal data on efficacy of CV drugs in children with T1DM[1]. Previous studies have shown beneficial effects of angiotensin converting enzyme (ACE) inhibitors in adolescents with microalbuminuria [18-22], but data on cholesterol lowering therapy in this population is limited [23,24]. The results of the Adolescent T1DM cardio-renal intervention trial (AdDIT) which is an ongoing multi-center, randomized, double-blind, placebo-controlled trial will provide important data on the potential renal and CV protective effects of ACEI and statins in high-risk adolescents [25,26].

Given this situation, it is of interest to study the use of CV medication and disease in type 1 diabetic children in daily clinical practice. Therefore, the aim of our study was to calculate the prevalence and incidence rates of CV medication use and the incidence rates of hospital admissions for CVD in children and adolescents with T1DM before and after the diagnosis of diabetes and to compare these rates with a group of age- and sex-matched diabetes-free children and adolescents in the Netherlands.

METHODS

Setting

Data for this retrospective cohort study was obtained from the Dutch PHARMO Record Linkage System (RLS) (http://www.pharmo.nlhttp://www.pharmo.nl). PHARMO RLS is a population-based patient centric data network including high quality and complete information linked on a patient level of, among other data, patient demographics, drug dispensing records from community pharmacies and hospital discharge records of more than four million individuals throughout the Netherlands (approximately 24% of the Dutch population) [27,28]. The drug-dispensing database contains detailed information on the dispensed drug, the type of prescriber, the dispensing date, the amount dispensed, and the written dose instructions. The hospital records are obtained from the Dutch National Medical Register (LMR), which comprises all hospital admissions in the Netherlands. Date of hospital admissions and discharges, together with primary and secondary diagnoses, are

documented in the hospital records. Diagnoses are coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) (http://icd-9cm.chrisendres.com), whereas the drugs are coded according to the Anatomical Therapeutic Chemical codes (ATC codes) (http://www.whocc.no/atc_ddd_index).

Study population

A population-based cohort study was conducted within PHARMO RLS. The T1DM cohort consisted of children and adolescents younger than 19 years of age that received at least two insulin prescriptions (based on the ATC codes for insulin preparations [A10A]) between 1999 and 2009 [29]. The date of the first insulin dispensing was selected as the cohort entry date (index date). Up to four diabetes-free children and adolescents from the PHARMO RLS (without any prescription of glucose lowering medications (ATC code: A10) or hospitalization for diabetes (ICD-9-CM code: 250) during the study period were matched to each child in the T1DM cohort by gender, age, and calendar time distribution (reference cohort). Patients in both cohorts were eligible for inclusion in the study if they had at least 12 months of exposure history before and at least 12 months follow- up after the index date. Patients in the T1DM cohort were excluded in case of ever use of oral anti-diabetic agents or use of glucagon prior to insulin.

Glucagon is mainly used to treat diabetic patients for the management of hypoglycemia. Therefore the index date was not clear for those patients who had a prescription of glucagon before starting insulin therapy. For both cohorts, data for a maximum of five years before and after the index date were retrieved.

CV medication use and hospital admissions due to CVD

For both cohorts, exposure to CV medication was defined as a recorded receipt of a prescription for CV medication categorized into the following groups: cardiac drugs (C01), anti-hypertensive drugs (C02) including ((diuretics (C03), peripheral vasodilators (C04), beta-blocking agents (C07), calcium channel blockers (C08), agents acting on the renin-angiotensin system (C09)), lipid modifying agents (C10), and anti-thrombotic agents (B01) as listed in **Table S1**. Children and adolescents who received at least one prescription for CV medication during the study period were defined as CV medication users. All hospital admissions due to CVD were extracted from the database using ICD-9-CM codes as listed in **Table S2**.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of both cohorts. Overall prevalence rates of CV medication use in the period before and after the index date were calculated for both cohorts. Prevalence rate ratios (PRRs) were calculated by dividing the prevalence rates in children with T1DM by the prevalence rates in the reference cohort. Annual prevalence rates of CV medication use in each cohort were defined by dividing the number of CV medication users in a specific year by the total number of children and adolescents in the same year. In subgroup analyses, prevalence rates of CV medication use stratified by gender and age categories (using age bands: 0-4, 5-9, 10-14, and 15-

18 years) were compared using the ordinal chi square test. Annual incidence rates of CV medication use and hospital admission for CVD in each cohort were defined as the number of incident CV medication users and incident hospitalized CVD cases during a given time period divided by the person time at risk. For calculating annual incidence rates, to exclude prevalent cases in each year, subjects were required to have at least 12 months prior history (either a drug prescription or the occurrence of a hospital admission due to CVD) in the database. Incidence rate ratios (IRRs) were calculated to compare the incidence rates between different cohorts by dividing incidence rates in the T1DM cohort by the rates in the reference cohort. Data analyses were performed using SPSS version 20.0 (SPSS, Chicago,IL).

RESULTS

A total of 925 children and adolescents with T1DM cohort were identified from PHARMO RLS in the period 1999 to 2009 and compared with a group of 3,591 diabetes-free children and adolescents in the reference cohort. At the index date, almost 49% of the study participants were girls with a median age of 10 years (interquartile range [IQR] 7-14 years) (**Table1**).

Table 1. Baseline characteristics of patients with T1DM and diabetes-free subjects

		T1DM Cohort (n=925)	Reference cohort (n=3,591)
Gender, n (%)	Boys	469 (50.7)	1,817 (50.6)
	Girls	456 (49.3)	1,774 (49.4)
Age at diagnosis (Index date), n (%)	0-4 y	132 (14.3)	537 (15.0)
	5-9 y	269 (29.1)	1,043 (29.0)
	10-14 y	338 (36.5)	1,295 (36.1)
	15-18 y	186 (20.1)	716 (19.9)
Age (median, IQR), Y		10 (7-14)	10 (7-14)
Follow-up before the index date (median , IQR), Y		2.7 (1.8-3.7)	2.9 (1.9-3.9)
Follow-up after the index date (median , IQR), Y		3.0 (2.0-4.0)	3.0 (2.0-4.0)

Abbreviations: T1DM: type 1 diabetes mellitus; y: years; IQR: interquartile range
Index date is the date of first insulin dispensing.

Prevalence rates of CV medication use

The overall prevalence rate of CV medication use before the index date in the T1DM cohort (2.2%, 95% confidence interval (CI): 1.3-3.5) was two times higher than in the diabetes-free cohort (1.0%, 95% CI: 0.7-1.4). For the period after the index date the prevalence rate in the T1DM cohort (9.2%, 95% CI: 7.4-11.4) was almost three times higher than in the reference cohort (3.2%, 95% CI: 2.6-3.8) (**Table 2**).

Table 2. Overall prevalence rates of CV medication use in the T1DM and the reference cohorts

	Cohort	Total population	CV medication users	Prevalence rate (%), 95% CI	PRR, 95% CI
5 years before the index date	T1DM	737	16	2.2 (1.3-3.5)	2.2 (1.2-4.1)
	Reference	2,875	28	1.0 (0.7-1.4)	
5 years after the index date	T1DM	794	73	9.2 (7.4-11.4)	2.9 (2.2-3.9)
	Reference	3,068	97	3.2 (2.6-3.8)	

Abbreviations: T1DM: type 1 diabetes mellitus; CV: cardiovascular; CI: confidence interval; PRR: prevalence rate ratio
Index date is the date of first insulin dispensing.

The annual prevalence rates showed that the statistically significant higher consumption of CV medication in the T1DM cohort compared with the reference cohort started from one year before the index date (1.3%, 95% CI: 0.7-2.3 vs. 0.5%, 95% CI: 0.3-0.7) and continued in the five years after the index date. After the index date there was an increasing trend and the highest prevalence rate appeared in patients with T1DM in the period of 4-5 years after the index date with the prevalence rate of 5.1% (95% CI: 3.6-7.2) versus 1.2% (95% CI, 0.8-1.7) in the reference cohort (**Fig 1 and Table S3**).

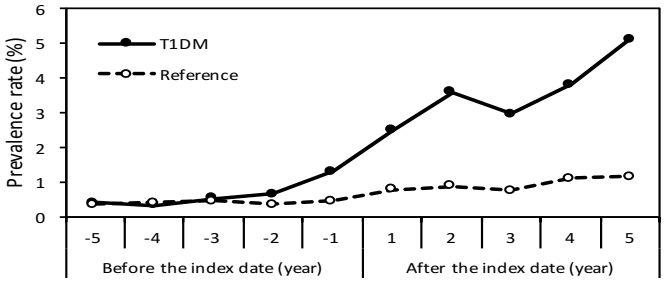

Fig 1. Annual prevalence rates of cardiovascular medication use in the type 1 diabetes and the reference cohorts
Index date is the date of first insulin dispensing.

Children and adolescents in the T1DM cohort had a four times higher prevalence rate of CV medication use in the period after the onset of diabetes compared with the years before. The same pattern was observed in the reference cohort.
There was no statistically significant difference between boys and girls in the prevalence rates of CV medication use in both cohorts. As shown in **Figure 2**, the number of CV prescriptions increased with increasing age. Diabetic adolescents aged 15-18 years used CV medication more frequently in the period five years after the index date (5.4%, 95% CI: 4.2-7.0) compared with the reference cohort (1.2%, 95% CI: 0.9-1.6**)**.
In the period after the index date, ACE inhibitors were the most commonly used CV medications in patients with T1DM with a prevalence rate of 2.0%, 95% CI: 1.2-3.2 compared with the reference cohort (0.2%, 95% CI: 0.1-0.4). Statins (1.5% vs. 0.1%; p< 0.001), heparin (1.3% vs. 0.5%; p=0.02), diuretics and potassium sparing agents (1.1% vs. 0.1%;

p< 0.001), and selective beta blocking agents (1.0% vs. 0.2%; p< 0.001) were also significantly more often prescribed in diabetic patients compared with the reference cohort.

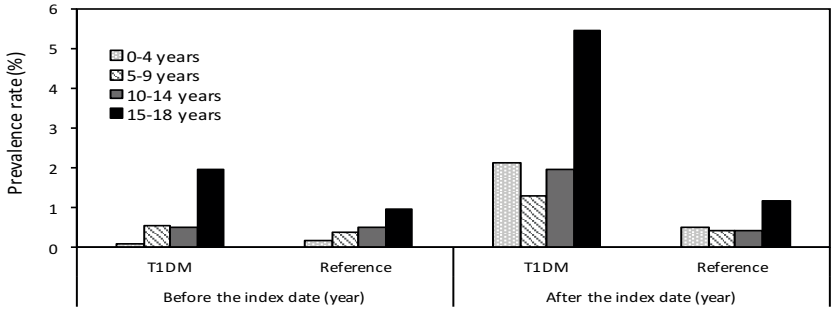

Fig 2. Overall prevalence rate of cardiovascular medication use in the type 1 diabetes and the reference cohorts, stratified by age
Index date is the date of first insulin dispensing.

Incidence rates of CV medication use

Annual incidence rates of CV medication use during the five years before and after the index date are presented in **Figure 3**. The highest annual incidence rate of CV medication use in the T1DM cohort was observed in the first year after the index date which was 5.5 times higher than the reference cohort in the same time period with the incidence rate of 28.1 per 1000 person years (PY) (95% CI: 19.1-41.2) compared with 5.1 per 1000 PY (95% CI: 3.2-8.0) in the reference cohort. This annual incidence rate of CV medication use in the T1DM cohort gradually decreased to 23.1 per 1000 PY in the second year after the index date and had a sharp decline in the third year after the index date which followed by an increase to 23.6 per 1,000 PY at the end of follow- up (**Table S4**). Because of the low numbers it was not possible to stratify the incidence rates of CV medication use by age and gender.

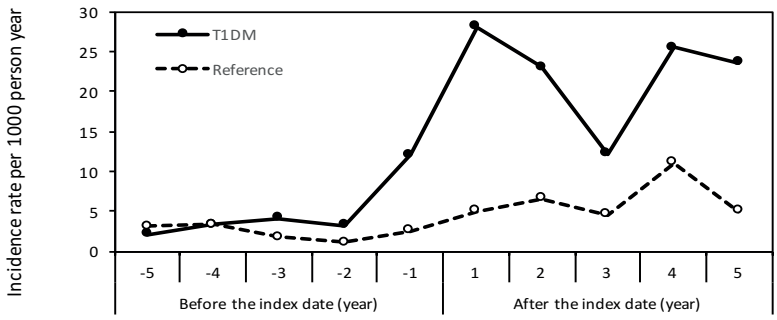

Fig3. Annual incidence rates of cardiovascular medication use (per 1000 person year) in the type 1 diabetes and the reference cohorts
Index date is the date of first insulin dispensing.

Incidence rates of hospital admission due to CVD

In the T1DM cohort, three patients were hospitalized due to cardiomyopathy (n=2) and heart failure (n=1), while in the reference group only one child was hospitalized with cardiomyopathy in the five years after the index date.

DISCUSSION

This study demonstrated that the overall prevalence rates of CV prescriptions among type 1 diabetic children and adolescents were significantly higher than among age and gender-matched diabetes-free individuals in the periods both before and after the onset of diabetes. The statistically significant difference between the two cohorts already started one year before the onset of diabetes and further increased during the follow-up. There was no significant difference between boys and girls in the prevalence rate of CV medication use. The oldest age group (15-18 years) had the highest prevalence rate of CV medication use (5.4%) in the T1DM cohort. The highest incidence rate of CV medication use was observed for children and adolescents with T1DM in the first year after the diagnosis which was 5.5 times higher than what we observed for the reference cohort. Furthermore, the number of CV hospitalizations, as expected, was low in this age category.

The use of CV medication which was already higher before the diagnosis of T1DM can be explained in two ways. Firstly, it is possible that the variable asymptomatic period of beta cell destruction prior to the clinical presentation of T1DM[30] is associated with a higher occurrence of CVD risk factors. However it is not clear how these pancreatic changes could influence the occurrence of CVD risk factors. Secondly, it is clear that some of the CV drugs may trigger the clinical manifestation of T1DM, for instance beta blockers and thiazide diuretics have diabetogenic properties[31,32]. Beta-blockers increase insulin resistance and thiazide diuretics reduce insulin secretion [33-35]. Due to the very low frequency of CV medication use before the onset of diabetes we could not formally test the association between drugs with diabetogenic properties and the occurrence of T1DM. Further research is needed to understand the increased use of CV medication prior to the clinical onset of T1DM**.**

The higher use of CV medication in children and adolescents with T1DM compared with the reference cohort is in line with earlier findings that these patients have higher prevalence rates of CVD risk factors[3-7] and have an increased risk of carotid intima-media thickness, reduced endothelium-dependent arterial flow-mediated dilation (FMD), and increased arterial stiffness [8-11]. The highest incidence rate of CV medication use in the T1DM cohort in the first year after the diabetes diagnosis (28.1 per 1000 PY; IRR: 5.5) is probably caused by active screening for CVD risk factors [16,17,36,37]. There is an unexpected dip in the data for the year 2-3 in the Figures 1 and 2.

Although there was a higher consumption of CV medication among the T1DM cohort, still only 9% of these patients were on CV medications during the five years follow-up. Based on the published prevalence rates of hypertension and dyslipidemia in children and adolescents with T1DM (ranging between 8.1 to 16.7% and 16.9 to 28.7%, respectively)[3,5,17]

and the recommendations of the American Diabetes Association (ADA) [16] to treat these risk factors there is probably under treatment in children with T1DM in the Netherlands. Such under treatment was shown in several other population based studies [4-6,23,38]. One explanation might be that there is no evidence that pharmacological treatment of hypertension and dyslipidemia in children with T1DM prevents CV morbidity and mortality. Another explanation might be that clinically apparent vascular complications are rarely manifested in children and adolescents [39] and there is a general reluctance to use drug therapy to treat hypertension and lipid abnormalities in children and adolescents. Finally, it is possible that the first step in first line management of these risk factors has been through lifestyle modifications.

During recent years guidelines have published detailed recommendations for the management of diabetes and related complications including CVD risk factors. Accordingly, more attention should be given to early treatment of CVD risk factors to reduce long-term morbidity and mortality from diabetes complications [16,17,40,41].

The relatively high prevalence rate of ACE inhibitors use is in accordance with the guideline for diabetes in children and adolescents (www.ispad.org/forums/2014-consensus-guidelines), ADA and American heart association (AHA) [17] to prescribe ACE inhibitors or angiotensin II receptor blockers (ARB) in young children. Daniels and colleagues showed that 36% of children with T1DM used ACE inhibitors/ARB medications for microalbuminuria [42].

The observed higher consumption of heparin as a third most prevalent CV medication in T1DM compared to the reference cohort was unexpected. It is not directly clear why children with T1DM have more (or increased risk for) thromboembolic events.

A main strength of our study is that we used PHARMO RLS which is a large, population-based data set (including almost 24% of the Dutch population) providing accurate data on medications dispensing and hospital admissions which is representative of the general population [27,28,43]. As all children and adolescents with T1DM are treated with insulin we are sure that all of them living in the catchment area of PHARMO will be included in the database. Routinely collected detailed data on medication use reduces the probability of information bias and recall bias. Insulin prescriptions can be used as a proxy for T1DM since hyperglycemia is the only indication for insulin [29,44,45] and other types of diabetes in which insulin is indicated e.g. mitochondrial diabetes have low prevalence rates compared with T1DM [46,47].

An important limitation of our study is that the reference cohort was randomly captured from the PHARMO RLS which only includes individuals who have obtained at least one prescription from the community pharmacy. Children and adolescents for whom never medication was prescribed (varying between 28-58% for different age categories) are not in this database compromising the representativeness of our reference cohort. Therefore, the gap in CV medication use between the two cohorts will be even larger than observed in our study. Another limitation in this study might be ascertainment bias of CVD risk factors due to increased screening in patients with T1DM compared to the general population. We did not have information on the indication for prescribing CV medication which might lead to some misclassification of the pharmacological treatment of CV risk factors. For instance our study included adolescents that might use beta-blockers for test

anxiety before school exams or driving tests (Sage, Weld. “Drugs for Test Anxiety). There is also a lack of correction for multiple comparisons in our study. Finally, in our database we missed information on important CVD risk factors like BMI, genetic related risk factors, and family history of CVD which might also influence the choice for yes or no pharmacological treatment.

In summary, our results showed that there is an increased risk for CV medication use in children and adolescents with T1DM compared with the age- and gender-matched diabetes-free population both before and after the onset of diabetes. More comprehensive data is needed to fully address and draw conclusions about under treatment of CVD risk factors in children and adolescents with T1DM. Furthermore, our study emphasizes the need to pay more attention to screening for risk factors and to start programs that implement prevention strategies to lower CVD risk in the pediatric population with diabetes. Future observational studies are needed to study the long term CVD outcomes in diabetic children and adolescents, and also the influence of the use of CV medication early in life on these outcomes.

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SUPPORTING INFORMATION

Table S1. Codes used to identify CV medications

CV medication (ATC code)	Subgroup 1 (ATC code)	Subgroup 2 (ATC code)
Cardiac therapy (C01)	Cardiac glycosides (C01A)	Digital glycosides (C01AA)
	Antiarrhythmic, class I and III (C01B)	Antiarrhythmic, class Ia (C01BA) class Ib (C01BB) class Ic (C01BC) Antiarrhythmic class III (C01BD) Other antiarrhythmic class I and III
	Cardiac stimulants excl. cardiac glycosides (C01C)	Adrenergic and dopaminergic agents (C01CA) Phosphodiesterase inhibitors (C01CE) Other cardiac stimulants (C01CX)
	Vasodilators used in cardiac diseases (C01D)	Organic nitrates (C01DA) Quinolon vasodilators (C01DB) Other vasodilators used in cardiac diseases (C01DX)
	Other cardiac preparations (C01E)	Prostaglandins (C01EA) Other cardiac preparations (C01EB) Other cardiac combination products
Antihypertensives (C02)	Antiadrenergic agents, centrally acting (C02A)	Rauwolfia alkaloids (C02AA) Methyldopa (C02AB) Imidazoline receptor agonists (C02AC)
	Antiadrenergic agents, ganglion-blocking (C02B)	Sulfonium derivatives (C02BA) Secondary and tertiary amines (C02BB) Bisquaternary ammonium compounds (C02BC)
	Antiadrenergic agents, peripherally acting (C02C)	Alpha-adernoreceptor antagonists (C02CA) Guanidine derivatives (C02CC)
	Arteriolar smooth muscle, agents acting on (C02D)	Thiazide derivatives (C02DA) Hydrazinophthalazine derivatives (C02DB) Pyrimidine derivatives (C02DC) Nitroferricyanide derivatives (C02DD) Guanidine derivatives (C02DG)
	Other antihypertensives (C02K)	Alkaloids, excl. rauwolfia (C02KA) Tyrosine hydroxylase inhibitors (C02KB) MAO inhibitors (C02KC) Serotonin antagonists (C02KD) Other antihypertensives (C02KX)
	Antihypertensives and diuretics in combination (C02L)	Rauwolfia alkaloids and diuretics in combination (C02LA) Methyldopa and diuretics in combination (C02LB) Imidazoline receptor agonists in combination with diuretics (C02LC) Alpha-adrenoreceptor antagonists and diuretics (C02LE) Guanidine derivatives and diuretics (C02LF) Hydrazinophthalazine derivatives and diuretics (C02LG) MAO inhibitors and diuretics (C02LL) Serotonin antagonists and diuretics (C02LN) Other antihypertensives and diuretics (C02LX)
	Combinations of antihypertensives in ATC-GR.C02 (C02N)	

CV medication (ATC code)	Subgroup 1 (ATC code)	Subgroup 2 (ATC code)
Diuretics (C03)	Low-ceiling diuretics, Thiazides (C03A)	Thiazides, plain (C03AA) Thiazides and potassium in combination (C03AB) Thiazides, combinations with psycholeptics and/or analgesics (C03AH) Thiazides, combinations with other drugs (C03AX)
	Low-ceiling diuretics, excl. Thiazides (C03B)	Sulfonamides, plain (C03BA) Sulfonamides and potassium in combination (C03BB) Mercurial diuretics (C03BC) Xanthine derivatives (C03BD) Sulfonamides, combinations with other drugs (C03BK) Other low-ceiling diuretics (C03BX)
	High-ceiling diuretics, Thiazides (C03C)	HIGH-CEILING DIURETICS (C03C) Sulfonamides, plain (C03CA) Sulfonamides and potassium in combination (C03CB) Aryloxyacetic acid derivatives (C03CC) Pyrazolone derivatives (C03CD) Other high-ceiling diuretics (C03CX)
	Potassium-sparing agents (C03D)	Aldosterone antagonists (C03DA) Other potassium-sparing agents (C03DB)
	Diuretics and potassium-sparing agents in combination (C03E)	Low-ceiling diuretics and potassium-sparing agents (C03EA) High-ceiling diuretics and potassium-sparing agents (C03EB)
	Other diuretics (C03X)	
Peripheral vasodilators (C04)	Peripheral vasodilators (C04A)	2-amino-1-phenylethanol derivatives (C04AA), Imidazoline derivatives (C04AB) Nicotinic acid and derivatives (C04AC) Purine derivatives (C04AD) Ergot alkaloids (C04AE) Enzymes (C04AF) Other peripheral vasodilators (C04AX)
Beta blocking agents (C07)	Beta blocking agents (C07A)	Beta blocking agents, non-selective C07AA, Beta blocking agents, selective C07AB C07AG Alpha and beta blocking agents
	Beta blocking agents and thiazides (C07B)	Beta blocking agents, non-selective, and thiazides C07BABeta blocking agents, selective, and thiazides C07BBAlpha and beta blocking agents and thiazides C07BG
	Beta blocking agents and other diuretics (C07C)	Beta blocking agents, non-selective, and other diuretics C07CABeta blocking agents, selective, and other diuretics C07CBAlpha and beta blocking agents and other diuretics C07CG
	Beta blocking agents, thiazides and other diuretics (C07D)	Beta blocking agents, non-selective, thiazides and other diuretics C07DA Beta blocking agents, selective, thiazides and other diuretics C07DB
	Beta blocking agents and vasodilators (C07E)	Beta blocking agents, non-selective, and vasodilators C07EA Beta blocking agents, selective, and vasodilators C07EB
	Beta blocking agents and other antihypertensives (C07F)	Beta blocking agents, non-selective, and other antihypertensives C07FABeta blocking agents, selective, and other antihypertensives C07FB

CV medication (ATC code)	Subgroup 1 (ATC code)	Subgroup 2 (ATC code)
	Selective calcium channel blockers with mainly vascular effects (C08C)	Dihydropyridine derivatives C08CAOther selective calcium channel blockers with mainly vascular effects C08CX
	Selective calcium channel blockers with direct cardiac effects (C08D)	Phenylalkylamine derivatives C08DABenzothiazepine derivatives C08DB
	Non-selective calcium channel blockers (C08E)	Phenylalkylamine derivatives C08EAOther non-selective calcium channel blockers C08EX
	Calcium channel blockers and diuretics (C08G)	Calcium channel blockers and diuretics C08GA
Agents acting on the renin angiotensin system (C09)	ACE inhibitors, plain (C09A)	ACE inhibitors, plain C09AA
	ACE inhibitors, combinations (C09B)	ACE inhibitors and diuretics C09BA ACE inhibitors and calcium channel blockers C09BB ACE inhibitors, other combinations C09BX
	Angiotensin II antagonists, plain (C09C)	Angiotensin II antagonists, plain C09CA
	Angiotensin II antagonists, combinations (C09D)	Angiotensin II antagonists and diuretics C09DAAngiotensin II antagonists and calcium channel blockers C09DBAngiotensin II antagonists, other combinations C09DX
	Other Agents acting on the renin angiotensin system (C09X)	Renin-inhibitors C09XA
Lipid modifying agents (C10)	Lipid modifying agents, plain (C10A)	HMG CoA reductase inhibitors (C10AA) Fibrates (C10AB) Bile acid sequestrants (C10AC) Nicotinic acid and derivatives (C10AD) Other lipid modifying agents (C10AX)
	Lipid modifying agents, combinations (C10B)	HMG CoA reductase inhibitors in combination with other lipid modifying agents C10BAHMG CoA reductase inhibitors, other combinations C10BX
Antithrombotic agents (B01)	Antithrombotic agents (B01A)	Vitamin K antagonists (B01AA) Heparin group (B01AB) Platelet aggregation inhibitors excl. heparin (B01AC) Enzymes (B01AD) Direct thrombin inhibitors (B01AE) Direct factor Xa inhibitors (B01AF) Other antithrombotic (B01AX)

Abbreviations: CV: cardiovascular; ATC: Anatomical Therapeutic Chemical

Table S2. Codes used to identify CVD

CVD	(ICD-9-CM codes)
Hypertensive disease	401-405
Ischemic heart disease	410-414
Cardiomyopathy, conduction disorders, cardiac dysrhythmias, heart failure, defined descriptions and complications of heart disease, subarachnoid hemorrhage, intracerebral hemorrhage, other and unspecified intracranial hemorrhage, occlusion and stenosis of precerebral arteries, occlusion of cerebral arteries, transient cerebral ischemia, acute but ill-defined, cerebrovascular disease, other and ill-defined cerebrovascular disease, late effects of cerebrovascular disease	425-438
Atherosclerosis, arotic aneurysm and dissection	440-441
Arterial embolism and thrombosis, atheroembolism	404-405
Other disorders of arteries and arterioles, disease of capillaries	447-408

Abbreviations: CVD: cardiovascular disease, ICD-9-CM: International Classification of Disease, 9th edition, Clinical Modifications;

Table S3. Annual prevalence rate of CV medication use

	Cohort	Population	Cases	Prevalence rate (%)	95% CI	PRR	P-value
			Before the index date				
4-5 year	T1DM	487	2	0.41	0.11-1.48	1.12	0.90
	Reference	1,912	7	0.37	0.18-0.75		
3-4 year	T1DM	610	2	0.33	0.09-1.19	0.79	0.74
	Reference	2,379	10	0.42	0.23-0.77		
2-3 year	T1DM	741	4	0.54	0.21-1.38	1.20	0.76
	Reference	2,909	13	0.45	0.26-0.76		
1-2 year	T1DM	924	6	0.65	0.30-1.41	1.81	0.23
	Reference	3,583	13	0.36	0.21-0.62		
0-1 year	T1DM	925	12	1.30	0.74-2.25	2.90	0.00
	Reference	3,591	16	0.45	0.27-0.72		
			After the index date				
0-1 year	T1DM	925	23	2.49	1.66-3.70	3.19	0.00
	Reference	3,591	28	0.78	0.54-1.12		
1-2 year	T1DM	923	33	3.58	2.55-4.97	4.01	0.00
	Reference	3,579	32	0.89	0.63-1.26		
2-3 year	T1DM	810	24	2.96	1.99-4.36	3.89	0.00
	Reference	3,131	24	0.77	0.51-1.14		
3-4 year	T1DM	708	27	3.81	2.63-5.49	3.46	0.00
	Reference	2,726	30	1.10	0.77-1.57		
4-5 year	T1DM	605	31	5.12	3.63-7.18	4.38	0.00
	Reference	2,315	27	1.17	0.80-1.69		

Abbreviations: T1DM: type 1 diabetes mellitus; CV: cardiovascular; CI: confidence interval; PRR: prevalence rate ratio

Index date is the date of first insulin dispensing.

Table S4. Annual incidence rate of CV medications use

	Cohort	Population	Events	PY	Incidence rate per 1,000 PY	(95% CI)	IRR	(95% CI)
				Before the index date				
4-5 year	T1DM	487	1	486.91	2.05	0.36-11.54	0.65	0.08-5.42
	Reference	1,913	6	1911.38	3.14	1.44-6.83		
3-4 year	T1DM	610	2	607.10	3.29	0.90-11.93	0.98	0.21-4.59
	Reference	2,381	8	2371.88	3.38	1.71-6.64		
2-3 year	T1DM	741	3	736.3	4.07	1.39-11.91	2.36	0.56-9.84
	Reference	2,910	5	2893.54	1.73	0.74-4.04		
1-2 year	T1DM	924	3	916.22	3.27	1.11-9.58	2.92	0.65-13.02
	Reference	3,587	4	3565.66	1.12	0.44-2.88		
0-1 year	T1DM	925	11	909.25	12.10	6.77-21.53	4.79	1.99-11.53
	Reference	3,591	9	3564.18	2.53	1.33-4.79		
				After the index date				
0-1 year	T1DM	925	25	889.78	28.10	19.10-41.15	5.54	3.04-10.12
	Reference	3,591	18	3551.62	5.07	3.21-8.00		
1-2 year	T1DM	925	20	869.66	23.05	14.94-35.25	3.53	1.95-6.39
	Reference	3,587	23	3527.98	6.53	4.35-9.76		
2-3 year	T1DM	812	9	743.23	12.14	6.38-22.85	2.64	1.15-6.09
	Reference	3,139	14	3057.23	4.59	2.73-7.67		
3-4 year	T1DM	708	16	626.82	25.53	15.77-41.06	2.31	1.26-4.22
	Reference	2,726	29	2620.91	11.06	7.72-15.85		
4-5 year	T1DM	605	12	507.62	23.64	13.57-40.86	4.71	2.09-10.62
	Reference	2,315	11	2193.62	5.01	2.80-8.96		

Abbreviations: T1DM: type 1 diabetes mellitus; PY: person-years; CI: confidence interval; IRR: Incidence rate ratio
Index date is the date of first insulin dispensing.

Chapter 2.2

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Time trends in the prevalence of cardiovascular risk factors, diseases and medication use in children and adolescents with type 1 diabetes: a CPRD study

Submitted for publication

ABSTRACT

Objectives: To evaluate long-term trends in the occurrence and (under) treatment of cardiovascular disease (CVD) risk factors and occurrence of CVD events in children with type 1 diabetes mellitus (T1DM). Furthermore, determinants of undertreatment of CVD risk factors were evaluated.

Methods: A retrospective cohort study was conducted in 3,728 children (<19 years) with T1DM and up to 5 age and gender-matched diabetes-free children (reference cohort) (n=18,513) using data from the Clinical Practice Research Datalink (CPRD).

Results: The annual prevalence rates of hypertension (0.64% vs. 0.34%, p=0.007 and 35.2% vs. 11.4%, p<0.001), hypercholesterolemia (0.91% vs. 0.05%, p<0.001 and 64.8% vs. 5.0%, p<0.001) and cardiovascular (CV) medication use (0.59% vs. 0.27%, p=0.002 and 37.0% vs. 3.6%, p<0.001) were substantially higher in the T1DM cohort compared with the reference cohort one year before and 20 years after the index date, respectively. Furthermore, 70% of the children in the T1DM cohort with hypertension and 98% with hypercholesterolemia remained untreated with CV drugs for at least a period of one year during a 20-year follow-up.

Conclusions: Children with T1DM had substantial higher prevalence rates of hypertension and hypercholesterolemia one year before up to 20 years after the onset of diabetes compared to non-diabetics. There is a substantial undertreatment of CVD risk factors with CV drugs. Screening for CVD risk factors in children with T1DM and adequate treatment is of utmost importance to prevent CVD later in life.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is associated with an increased risk of cardiovascular disease (CVD), which is evident in all age groups including children[1,2]. Already in childhood, T1DM is associated with vascular smooth muscle dysfunction and increased intima media thickness[3-6]. There is extensive evidence to support the role of atherosclerosis in developing CVD which starts in childhood and further increases through life[7]. Though the pathogenesis of CVD begins in childhood, clinical manifestations of CVD are not common before adulthood[8].

Several studies have shown that children with T1DM are almost twice as likely to have CVD risk factors compared to the general population, although the use of CV medication to treat these risk factors is low[9-15]. Previous studies on CVD risk factors in T1DM have some important limitations. Firstly, most of these studies used a cross-sectional study design or had a relatively short follow-up time not adequately taking into account the dynamics of occurrence of CVD risk factors during aging[10-14,16]. Secondly, in most studies CVD risk factors like body mass index (BMI), smoking status and family history of CVD were not evaluated.

Therefore, in this study we aimed to calculate the long-term trends in prevalence and incidence rates of CVD risk factors, CVD events and use of CV medication before and after the onset of T1DM in children and adolescents, and to compare these with prevalence and incidence rates in a group of age-and gender-matched diabetes-free children and adolescents. We also evaluated the percentages of untreated children with hypertension and hypercholesterolemia, as well as determinants associated with undertreatment.

METHODS

Data Source

Data for this study was obtained from the Clinical Practice Research Datalink (CPRD). CPRD data are collected from anonymized patient records from participating general practitioners in the United Kingdom (UK). The population in the CPRD reflects the wide distribution of participating general practices across the UK (providing around 6.9% national coverage), rather than individualized recruitment, and has been shown to be broadly representative of the UK population as a whole[17]. Data available from the CPRD include patient demographic data, patient registration details, practice details, medication records (including medicines prescribed for patients), consultation details, clinical records, laboratory test results, and referrals. Medication records in the CPRD are based on prescriptions and do not provide information regarding the filling of prescriptions. Ethics approval was granted by the CPRD Scientific and Ethical Advisory Group and the protocol (15_133R2) was reviewed and approved by the Independent Scientific Advisory Committee for Medicines and Healthcare Products Regulatory Agency database research. Further details on the CPRD have been published elsewhere[17-19].

Study design and study population

Using a retrospective cohort study design, we identified all children and adolescents in CPRD that started insulin therapy before they were 19 years of age (T1DM cohort) between 01/01/1987 and 04/11/2015. Within this time period, patients were included in the study if they had a first ever diagnosis of diabetes and first ever insulin prescription. Insulin use was defined as at least two prescriptions during the study period and the date of first insulin prescription was termed as the index date. Exclusion criteria for the T1DM cohort were having a glucagon prescription before the index date and if children had already ever used any type of oral anti-diabetic agents. Furthermore, patients with a history of cystic fibrosis were excluded from the study.

Up to five diabetes-free subjects (without diagnosis of T1DM and any prescription of insulin before the index date and during the follow-up) matched on the year of birth (age), gender, general practice and being registered in the CPRD in the index date was sampled at random as a reference cohort. The index date for the reference cohort was set as the index date for the matched T1DM cohort. Baseline characteristics were recorded at the index date.

To ensure all subjects were active within their general practice, they were required to have had at least a12 months contact recording history in CPRD before and after the index date. Subjects were followed until the end of study period (04/11/2015), death or date of transfer out of the practice whichever came first.

Cardiovascular disease, risk factors and treatment

Presence of CVD risk factors including hypertension, hypercholesterolemia, poor glucose level (measured by glycosylated hemoglobin (Hb)A1c level), and family history of CVD before and after the index date was identified by Read codes.

Hypertension was identified by either a GP diagnosis, systolic and diastolic blood pressure values (during follow-up three times an elevated blood pressure (SBP≥140 and DBP≥90 mmHg) or by antihypertensive medication use[20].

Hypercholesterolemia was identified based on GP diagnoses or lipid values (LDL cholesterol ≥130 mg/dL (3.4 mmol/L) and/or total cholesterol≥200 (5.2 mmol/L)) or lipid-lowering medication[21].

The first time hypertension or hypercholesterolemia was identified during the 2nd year prior to the index date up to 20 years after the index date, we assumed that this risk factor remained present during the rest of the follow-up.

Poor glycemic control was defined as HbA1c>7.5% (58 mmol/mol) which is a determinant for CVD risk factors including hypertension[22]. Any record of HbA1c>7.5% (58 mmol/mol) during the years after the index date was defined as poor glycemic control.

Ever family history of CVD was identified by GP's recording.

Smoking habits in children during the complete follow-up were categorized as ever versus never smokers or unknown.

Obesity was defined as BMI percentile≥95th and categorized as ever versus never obesity or unknown. We calculated age- and gender-adjusted BMI percentiles (for children aged 2-20 years at the time of BMI measurement) using height and weight measures as de-

fined by the CDC standardized gender- and age-specific growth charts (http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm).
Treatment with CV medication was identified using CPRD product codes. CV medication was defined as a recorded receipt of a prescription for CV medication categorized into the following groups: nitrates, anti-hypertensive medications (diuretics, beta blockers, angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and calcium channel blockers), lipid-lowering medications (statins, nicotinic acid, fibrates, and bile acid sequestrants), and antithrombotics. Children and adolescents who received at least one prescription for CV medication during the study period were defined as CV medication users.
CVD events including stable/unstable angina, myocardial infarction, heart failure, atrial fibrillation and peripheral artery diseases were identified by CPRD diagnosis.

Undertreatment of hypertension and hypercholesterolemia
Children were classified as being undertreated if they were identified as hypertensives without at least one antihypertensive agent in their medication regimen during the same year after the index date. For hypercholesterolemia we used the same definition. For the evaluation of determinants of undertreatment of hypertension and hypercholesterolemia, age, gender and family history of CVD were considered. Due to the high rates of missing values, we did not use the data on BMI and smoking status as possible determinants of medication undertreatment.

Statistical analysis
Descriptive statistics were used to summarize the characteristics of children in both the T1DM and the reference cohorts. The annual prevalence rates for hypertension, hypercholesterolemia, and CV medication use during two years before and maximal 20 years after the index date were calculated. To compare the rates of CVD risk factors and CV medication use between the two cohorts, chi square tests were used. To compare cumulative incidence rates of events in the two cohorts, Kaplan-Meier survival analyses were used. For this analysis, the date of first diagnosis of a CVD event or a CVD risk factor or the date of the first prescription of a CV medication after the index date (t=0) was counted as the first event. Prevalent cases at index date (t=0) were excluded from the denominator. In subgroup analyses, the rates were stratified by age (being split into 4 bands: 0-4, 5-9, 10-14 and 15-18 years) and gender. In the T1DM cohort, undertreated children for hypertension and hypercholesterolemia were further stratified by the years being undertreated; being split into 4 bands: ≤1 year, 2-5, 6-10 and ≥11years. Chi square statistics and Fisher's test were used to test determinants of undertreatment of hypertension and hypercholesterolemia. All statistical analyses were carried out using statistical package R version 3.2.3 and SPSS 23.0.

RESULTS

A total of 22,241 children met the inclusion criteria. **Table 1** shows the demographic features of 3,728 children aged younger than 19 years with diagnosed diabetes and at least two insulin prescriptions (T1DM cohort) and 18,513 age-and gender-matched diabetes free peers (reference cohort). The mean age at the index date was almost 11 years with standard deviation (SD), 4.5 in the T1DM and 4.4 in the reference cohort. Males comprised over half of the population (55%) in both cohorts. The median duration of follow-up was almost 6 (interquartile range: 3-10) years in both cohorts.
In the T1DM cohort, obesity (any moment during follow-up) was found in 8.8% of children aged 2-20 years (BMI percentile ≥95th) and in 0.24% aged older than 20 years (BMI≥30 mg/kg^2). The rates of obesity in the reference cohort were 8.1% and 0.53%, respectively. Compared with children in the T1DM cohort, those in the reference cohort were more likely to ever smoke (8.9% vs 6.9%, p<0.001) during the years before and after the index date. Almost 5% of both study cohorts had a family history of CVD.

Time trends in CVD risk factors

Annual prevalence rates of CVD risk factors in the T1DM cohort compared with the reference cohort are shown in **Figure 1.** Prevalence rates of hypertension and hypercholesterolemia were statistically significantly higher in the T1DM cohort compared to the reference cohort after the index date. The rates of hypertension in the T1DM cohort compared with the reference cohort started from 0.27% vs. 0.18% (p=0.29) in the 2nd year before the onset of diabetes to 35.2% vs. 11.4% (p<0.001) in the 20th year after the index date. Already in the 1st year before the onset of diabetes there was a statistically significant higher rate of hypertension in the T1DM cohort (0.54% vs. 0.24%; p=0.002). The prevalence rate of hypercholesterolemia was 0.11% in the T1DM cohort vs. 0.03% (p<0.001) in the reference cohort in the 2nd year before the onset of diabetes and the difference in prevalence rate between the cohorts increased further during follow-up; 64.8% vs. 5.0%, p<0.001 in 20th year after the index date.
Time to hypertension and to hypercholesterolemia occurrence comparing the T1DM and the reference cohort is presented in **Figure S1**. The probability of developing hypertension (log-rank p<0.001) or hypercholesterolemia (log-rank p<0.001) was statistically significantly higher in children with T1DM compared with children in the reference cohort. Age-stratified prevalence rates of hypertension and hypercholesterolemia in the T1DM cohort and the reference cohort are presented in **Figure S2**. In both cohorts, children aged 10-14 and 15-18 years at the index date had a higher risk of both hypertension and hypercholesterolemia compared with younger children.
The same trends were shown by Kaplan-Meier analysis in which the probability of hypertension (log-rank p<0.001) and hypercholesterolemia (log-rank p<0.001) after the index date was much higher in children with T1DM aged 15-18 years (at index date) compared with younger children (**Fig S3**).
In the T1DM cohort, trends in prevalence rates of hypertension were similar in males and females for most of the time during follow-up. While in the reference cohort, females were

Table 1. Baseline characteristics of patients in the T1DM and reference cohorts

		T1DM Cohort (n=3,728)	Reference Cohort (n=18,513)
Gender, n (%)	Females	1,685 (45.2)	8,375 (45.2)
	Males	2,043 (54.8)	10,138 (54.8)
Age at index date, n (%)	0-4 y	494 (13.3)	2,240 (12.1)
	5-9 y	1,052 (28.2)	5,212 (28.2)
	10-14 y	1,434 (38.5)	7,331 (39.6)
	15-19 y	748 (20.1)	3,730 (20.1)
Age at index date (mean, SD), Y		10.7 (4.5)	10.9 (4.4)
BMI available, n (%)		2,967 (79.6)	5,276 (28.5)
Unknown BMI, n (%)		761 (20.4)	13,243 (71.5)
BMI percentile category (aged 2-20 years at time of measurement)*, n (%)	Overweight (≥85th to 95th percentile)	488 (13.1)	984 (5.3)
	Obese (≥95th percentile)	328 (8.8)	1491 (8.1)
BMI category (aged >20 years at time of measurement), n (%)	Overweight (≥25-30 kg/m^2)	11 (0.30)	131 (0.71)
	Obese (≥30 kg/m^2)	9 (0.24)	99 (0.53)
Smoking status, n (%)	Ever-smoker	251 (6.9)	1599 (8.9)
	Never-smoker	2,234 (61.0)	7645 (42.5)
	Unknown	1,178 (32.1)	8,730 (48.6)
Family history of CVD		182 (4.9)	834 (4.5)
Number of participants in each year of the study period, before the index date	2 years	3,726	18,506
	1 year	3,728	18,513
Number of participants in each year of the study period, after the index date	1 year	3,728	18,,513
	2 years	3,727	18506
	3 years	3,302	16,358
	4 years	2,910	14,227
	5 years	2,535	12,324
	6 years	2,198	10,664
	7 years	1,896	9,207
	8 years	1,635	7,823
	9 years	1,400	6,621
	10 years	1,180	5,507
	11 years	963	4,470
	12 years	732	3,450
	13 years	571	2,660
	14 years	436	2,064
	15 years	326	1,550
	16 years	226	1,065
	17 years	146	759
	18 years	109	570
	19 years	82	419
	20 years	54	280
Median follow-up, y (IQR)	After the index date	6.1 (3.3-10.2)	6.0 (3.2-9.9)

Abbreviations: SD: standard deviation; CVD: cardiovascular disease; y:years; BMI: body mass index; IQR: interquartile range * The CDC growth-chart data set has not included children younger than age 2 years in BMI percentile calculation, however none of children younger than two years had data available on BMI in this study.
Index date is the time of first insulin prescription.

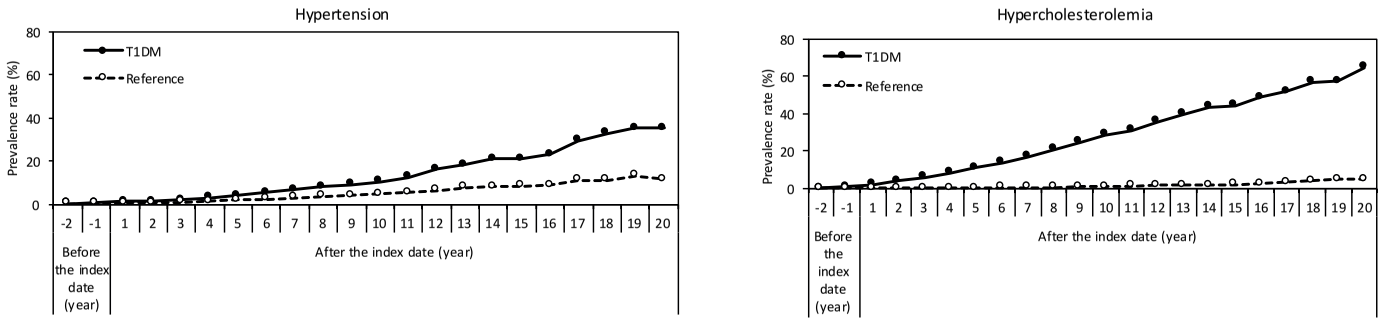

Fig 1. Prevalence rates of hypertension and hypercholesterolemia comparing the type 1 diabetes and the reference cohorts
Index date is the time of first insulin prescription.

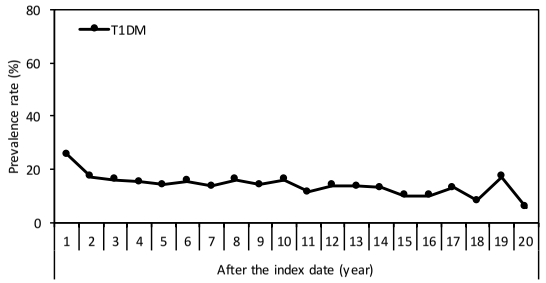

Fig 2. Prevalence rate of poor glycemic control (HbA1C>58 mmol/mol) among the type 1 diabetes cohort after the index date
Index date is the time of first insulin prescription.

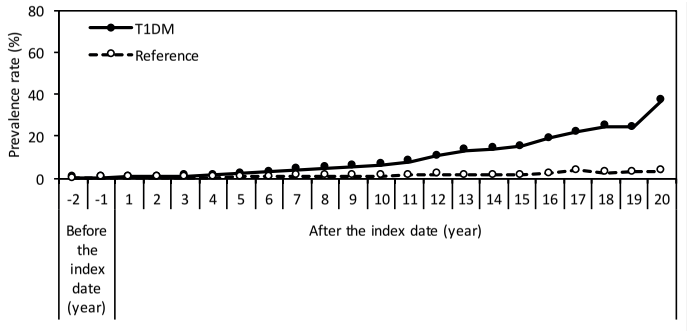

Fig 3. Prevalence rate of CV medication use comparing type 1 diabetes and the reference cohorts
Index date is the time of first insulin prescription.

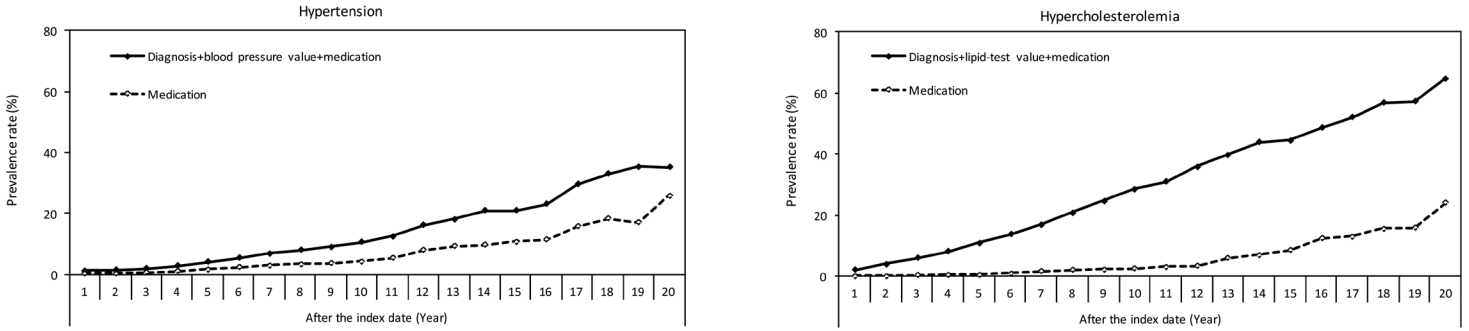

Fig 4. Undertreated hypertension and hypercholesterolemia (undertreatment for at least one year) in the type 1 diabetes cohort
Index date is the time of first insulin prescription.

more likely to have hypertension. Females in the T1DM cohort were statistically significantly more likely to have hypercholesterolemia while no significant difference between males and females was observed in the reference cohort (**Fig S4**).
The highest prevalence rate of a HbA1c level>7.5% (58 mmol/mol) was observed one year after the index date (26%) followed by a sharp decrease in the 5th year (14%) (**Fig 2**). After that it fluctuated and decreased to almost 6% at the end of follow-up (average rate from the second year after the index date on was 13%). Children aged 10-14 and 15-18 years at the index date were statistically significantly more likely to have poor glycemic control during the follow-up time compared to children in younger age groups. No statistically significant difference between males and females was found regarding glycemic control during follow-up (data not shown).

Time trends in CVD events and medication use

The number of children with CVD events during a maximum of 20 years follow-up was extremely low in both T1DM (n=5) and the reference cohort (n=9). The Kaplan–Meier survival curves for CVD events showed no statistically significant difference between the two cohorts (log-rank p=0.07).
The annual prevalence rate of CV medication use in the T1DM cohort compared with the reference cohort started from 0.40% vs. 0.18% (p=0.009) in the 2nd year before the index date and increased up to 37.0% vs. 3.6% (p<0.001) in the 20th year after the index date (**Fig 3**). The corresponding Kaplan-Meier analysis after the index date showed a statistically significantly higher risk of CV medication use in the T1DM cohort compared with the reference cohort (log-rank p<0.001).
Age-stratified prevalence and cumulative incidence rates of CV medication use showed that increasing age was associated with a higher risk of CV medication use. Children with T1DM in the age group of 15-18 at index date were most likely to receive these medications during follow-up (**Fig S5**).
Neither the prevalence nor the incidence rates of CV medication use differed between males and females, in the T1DM cohort. While in the reference cohort, females were statistically significantly more likely to use CV medication compared to males during the follow-up (data not shown).
Prevalence rates of antihypertensive medications and lipid-lowering medications are shown in **Figure S6**. Children in the T1DM cohort compared to the reference cohort were statistically significantly more often treated with antihypertensive medication and lipid-lowering drugs over the follow-up time; the highest rates were shown at the end of follow-up for both antihypertensive medication (25.9% vs. 3.2%, p<0.001) and for lipid-lowering drugs (24.1% vs. 0.0%, p<0.001), respectively. ACEIs and statins were the most commonly used CV medications in patients with T1DM. Prevalence rates of ACEIs in the T1DM and the reference cohorts started at the same rate (0.03%) in the 2nd year before the index date and increased to 18.5% vs. 1.1% (p<0.001) at the end of follow-up. Statins were also statistically significantly more often prescribed in diabetic children compared with the children in the reference cohort. Prevalence rates started from 0.0% vs.

0.01%, (p=0.65) at the 2nd year before the index date and increased to 24.1% vs. 0.0% (p<0.001) at the 20th year after the index date (**Fig S7**).

Undertreatment of hypertension or hypercholesterolemia in children with T1DM

In children with hypertension (based on physician's diagnosis and/or BP measurement and/or antihypertensive medication) (n=294), 205 (70%) were undertreated for at least one year after the index date (**Fig 4**). Chi square statistics showed that age was statistically significantly associated with undertreatment in this group (p=0.04) (**Table S1**). In the undertreated children (n=205), stratified analyses by the years being undertreated showed that 16 children (7.8%) were untreated for a period of at least 11 years during the follow-up. The majority (50%) of undertreated children in this group did not take anti-hypertensive medication for a period of 2-5 years (**Table S2**).

In children with hypercholesterolemia, 721 out of 739 diabetic children (98%) who were diagnosed with hypercholesterolemia (based on physician's diagnosis and/or laboratory test results and/or lipid-lowering medications) were not treated pharmacologically for at least one year during follow-up (**Fig 4**). Age, gender and family history of CVD were not statistically significant associated with undertreatment of hypercholesterolemia (**Table S1**). In the undertreated group (n=721), 43 (5.9%) children were shown to be undertreated for hypercholesterolemia for at least 11 years during the follow-up. Most children were undertreated for a period ranging between 2-5 years (53%) (**Table S2**).

DISCUSSION

To the best our knowledge this study provides the first large population-based study with a long follow-up time to quantify the rates of CVD risk factors, CVD events and CV medication use in children with T1DM. Children with T1DM had statistically significantly higher rates of hypertension, hypercholesterolemia and used more CV medication compared with a matched diabetes-free reference cohort in the period after the onset of diabetes. However, the percentage of children diagnosed with CVD events, as expected, was low (and not different) in both cohorts. The statistically significantly higher rates of hypertension, hypercholesterolemia and CV medication already started one year before the onset of diabetes and further increased during the 20-year follow-up. Rates of hypertension were similar among males and females in both cohorts. Females were more likely to have hypercholesterolemia in the T1DM cohort and to use CV medication in the reference cohort. Older children (15-18 years at index date) were more likely to have hypertension and hypercholesterolemia compared with younger peers. Our data indicated that a substantial number of diabetic children with hypertension (70%) and hypercholesterolemia (98%) were undertreated at least for a period of one year during the 20-year follow-up. Age was the only determinant that appeared to be associated with undertreated hypertension in the T1DM cohort.

Although, it is not clear yet what causes the higher rates of hypertension and hypercholesterolemia in the year prior to the onset of diabetes, we speculate that beta cell destruction or the underlying factors causing this destruction before the clinical presentation of diabetes could be possible reasons[23]. Further research is warranted to interpret and understand the increased risk of CVD risk factors prior to the clinical onset of T1DM. The higher rates of hypertension and hypercholesterolemia in diabetic children compared with the reference cohort, is in line with previous studies[10-15]. Previous studies also showed an increased risk of CVD abnormalities e.g. carotid intima-media thickness and increased arterial stiffness in children with T1DM[3-5]. Elevated HbA1c in diabetic patients plays a role in the pathogenesis of CVD risk factors particularly hypertension[24] and predicts long-term CVD outcomes[22,25].

Recently, researchers showed that adolescent females compared to males with T1DM had a significantly worse CVD risk profile (higher BMI, HbA1c and cholesterol level)[26]. In our study, in the T1DM cohort, there were no clear differences between males and females with respect to hypertension, however, females were statistically significantly more likely to have hypercholesterolemia during the follow-up.

The high percentages of children with T1DM undertreated for at least one year for hypertension (70%) and hypercholesterolemia (98%) in our study is in line with previous findings[10-16]. For instance, in pediatric diabetes clinics next to under-diagnosis undertreatment of hypertension was reported[15]. Also Zgibor et al. showed that CVD risk factors, particularly hypercholesterolemia are not adequately treated in patients with childhood onset of T1DM[16]. This is a reason for concern because hypertension and hypercholesterolemia are the most important modifiable risk factors for CVD events[27-29].

Current guidelines[20,30,31] highlight the importance of screening, diagnosis and treatment of hypertension and hypercholesterolemia in children with T1DM. Based on these guidelines, pharmacological treatment of hypertension in diabetic children should be considered as soon as the diagnosis is confirmed[31,32]. In children with dyslipidemia, after the age of 10 years, addition of a statin is suggested in those who, despite lipid-lowering diet and lifestyle changes, continue to have LDL cholesterol ≥130 mg/dL (3.4 mmol/L) or in children with multiple CVD risk factors[31].

Since patients with T1DM are at excessive risk of CVD[8], the high proportion of diabetic children not treated for hypertension and hypercholesterolemia indicates an area for improving care in this population. Implementation of guidelines on the management of CVD risk factors in diabetic patients should therefore be reinforced. A recent meta-analysis showed the beneficial effect of blood pressure-lowering treatment on CVD morbidity in adults with diabetes[33], but studies in children are limited. Although the rate of vascular progression in children is still slow[8] intensive treatment of CVD risk factors should be implemented in pediatric population to prevent CVD later in life[34].

The evaluation of glycemic control in the T1DM cohort showed that 13% of the children had a HbA1c level above 7.5% (58 mmol/mol) at least once during follow-up from the second year after the index date. Although this is obviously undesirable we realize there are many reasons for disturbance of glycemic control including the occurrence of infections, diet changes, stress, life style changes etc[35-37]. Previous studies have shown that a

relatively low percentage of children and adolescents with T1DM attained target HbA1c levels[1,9]. The reason for this discrepancy might be due to different data collection and different methods applied.

We used the CPRD database, which is a population-based database with a high quality data collection. Children in the T1DM cohort were all diagnosed with diabetes and treated with insulin, and probably are representative for the children with T1DM in the UK. The current estimated number of children and young people with diabetes, under the age of 19 in the UK is 42,000 where the vast majority (around 95%) of this population has T1DM[38].

Despite positive aspects of using CPRD for this study, there are some limitations that should be addressed. An important limitation could be a possibility of ascertainment bias in which CVD risk factors were more diagnosed and treated in the T1DM cohort due to increased screening in this population compared to the reference cohort.

Lack of information on the indication for prescribed medications is another important issue, which might lead to misclassification bias. For instance, beta-blockers might also be indicated for test anxiety before school exams or driving tests. Another limitation was the high amount of missing information about smoking, height and weight (and therefore BMI) and the family history of CVD. This limited us in the evaluation of the prevalence rates of these risk factors but also in the evaluation of determinants of CVD risk factors. Furthermore, it limited us in the classification of hypertension since hypertension in children is defined as systolic and/or diastolic blood pressure that is ≥95th percentile for the corresponding age, gender, and height group. Moreover, since the data for this study was collected from 1987 to 2015 guidelines and recommendations for treatment of children and youth have changed during this period. Earlier guidelines did not recommend treatment with lipid-lowering and antihypertensive drugs before the age of 18 years and the recommended duration of life-style changes (diet and exercise) before considering initiation of medication was much longer. Finally, the lack of information on lifestyle modifications and dietary changes did not allow us to evaluate the first treatment steps of hypertension and hypercholesterolemia.

In summary, our findings confirm that children with T1DM are at increased risk for hypertension and hypercholesterolemia both before and after the onset of diabetes and that there is a substantial amount of undertreatment of these risk factors. The key message of our study is that there should be more attention for the pharmacological treatment of hypertension and hypercholesterolemia in children with T1DM based on available guidelines to prevent CVD later in life.

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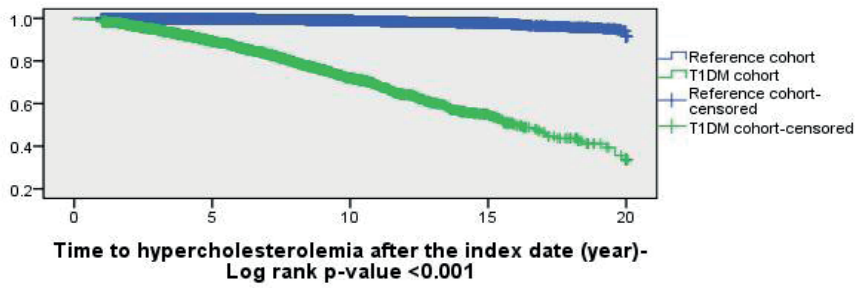
SUPPORTING INFORMATION



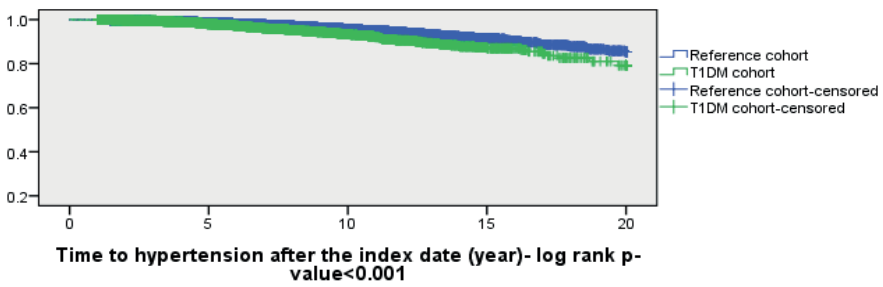


Fig S1. Time to hypertension and hypercholesterolemia comparing the type 1 diabetes and the reference cohorts. *Index date is the time of first insulin prescription.*

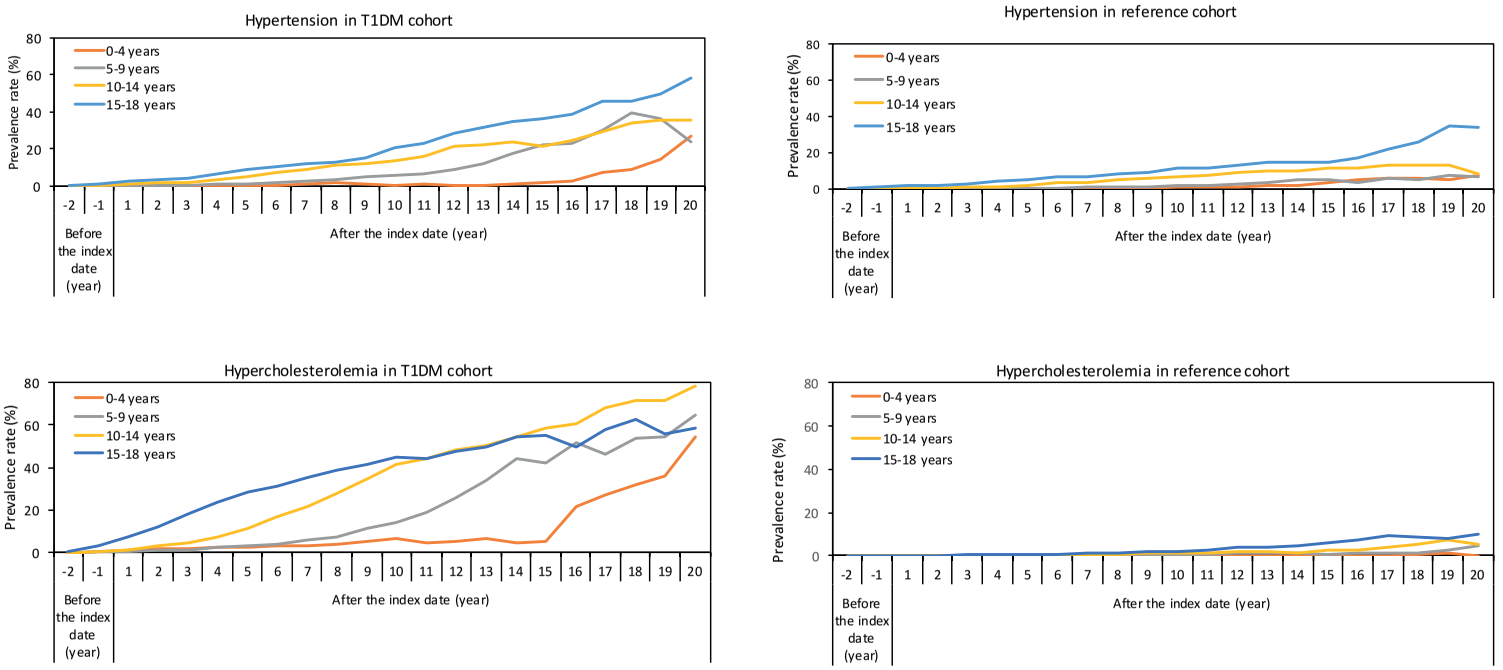
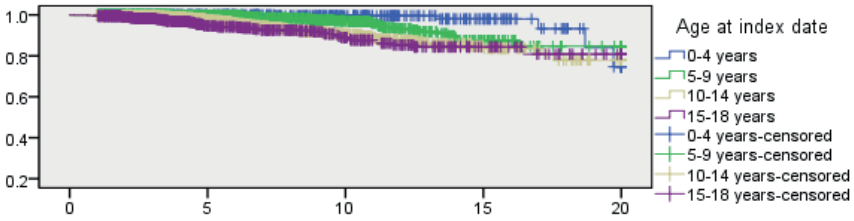
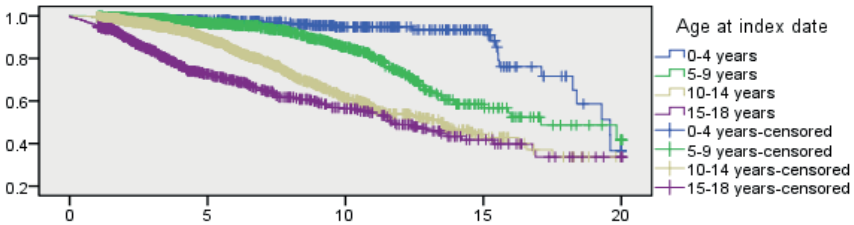


Fig S2. Index date age stratified prevalence rates of hypertension and hypercholesterolemia
Index date is the time of first insulin prescription.



Time to hypertension in the T1DM cohort after the index date (year)- Log rank p-value<0.001



Time to hypercholesterolemia in the T1DM cohort after the index date (year)-Log rank p-value<0.001

Fig S3. Time to hypertension and hypercholesterolemia in the type 1 diabetes cohort comparing different age categories.
Index date is the time of first insulin prescription.

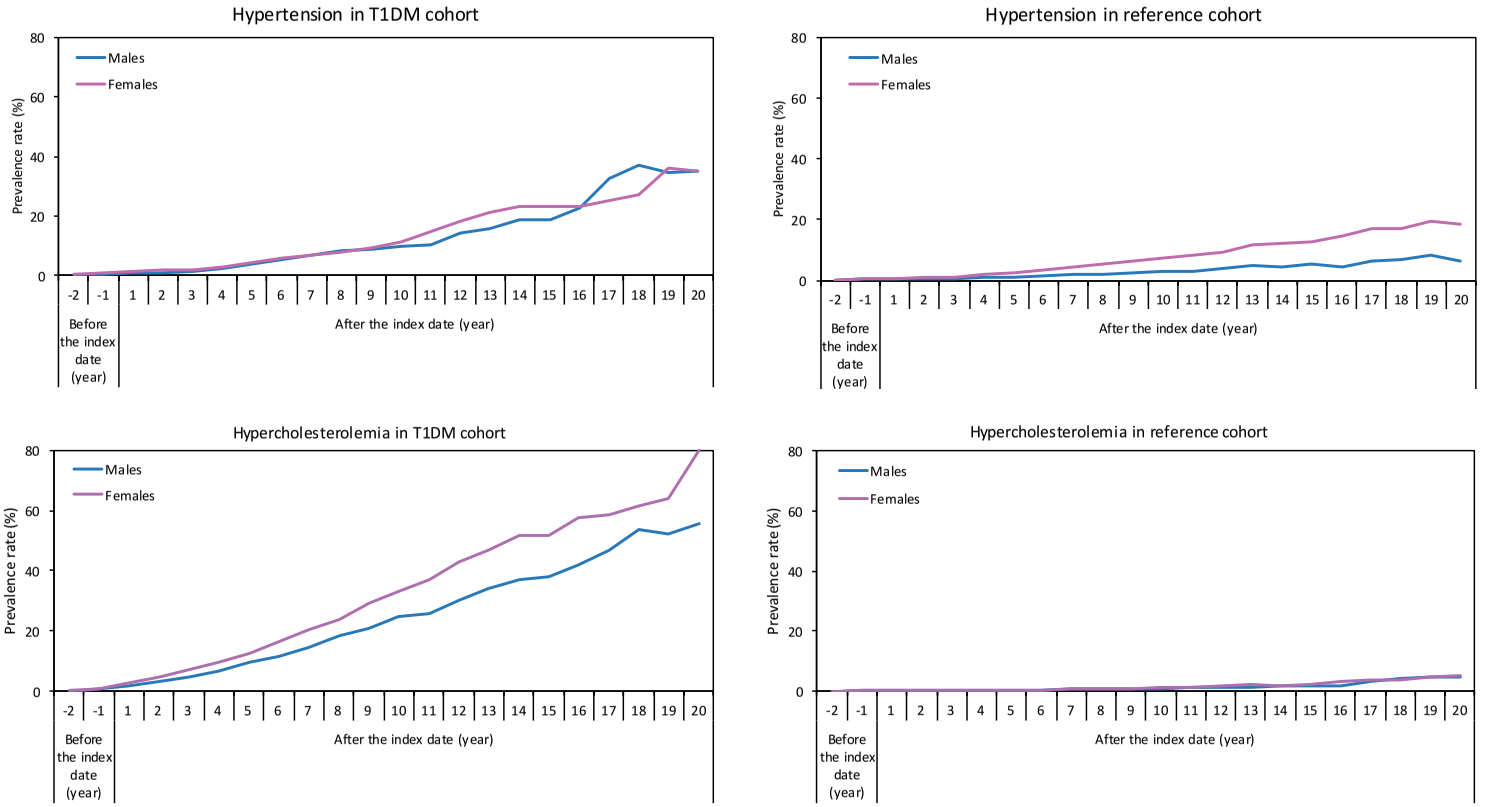


Fig S4. Gender-stratified prevalence rates of hypertension and hypercholesterolemia
Index date is the time of first insulin prescription.

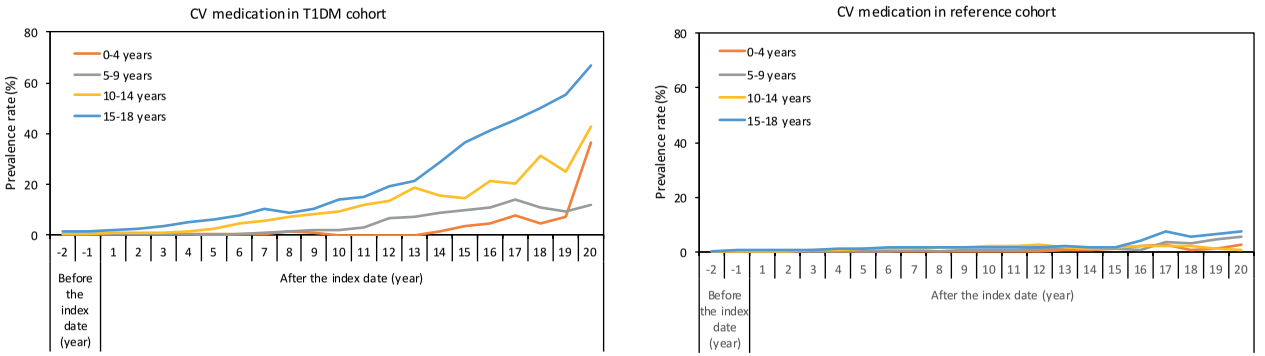


Fig S5. Index date age-stratified prevalence rates of cardiovascular medication use.
Index date is the time of first insulin prescription.

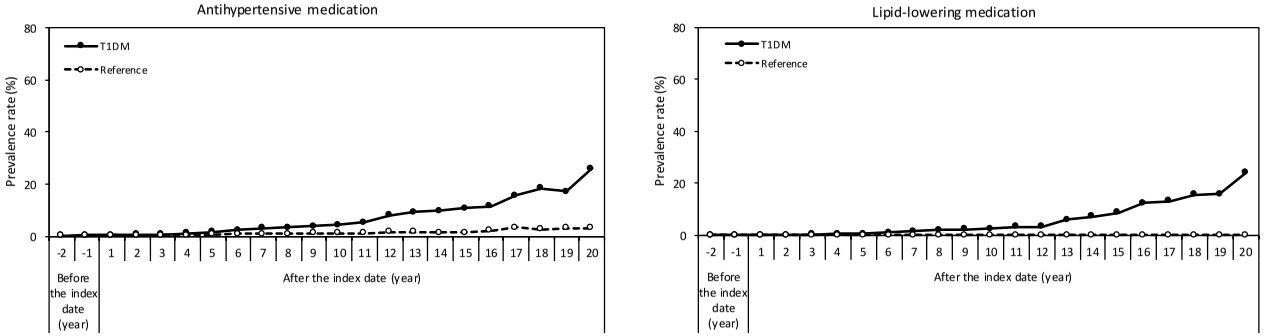


Fig S6. Prevalence rates of anti-hypertensives and lipid-lowering drugs comparing type 1 diabetes and the reference cohorts
ndex date is the time of first insulin prescription.

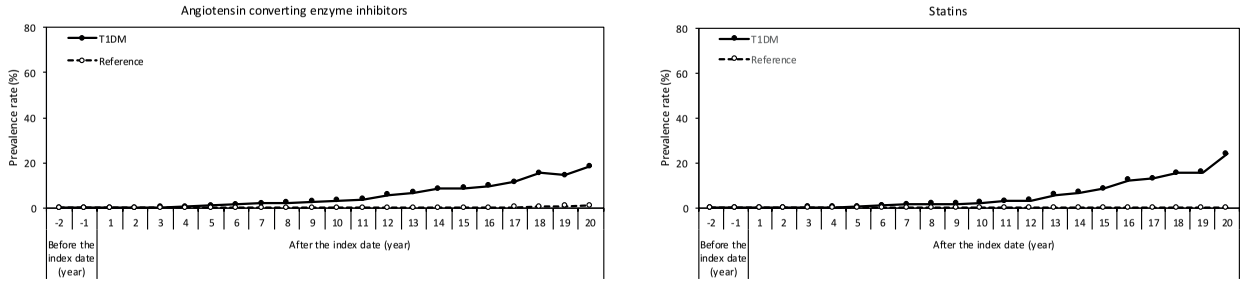


Fig S7. Prevalence rates of angiotensin converting enzyme inhibitors and statins use comparing type 1 diabetes and the reference cohorts.
Index date is the time of first insulin prescription.

Table S1. Determinants of undertreated children (at least one year no medication) for hypertension and hypercholesterolemia in the T1DM cohort

		Hypertension (n=294)				Hypercholesterolemia (n=739)		
		Treated (N=89)	Not-treated (N=205)	P-value		Treated (N=18)	Not-treated (N=721)	P-value
Gender, n (%)	Males (n=147)	52 (58.4)	95 (46.3)	0.06	Males (n=350)	10 (55.6)	340 (47.2)	0.48
	Females (n=147)	37 (41.6)	110 (53.7)		Females (n=389)	8 (44.4)	381 (52.8)	
Age at index date, n (%)	0-4 y (n=9)	4 (4.5)	5 (2.4)	**0.04**	0-4 y (n=31)	1 (5.6)	30 (4.2)	0.92
	5-9 y (n=52)	8 (9.0)	44 (21.5)		5-9 y (n=125)	3 (16.7)	122 (16.9)	
	10-14 y (n=143)	51 (57.3)	92 (44.9)		10-14 y (n=340)	9 (50.0)	331 (45.9)	
	15-19 y (n=90)	26 (29.2)	64 (31.2)		15-19 y (n=243)	5 (27.8)	238 (33.0)	
Family history of CVD, n (%)	Yes (n=35)	9 (10.1)	26 (12.7)	0.53	Yes (n=64)	2 (11.1)	62 (8.6)	0.68
	No (n=259)	80 (89.9)	179 (87.3)		No (n=675)	17 (88.9)	659 (91.4)	

Abbreviations: T1DM: type 1 diabetes mellitus; y: years; CVD: cardiovascular disease

Table S2. Stratified analyses in undertreated children for hypertension and hypercholesterolemia in the T1DM cohort, by the years of undertreatment

Years of undertreatment	Undertreated for hypertension (n=205)	Undertreated for hypercholesterolemia (n=721)
≤ 1 y, n (%)	40 (19.5)	59 (8.2)
2-5 y, n (%)	102 (49.8)	384 (53.3)
6-10 y, n (%)	47 (22.9)	235 (32.6)
≥ 11 y, n (%)	16 (7.8)	43 (5.9)

Abbreviations: T1DM: type 1 diabetes mellitus; y: years

Chapter 2.3

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Asthma related medication use and exacerbations in children and adolescents with type 1 diabetes

Pediatr Pulmonol (2016); PMID: 27132537

ABSTRACT

Objectives: To investigate the use of asthma medication and occurrence of asthma exacerbations up to 5 years before and after the onset of type 1 diabetes mellitus (T1DM) in children and adolescents.

Methods: Children and adolescents younger than 19 years with at least 2 insulin prescriptions between 1999 and 2009 classified as T1DM cohort (n=915) and a 4 times larger reference cohort (n=3,590) with the same age and gender were identified from the Dutch PHARMO Record Linkage System. The date of first insulin dispensing was selected as the index date.

Results: The 5-year prevalence rate of asthma medication use in the T1DM cohort (23.2%) was significantly higher than the reference cohort (18.3%) after the onset of diabetes. No statistically significant difference between the two cohorts was observed in the use of specific types of asthma medication except for short acting muscarinic antagonists that were significantly more used in the T1DM cohort (5.5%) compared with the reference cohort (0.62%) after the onset of diabetes. The incidence rate of asthma medication use declined over time with a peak in the T1DM cohort the first year after the onset of diabetes. Furthermore, one year after the index date there was a peak in incidence rate of asthma exacerbations in both T1DM (7.8 per 1000 person year) and reference (6.8 per 1000 person year) cohorts.

Conclusions: T1DM is associated with statistically significantly higher asthma medication use after the onset of T1DM, especially in the first year after the onset of diabetes.

INTRODUCTION

The association of type 1 diabetes (T1DM) and asthma in children has been studied with controversial results. Some studies have reported a significant reduction in the prevalence of asthma in children with T1DM[1,2], while other studies have shown a higher risk of asthma in this population[3-6]. Tosca et al. also showed no difference in the frequency of asthma between T1DM patients and control group[7]. It has also been reported that children with T1DM have an abnormal lung function in which forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1) and transfer factor for carbon monoxide (TLCO) were reported to be significantly lower compared with children in the general population[8-10]. Previous studies investigating the association between lung function and glucose regulation showed that dysregulated glucose was related with impaired lung function[3,11]. So far, no study has quantified asthma medication use in children and adolescents with T1DM before and after the onset of diabetes. Quantification of asthma medication use in children and adolescents before and after the onset of T1DM might provide further insight into the relation between T1DM and impaired lung function or asthma. Therefore, we conducted a population-based cohort study by using community pharmacy prescription records linked to hospital diagnoses to calculate the prevalence and incidence rates of asthma medication use and incidence rates of asthma exacerbations in children and adolescents with T1DM and we compared these rates with a group of age- and gender-matched diabetes-free children and adolescents in the Netherlands.

METHODS

Setting

A population-based cohort study was conducted using the Dutch PHARMO Record Linkage System (RLS) (http://www.pharmo.nl) that comprises community pharmacy dispensing records linked to hospital admissions. Nowadays data from more than 4 million residents (both rural and urban areas) of the Netherlands (approximately 24% of the Dutch population) are collected in PHARMO RLS[12,13]. The drug dispensing records consist of data on the dispensed drug, the type of prescriber, the dispensing date, the amount dispensed, and the written dose instructions. Dates of hospital admissions and discharges, together with primary and secondary diagnoses are documented in the hospital records. Diagnoses are coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) (http://icd9cm.chrisendres.com), whereas the drugs are coded according to the Anatomical Therapeutic Chemical codes (ATC codes) (http://www.whocc.no/atc_ddd_index). Information is recorded since 1986 and has been used in many pharmacoepidemiologic studies[12,13]. Hospital diagnoses and drug exposures retrieved from the prescription records in the PHARMO RLS have been validated in several studies[14-16].

Study population

In this study, the T1DM cohort was defined as a cohort including children and adolescents (<19 years old) with at least 2 insulin prescriptions between 1999 and 2009. The date of first insulin prescription or first hospital admission for T1DM was selected as the entry date (index date). For each patient in the T1DM cohort, up to 4 diabetes-free children and adolescents (without any prescription of glucose lowering medications (ATC code: A10) or hospitalization for diabetes (ICD-9-CM code: 250) during the study period with the same gender, age, and calendar time distribution were randomly sampled from the PHARMO RLS (reference cohort). Patients in both cohorts were included in the study if they had at least 12 months of drug history before and at least 12 months follow-up after the index date. Exclusion criteria for the T1DM cohort were having a glucagon (ATC code H04AA01) prescription prior to the index date (glucagon is usually prescribed in patients with diabetes for the management of hypoglycemia, therefore in patients who had prescriptions of glucagon before the insulin prescriptions, the index date was not clear) and ever use of oral anti-diabetic agents (in order to exclude potential patients with type 2 diabetes, ATC code A10B). Also patients in both cohorts with a history of cystic fibrosis (CF) (ICD-9-CM code: 277) were excluded from the study in order to exclude CF-related diabetes and respiratory problems. Both cohorts were followed from a maximum of 5 years before until a maximum of 5 years after the index date.

Asthma medication use and exacerbations

For both cohorts, exposure to asthma medication was defined as a dispensing for any asthma medication categorized as ATC code R03 (drugs for obstructive airway disease) (**Table S1** in Appendix). Asthma exacerbations in both cohorts was defined as discharge diagnoses of asthma (ICD-9 code 493) and/or short courses of oral corticosteroids (OCS) (ATC code: H02AB), for a period ≤14 days that were dispensed in children using asthma (R03) medication.

Statistical analysis

To summarize the characteristics of both cohorts we used descriptive statistics. 5-year prevalence rates of asthma medication use in general and for specific subgroups in the period before and after the index date were calculated by dividing the total number of patients receiving prescriptions to treat asthma symptoms by the average number of children and adolescents studied 5 years before and after the index date. Annual prevalence rates of asthma medication use were also calculated. The 5-year prevalence rate and annual prevalence rate of asthma medication were stratified by different age categories (using age bands 0-4, 5-9, 10-14, and 15-18 years) and gender which was compared by an ordinal chi square test. To assess patterns over time, the 5-year prevalence rate of asthma medication use and the 5-year number of asthma prescriptions per child were calculated from 5 years prior to the index date up to 5 years after the index date in both cohorts and further stratified by age. Annual incidence rates of asthma medication use and asthma exacerbations in both cohorts were defined as the number of incident medication users and incident cases with asthma exacerbations during a given time period divided

by the person time at risk. For calculating annual incidence rates, to exclude prevalent cases in each year, subjects were required to have at least 12 months prior history (either a drug prescription or the occurrence of asthma exacerbations) in the database. Time to events (asthma medication consumption and exacerbations) in the two cohorts was compared using the Kaplan–Meier method followed by log-rank test. Two-tailed p-values were considered significant at 0.05. Data analyses in this study were performed using SPSS version 23.0 (SPSS, Chicago,IL).

RESULTS

We identified 915 children and adolescents with at least 2 insulin prescriptions (T1DM cohort) from the PHARMO RLS and compared them with a group of 3,590 age- and gender-matched diabetes-free individuals (reference cohort). At the index date, boys comprised just over half of the population in both T1DM (50.9%) and the reference (50.6%) cohorts and the median age was 10 years (interquartile range (IQR) 7-14 years) (**Table 1**).

Prevalence rates of asthma medication use

Before the index date there was no difference between the 5-year prevalence rate of asthma medication use in the T1DM cohort (28.3%, 95% CI: 25.2-31.7) compared to the reference cohort (27.6%, 95% CI: 26.0-29.2), while after the index date the 5-year prevalence rate was statistically significantly higher in the T1DM cohort (23.2%, 95% CI: 20.4-26.3 vs. 18.3%, 95% CI: 16.9-19.7). As shown in **Figure 1**, the prevalence rate of asthma medication use in the T1DM cohort slightly dropped from 28.3% (n=206 asthma medication users) during 5 years before the index date to 23.2% (n=182 asthma medication users) in the years after diagnosing T1DM. After the index date there were 100 prevalent users who also used asthma medication before the onset of diabetes and the percentage of incident asthma medication users was 45.0% (n=82). In the reference cohort the percentage of incident users was 23.0% in the period after the index date. The higher prevalence rate of the use of asthma medication in the T1DM cohort compared with the reference cohort was most pronounced in the first year after the index date ($p<0.001$) and disappeared afterwards ($p=0.47$ in the 5 years after the index date) (**Fig 2A**).

As shown in **Figure 3**, children aged 4 years and younger in both cohorts, had considerably higher prevalence rates of asthma medication use over the 5 year period before and after the index date (14.7% and 17.4% in the T1DM cohort and 15.3% and 11.4% in the reference cohort, respectively) compared with those in the other age categories (rates below 12%). Furthermore, substantially higher prevalence rates of asthma medication use in the T1DM cohort compared with the reference group in the year after the index date was observed only in children aged 0-4 ($p<0.001$) and 10-14 ($p=0.02$) years old (**Fig 4**).

Table 1. Baseline characteristics of patients with T1DM compared with diabetes-free subjects

		T1DM cohort (n=915)	Reference cohort (n=3,590)
Gender, n (%)	Boys	465 (50.9)	1,817 (50.6)
	Girls	450 (49.2)	1,773 (49.4)
Age at diagnosis (Index date), n (%)	0-4 y	132 (14.3)	537 (15.0)
	5-9 y	269 (29.1)	1,042 (29.0)
	10-14 y	333 (36.4)	1,295 (36.1)
	15-18 y	181 (19.8)	716 (19.9)
Age (median, IQR), y		10 (7-14)	10 (7-14)
Follow-up before the index date (median , IQR), y		2.8 (1.8-3.8)	2.9 (1.9-3.9)
Follow-up after the index date (median , IQR), y		3.1 (2.0-4.1)	3.1 (2.0-4.1)
Number of participants in each year of the study period	5 years before the index date	480	1,911
	4 years before the index date	600	2,378
	3 years before the index date	731	2,908
	2 years before the index date	914	3,582
	1 year before the index date	915	3,590
	First year of follow-up	915	3,590
	Second year of follow-up	913	3,578
	Third year of follow-up	800	3,130
	Fourth year of follow-up	698	2,725
	Fifth year of follow-up	595	2,314
Average number of participants during 5 years of the study period	Before the index date	728	2,874
	After the index date	784	3,067
Number of prescriptions per child before the index date, n (%)	One prescription	54 (26.2)	211 (26.6)
	2 prescriptions	49 (23.8)	144 (18.2)
	≥3 prescriptions	103 (50.0)	437 (55.2)
Number of prescriptions per child after the index date, n (%)	One prescription	57 (31.3)	161 (28.8)
	2 prescriptions	28 (15.4)	88 (15.7)
	≥3 prescriptions	97 (53.3)	311 (55.6)

Abbreviations: T1DM: type 1 diabetes mellitus; IQR: interquartile range; y: years
Index date is the time of first insulin dispensing.

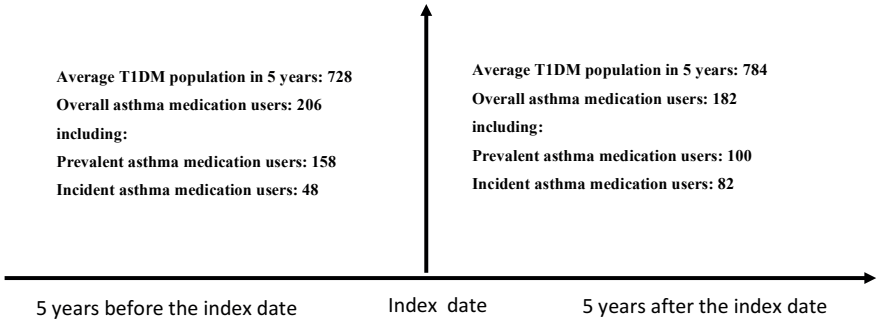

Fig 1. 5-year prevalence rate of asthma medication use, comparing T1DM cohort before and after the onset of diabetes
Index date is the date of first insulin dispensing.

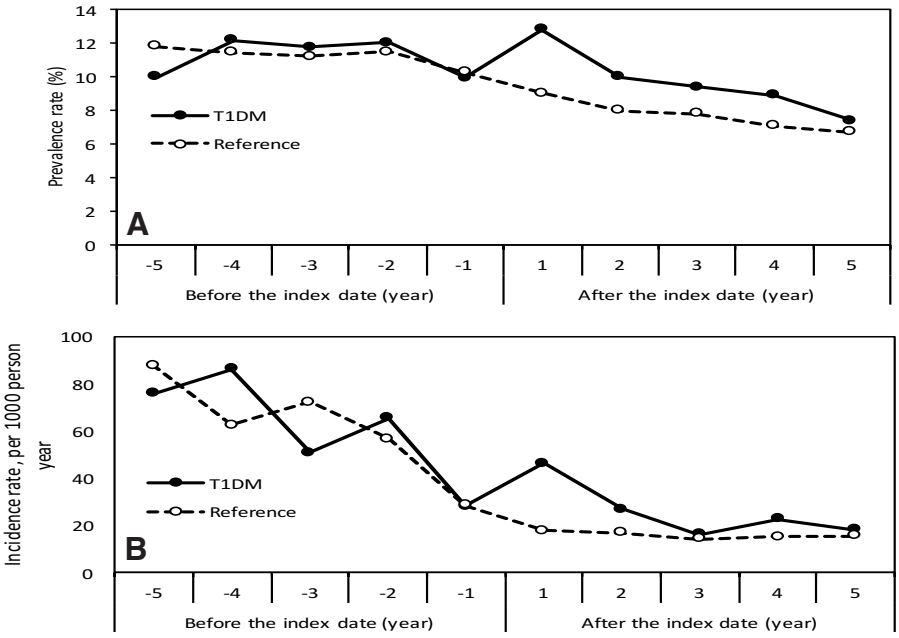

Fig 2. A: Annual prevalence rate of asthma medication use, comparing T1DM and reference cohorts before and after the index date. B: Annual incidence rate of asthma medication use, comparing T1DM and reference cohorts before and after the index date.

Index date is the date of first insulin dispensing.

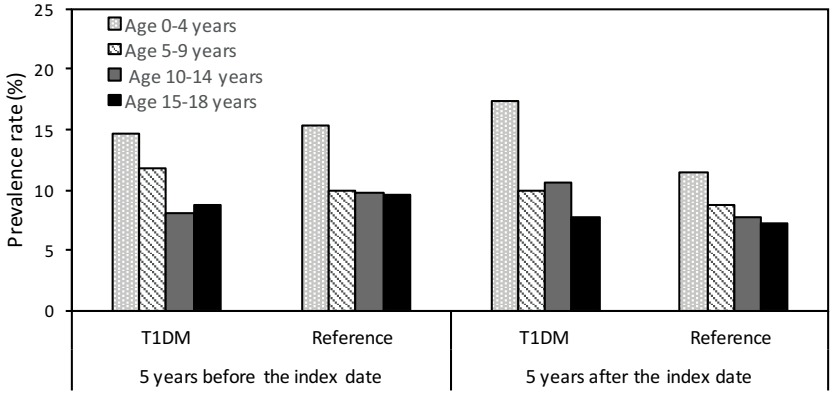

Fig 3. 5-year prevalence rate of asthma medication use, comparing T1DM and reference cohorts before and after the index date by age.

Index date is the date of first insulin dispensing.

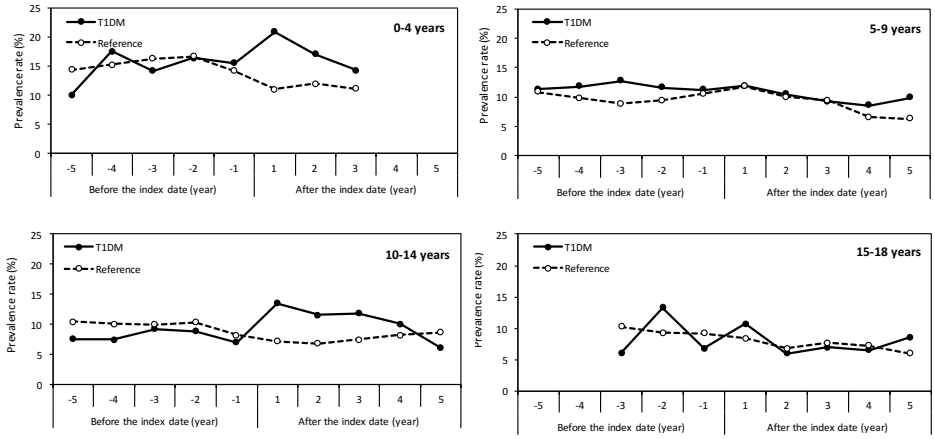

Fig 4. Annual prevalence rate of asthma medication use, comparing T1DM and reference cohorts before and after the index date by age.
Index date is the date of first insulin dispensing.

Asthma medication was more frequently used by boys in the T1DM cohort (32.7%) compared with girls (23.8%) before the index date (p=0.02). The same pattern was observed in the reference cohort (30.8% vs. 24.3%, respectively; p<0.001). After the index date, there was no statistically significant difference between girls and boys in both T1DM (p=0.29) and reference (p=0.07) cohorts.

Asthma medication utilization pattern

When investigating subgroups of medication, short acting beta agonists (SABAs) and inhaled corticosteroids (ICSs) were used most frequently in both cohorts. The 5-year prevalence rate of short acting muscarinic antagonists (SAMAs) was significantly higher in the T1DM cohort compared with the reference cohort (5.5% vs. 0.6%, p<0.001) after the onset of diabetes (**Table S2**). 43 out of 182 patients (almost 24%) who used asthma medications after the index date in the

T1DM cohort received SAMAs. Many of these patients were treated with SAMAs only or had a medication history of only using SABAs and ICSs before and after the index date. After the index date, children aged 4 years and younger were more likely to use SABAs and ICSs compared to the other age categories in both the T1DM cohort (36.2% and 14.9%, respectively; p=0.02) and reference cohort (8.9% and 7.3% respectively; p<0.001). A higher consumption of long acting beta agonists (LABAs) and SAMAs was found in 10-14 years old adolescents compared to the other age categories in T1DM cohort (**Fig S1**).

A majority of children among asthma medication users in the T1DM cohort (51.2%) and in the reference cohort (55%) received at least three asthma prescriptions while the percent-

age of children who received only one prescription was 28.8% and 27.7%, respectively during the complete follow-up. Children aged 4 years and younger received more frequently at least three prescriptions for asthma compared to only one prescription. SABAs and SAMAs were more often prescribed only once while the number of prescribed ICSs per child was higher; for these drugs children received at least 3 prescriptions.

Incidence rate of asthma medication use

Annual incidence rate of asthma medication use during the 5 years before and 5 years after the index date are presented in **Figure 2B**. The results show that the highest incidence rate of asthma medication use was observed in the period 4-5 year before the index date and further declined through follow-up in both cohorts with a peak in the T1DM cohort the first year after the onset of disease; 46.3 per 1000 person year (PY) (95% CI: 33.0-64.6) in the T1DM cohort compared to 17.9 per 1000 PY (95% CI: 13.6-23.6) in the reference group. The decreasing pattern in the T1DM cohort further continued until the end of follow-up (18.1 per 1000 PY). In the reference cohort, the incidence rate of asthma medication use shows a decrease during the years before and after the index date. The results of the Kaplan-Meier analyses showed a statistically significant difference for the incidence rate of asthma medication use between the two cohorts after the index date (p=0.001) (**Fig 5**).

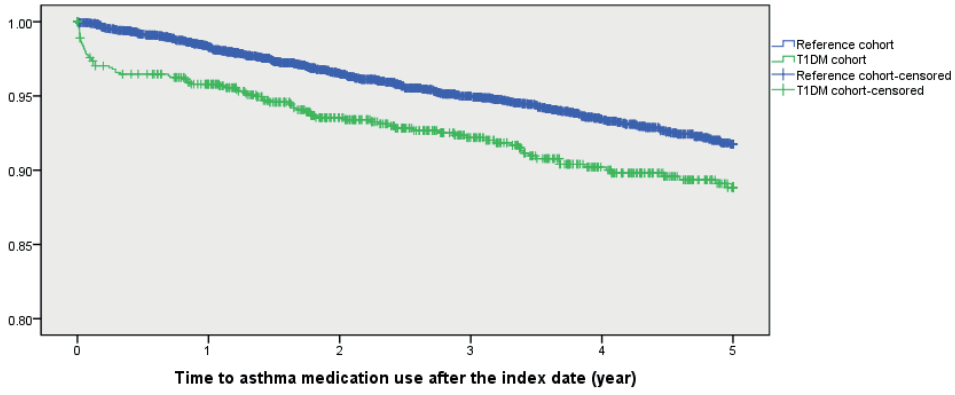

Fig 5. Kaplan-Meier analysis comparing time to asthma medication use in the T1DM and the reference cohorts after the index date (p-value<0.001)
Index date is the date of first insulin dispensing.

Incidence rate of asthma exacerbations

There was a peak in asthma exacerbations in the first year after the index date in both the T1DM (7.8 per 1000 PY) and the reference (6.8 per 1000 PY) cohorts. Although, the decreasing pattern after the index date was more pronounced in the T1DM cohort; at the end of follow-up both cohorts had almost the same incidence rates of exacerbations (5.2 per 1000 PY in the T1DM cohort and 5.4 per 1000 PY in the reference cohort) (**Fig 6**).

Kaplan-Meier analyses and log-rank tests showed no statistically significant difference in time to exacerbations between the two cohorts after the index date (p=0.49) (**Fig 7**).

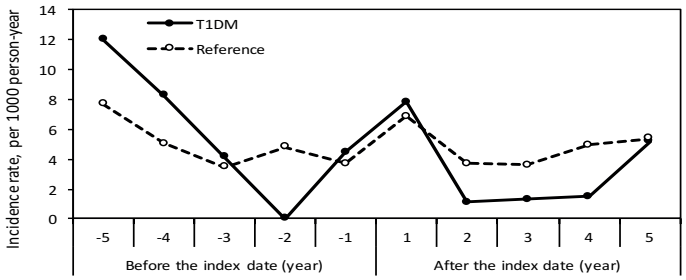

Fig 6. Annual incidence rate of asthma exacerbations, comparing T1DM and reference cohorts before and after the index date.
Index date is the date of first insulin dispensing.

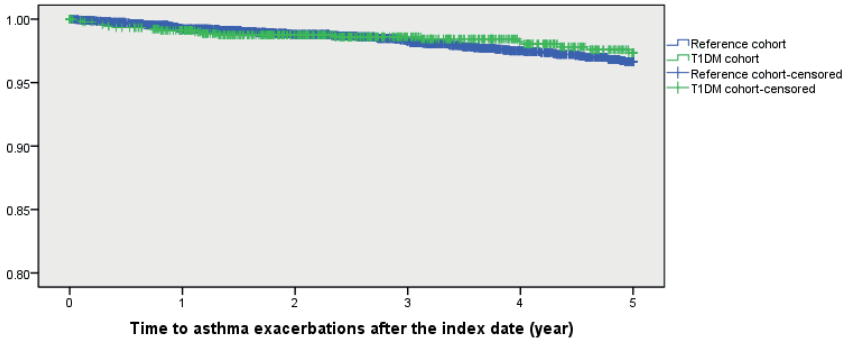

Fig 7. Kaplan-Meier analysis comparing time to asthma exacerbations in the T1DM and the reference cohorts after the index date (p-value=0.49).
Index date is the date of first insulin dispensing.

DISCUSSION

In this study, significantly more children and adolescents with new onset T1DM used asthma medication in the period after the onset of diabetes than diabetes-free individuals while no statistically significant difference in the 5-year prevalence rate of asthma medication use was observed before the index date. The number of children and adolescents using asthma medication decreased in both cohorts during follow-up time. Remarkably, in the T1DM cohort there was a peak in both the annual prevalence and incidence rates of asthma medication in the first year after the onset of diabetes; however the incidence rate of asthma exacerbations was comparable to the reference group.
Asthma is a chronic inflammatory airway disease with symptoms of cough, wheeze and shortness of breath usually related to some triggering events, which are not specific and

many other conditions can cause similar symptoms in children, especially preschool children. This makes the diagnosis of asthma difficult.

In our study we found that children aged 4 years and younger in both cohorts were more likely to have asthma medication prescriptions compared to the other age groups. Moreover, our results show that the higher prevalence rate of asthma prescriptions in recent onset diabetic patients compared to the reference group in the year after the index date was more pronounced in children aged 0-4 and 10-14 years old. Firstly, our finding strongly reflects the higher burden of respiratory complaints in younger age groups[17,18], not only in those with T1DM. Prevalence rate of asthma-like symptoms was shown to be 32.0% in the first years of life (children younger than 5 years) in a Danish population[19] and also higher in children aged 0-4 years (12.5%) than those in age of 5-7 (10.4%) in the Dutch population[20]. This could be explained by the smaller airways of young children (and more specific boys, premature and low for gestational age children) that are more prone to a relevant airway obstruction (and wheezing) during viral colds and after exposure to irritants. Therefore, the rise in asthma medication prevalence after T1DM onset might be partly related to a potential increase of asthmatic symptoms by the diabetes itself, but might also be related to a higher concern and awareness of parents and physicians to treat respiratory symptoms properly after the T1DM diagnosis was established.

The statistically significant higher prevalence rate of asthma medication use in boys confirms previous findings that boys are more often diagnosed and treated with asthma medication[20-22]. Lowered microbial exposure in early life might also contribute to the increasing prevalence of asthma[23]. When specifically considering a possible correlation of airway patency and glucose metabolism within this above mentioned pathophysiologic concept, an association between low body weight in the first years of life and impaired glycemic tolerance and decreased lung function can be considered[24-29]. Secondly, diabetes control might be influenced by hormonal changes in puberty in which puberty in diabetic adolescents (10-14 years) is associated with declined insulin sensitivity[30,31]. However, another explanation could be the increased therapy adherence triggered by the T1DM diagnosis in adolescents where treatment adherence is generally lower in this age group.

The annual prevalence and incidence rates showed a peak in the first year after the onset of diabetes followed by a gradual decline in asthma medication use during the follow-up time. The peak might be a result of regular contact with the physician after the diagnosis of T1DM which increases the probability of diagnosing and prescribing medications for respiratory symptoms (detection bias). The disappearance of the peak one year after the index date could be explained in two ways. Basically, part of the observed effect can be probably explained by increasing age. Besides, it might be because of the link between glucose regulation and lung function as mentioned above[3,11,32,33]. When diabetes is well regulated the asthma-like complaints disappear in these children. Therefore, we suggest physicians to pay more attention to control and treat diabetic children before starting medication for respiratory symptoms.

Our study showed no statistically significant difference between the two cohorts for the subgroups of asthma medication that were used except for SAMAs, that were statistically significantly more prescribed in the diabetic adolescents after the index date compared

with the reference cohort. Given the low numbers of SAMAs prevalence rate in general, it is not clear whether the difference in use is of clinical relevance. However, one possible explanation might be that it is a marker for the severity of respiratory problems in this cohort. In pediatric asthma exacerbations, use of SAMAs in combination with SABAs improves pulmonary function to a greater extent than the use of SABAs alone[34,35]. Although, most diabetic patients in our study were treated with SAMAs alone and the pattern of exacerbations in the reference cohort was almost the same.

To the best of our knowledge, this is the first population-based cohort study to investigate the prevalence and incidence rates of asthma medication use and exacerbations in children and adolescents with T1DM compared with diabetes-free subjects in the period both before and after the onset of this disease. An important strength of our study is the use of the PHARMO RLS database which is a large, population-based data set providing accurate data on medications dispensing and hospital admissions. Probability for occurrence of information bias and recall bias is therefore low. Another strength of this study is the low probability of T1DM misclassification. We used insulin prescription as a proxy for T1DM since hyperglycemia is the only indication for insulin and it has been validated in several studies[36-39]. Furthermore, children with CF were excluded at baseline from the study population and other types of diabetes in which insulin is indicated e.g. mitochondrial diabetes have reported low prevalence rates compared with T1DM [40,41].

An important limitation in this study is misclassification of asthma since the definition of asthma is based on the drug prescription information. Another limitation is the reference cohort which was randomly captured from the PHARMO database and it only includes children that filled prescriptions in the community pharmacies. Therefore the difference between asthma medication use in patients with T1DM and the general population might be larger than observed in our study. Another important limitation in this study is that there were no additional descriptive characteristics such as race/ethnicity, body mass index (BMI), or family history of asthma available in the database.

In summary, our study shows that children and adolescents with T1DM use more asthma medication than age- and gender-matched diabetes-free controls in the first year after the onset of diabetes. However, this difference disappears after the first year of diabetes diagnosis. As respiratory symptoms could be decreased by glycemic control, it might be worthwhile to re-evaluate asthma medication once glycemic control has been established.

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SUPPORTING INFORMATION

Table S1. ATC codes used to identify medications for obstructive airway disease

	Subgroup 1 (ATC code)	Subgroup 2 (ATC code)
R03: DRUGS FOR OBSTRUCTIVE AIRWAY DISEASES	R03A: ADRENERGICS, INHALANTS	R03AA: Alpha- and beta-adrenoreceptor agonists R03AB: Non-selective beta-adrenoreceptor agonists R03AC: Selective beta-2-adrenoreceptor agonists R03AH: Combinations of adrenergics R03AK: Adrenergics in combination with corticosteroids or other drugs, excl. anticholinergics R03AL: Adrenergics in combination with anticholinergics
	R03B: OTHER DRUGS FOR OBSTRUCTIVE AIRWAY DISEASES, INHALANTS	R03BA: Glucocorticoids R03BB: Anticholinergics R03BC: Antiallergic agents, excl. corticosteroids R03BX: Other drugs for obstructive airway diseases, inhalants
	R03C: ADRENERGICS FOR SYSTEMIC USE	R03CA: Alpha- and beta-adrenoreceptor agonists R03CB: Non-selective beta-adrenoreceptor agonists R03CC: Selective beta-2-adrenoreceptor agonists R03CK: Adrenergics and other drugs for obstructive airway diseases
	R03D: OTHER SYSTEMIC DRUGS FOR OBSTRUCTIVE AIRWAY DISEASES	R03DB: Xanthines and adrenergics R03DC: Leukotriene receptor antagonists R03DX: Other systemic drugs for obstructive airway diseases

Abbreviation: ATC, anatomical therapeutic chemical

Table S2. 5-year prevalence rate of asthma medication use before and after the index date (at least one prescription)

		5 years before the index date		5 years after the index date	
		Population (T1DM:728; Reference:2,874)		Population (T1DM:784; Reference:3067)	
		Events	Prevalence rate (%)	Events	Prevalence rate (%)
Short acting beta agonists (SABAs)	T1DM	161	22.12	128	16.33
	Reference	637	22.16	448	14.61
	P-value		0.92		0.23
Long acting beta agonists (LABAs)	T1DM	26	3.57	39	4.97
	Reference	98	3.41	137	4.47
	P-value		0.44		0.26
Short acting muscarinic antagonists (SAMAs)	T1DM	10	1.37	43	**5.48**
	Reference	39	1.36	19	**0.62**
	P-value		0.99		**0.00**
Long acting muscarinic antagonists (LAMAs)	T1DM	-	-	1	0.13
	Reference	-	-	1	0.03
	P-value	-	-		0.37
Inhaled corticosteroids (ICSs)	T1DM	131	17.99	88	11.34
	Reference	492	17.11	356	11.60
	P-value		0.64		0.79
Anti-allergic agents	T1DM	2	0.27	0	0
	Reference	14	0.49	4	0.13
	P-value		0.55		0.59
Systemic SABAs	T1DM	6	0.81	1	0.13
	Reference	35	1.22	5	0.16
	P-value		0.44		1.00
Montelukast	T1DM	11	1.49	11	1.39
	Reference	32	1.11	33	1.06
	P-value		0.45		0.45

Abbreviation: T1DM: type 1 diabetes mellitus
Index date is the date of first insulin dispensing.

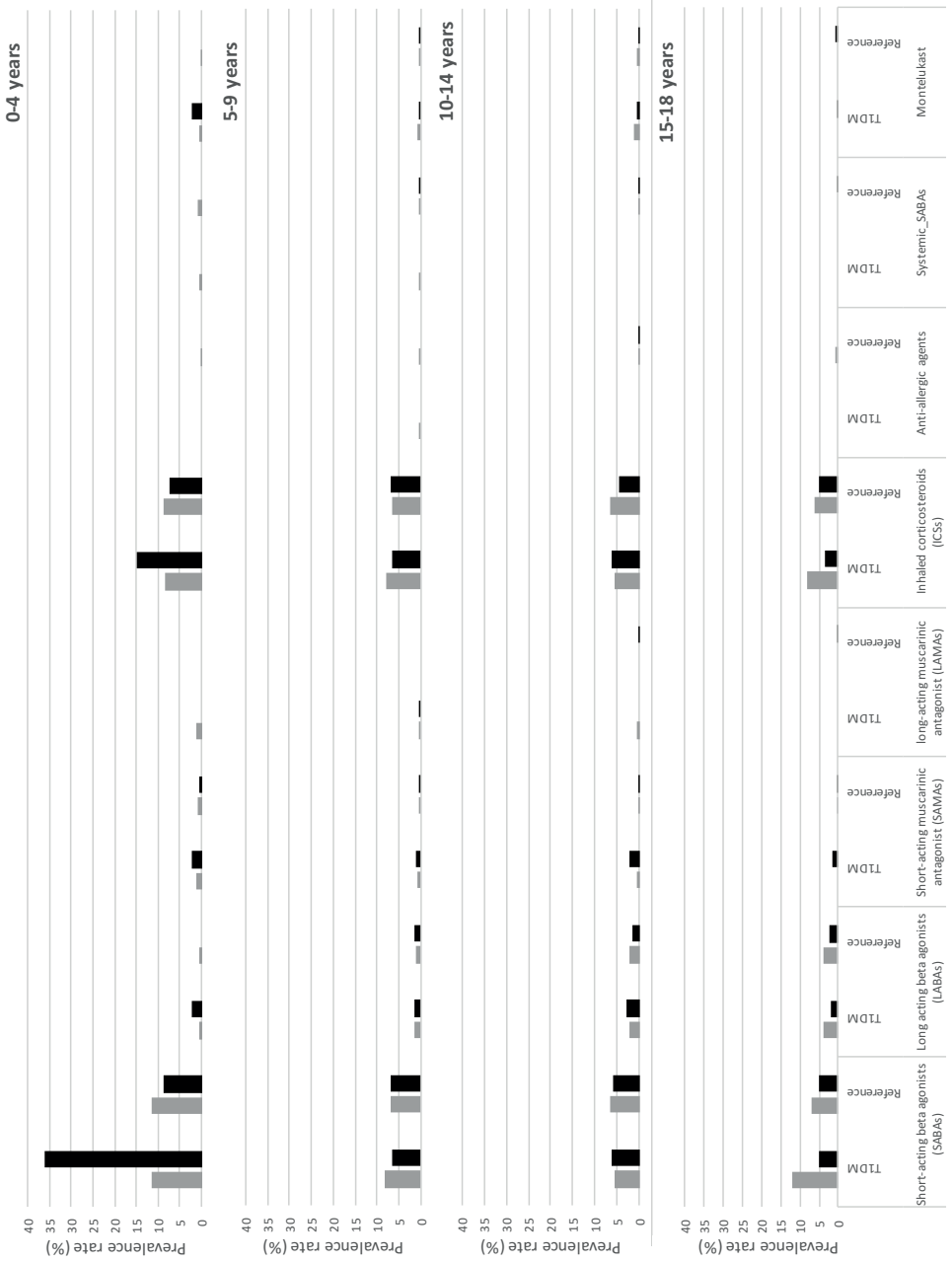

Fig S1. 5-year prevalence rate of asthma medication use, comparing T1DM and reference cohorts before and after the index date, stratified by age (children aged 0-4, 5-9, 10-14 and 15-18 years)

Chapter 3

Risk factors associated with asthma and allergy in children

Chapter 3.1

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Genetic variation and the association with asthma and lung function in children: results of the WHISTLER cohort study

Submitted for publication

ABSTRACT

Objectives: Genetic variations associated with asthma onset and lung function in adults have been identified by several large genome-wide association studies (GWAS). The aim of our study was to assess if these single nucleotide polymorphisms (SNPs) identified in adults are also associated with asthma and lung function in children.

Methods: Two genetic risk scores (GRSs) were constructed based on SNPs previously identified for adult asthma and lung function. In a prospective population-based birth cohort (WHISTLER) we tested the association between the asthma GRS and asthma diagnosis before the age of 8 years (n=1,249). We also studied the association between the lung function GRS and lung function measurements before the age of 2 months (airway resistance [R_{rs}] and lung compliance [C_{rs}]; n=1,126) and at the age of 5 years (interrupter resistance [Rint]; n=481).

Results: A higher asthma GRS was statistically significantly associated with the onset of asthma before the age of 8 years (hazard ratio: 1.71, 95% CI: 1.16-2.51). A better lung function according to the GRS developed in adults was associated with a lower lung function in neonates; C_{rs} (-6.32mL · kPa-1/score point, 95% CI: -11.5; -1.10). However, this association was no longer statistically significant after including age and gender in the model (-4.29 mL · kPa-1/score point, 95% CI: -9.36; 0.78). The lung function GRS was not associated with Rint at age 5 years.

Conclusions: In this study, an asthma GRS based on SNPs that have been previously associated with asthma in adults is also associated with asthma susceptibility in children. However a GRS based on SNPs associated with lung function in adults is not associated with lung function in children.

INTRODUCTION

Asthma is a common chronic inflammatory lung disease. The physiological state of the lungs and airways is represented by the lung function and lung function measurements play an important role in the diagnosis of asthma. In neonates, pulmonary compliance (C_{rs}) and resistance (R_{rs}) are used as measures of lung function[1]. Decreased neonatal lung function has been shown to be associated with wheezing illnesses during the first 5 years of life[2] and asthma by age of 7 years[3]. An increased neonatal airway R_{rs} and a lower neonatal pulmonary C_{rs} have both been associated with more wheezing illnesses during infancy. Both were also associated with the onset of asthma[2,4]. Additionally, asthma susceptibility at the age of 10 years has been associated with reduced lung function at birth[5]. Although both lung function and asthma have been linked to multiple environmental factors, a large body of evidence shows that genetic factors play an important role in both the occurrence of asthma and in lung function development[6-8].

Recent genome-wide association studies (GWAS) reported several high-risk alleles that are strongly associated with asthma[7,9-15]. Despite different inflammatory asthma phenotypes in children and adults[16], characteristics of asthma symptoms such as wheezing, coughing and airway hyperresponsiveness largely overlap[17]. We, therefore, hypothesized that overlapping genetic pathways might exist and that the single nucleotide polymorphism (SNPs) associated with asthma in adults might also be associated with childhood asthma.

To date, there have been several GWASs identifying genetic variation associated with lung function in adults[18-21]. Since neonates are naive to environmental exposure (aside from exposures during gestation), we hypothesized that genetic effects in lung function might have a relatively larger contribution to the observed phenotypic variation in children compared to adults.

A genetic risk score (GRS) - also called allele scores, gene scores or genotype scores - yields a quantitative index of genetic risk with a normal distribution. This can be used to summarize the total of genetic variants associated with an outcome, with a potentially larger effect size[22].

The use of a GRS can elucidate common genetic pathways between phenotypes and diseases. For example, a lung function GRS based on SNPs associated with lung function has been also associated with the development of asthma in adults[23]. Furthermore, asthma GRS in adults has been found to be associated with an increased risk of atopy in asthmatic children[8]. Belsky et al. showed that childhood-onset asthma cases that had a higher asthma GRS (above the cohort median) were more likely to miss school due to asthma as well as to become persistent asthmatic patients before age 13 years[8].

In this study, we developed two GRSs; one based on GWAS results for asthma in adults and one based on GWAS results of lung function in adults. Subsequently, we tested if these GRSs are associated with childhood asthma and lung function development in a large prospective birth cohort. Since previously a significant association between asthma GRS in adults and a higher risk of atopy in children with asthma has been reported[8], we

also assessed whether there was an association between asthma GRS and risk of allergy among asthmatic children.

METHODS

Study population

The study population consisted of children included in the Wheezing Illnesses Study Leidsche Rijn (WHISTLER) cohort, a prospective population-based birth cohort study on determinants and prediction of wheezing illnesses (including early life lung function). Study design and rationale of WHISTLER have been described in detail elsewhere[24].

Briefly, healthy neonates and infants born in a newly developed residential area in the Netherlands (i.e. Leidsche Rijn), were invited by telephone to participate in this study before the age of 2 months; before any respiratory illness was present (baseline). Exclusion criteria were gestational age younger than 36 weeks, major congenital abnormalities and neonatal respiratory disease. During this first visit lung function measurements were performed. At the age of 5 years, children were invited for a second visit, in which lung function measurements were performed again. Information about general health, allergic symptoms, asthma and respiratory symptoms, medication use, pre- and post-natal risk factors was obtained by questionnaires. Children were followed to the age of 9 years. During total follow-up, information on physician diagnoses, primary care consultations and medication prescriptions for respiratory symptoms was collected.

The pediatric medical ethics committee of the University Medical Center Utrecht, Utrecht, the Netherlands, approved the study. Written informed consent was obtained from the parents.

Genotyping and quality control

Genomic DNA was extracted from buccal cells of infants and DNA was extracted using the QIAamp DNA blood mini kit (Qiagen) and concentration was determined using PicoGreen (Molecular Probes)[24]. Genotyping was performed using the Infinium HumanExome chip (Illumina, San Diego, CA), version 1.1, which contains 242,902 variants[25].

Genotypes obtained from GenomeStudio were used for quality control (QC), and PLINK v1.07 was used for the downstream process[26]. Sample QC was performed on common SNPs (MAF ≥5%) of high quality (missingness<1%, Hardy-Weinberg equilibrium P >1e-4, and LD-pruned to leave no pairs with r^2 >0.2). We removed samples based on heterozygosity, keeping samples within 4 standard deviations. European ancestry was verified using EIGENSTRAT[27], and non-European samples were excluded for further analysis. Identity-by-descent estimates from PLINK[26] were used to identify siblings or otherwise related children (pi-hat >0.2), one of which was randomly excluded. As the calling algorithms in GenomeStudio are not designed for rare SNPs, genotypes from zCall[28] were used in subsequent analyses, where we excluded SNPs with a call rate less than 95% and a Hardy-Weinberg equilibrium p-value<1e-6.

Genetic risk scores

We constructed two separate GRSs; one based on SNPs previously associated with asthma susceptibility in adults[7,29-31] and one based on lung function[19] (specifically the Forced Expiratory Volume in the first second (FEV1)/Forced Vital Capacity (FVC) ratio, alternatively known as the FEV1%) in adults. FEV1 and FVC are the two most widely used measures of pulmonary function in adults[32]. Only single SNPs found at the genome-wide significance threshold of p<5e-8 were considered for inclusion; if several correlated SNPs (r^2>0.8) were reported, the SNPs with the lowest reported p-value was chosen. This process resulted in 12 SNPs for asthma and 20 SNPs for lung function. None of the SNPs were selected for both GRSs. All 12 asthma SNPs were available and passed quality control for the asthma GRS while of the 20 lung function's SNPs, 18 SNPs were available for the lung function GRS and passed quality control.

We weighted the SNPs in proportion to their effect size, as these varied considerably between the SNPs. The weighting was based on the linear regression coefficient (beta) reported for the lung function SNPs in the most recent and largest GWAS[19], and on the natural logarithm of the reported odds ratios (ORs) for the asthma SNPs[7,29-31,33]. The GRS was weighted such that an increase in the GRS in adults would be associated with an increase in lung function in the original GWAS. For the binary outcome, we weighted according to the alleles associated with an increased risk resulting in an OR above 1 for the association with asthma in the original studies. SNPs included in each of the GRSs, as well as effect sizes, effect alleles, and references to studies identifying them can be found in the supplementary material (**Tables S1-S3**). To reduce the effect of outliers in the lung function data, we calculated the mean±3 standard deviations, and limited more extreme measurements to this value (winsorization). Additionally, the data for the R_{rs} in neonates as well as Rint in 5-year olds was log-transformed so the residuals of the linear regression better resembled a normal distribution. Histograms of the transformed outcomes are included in the supplementary material (**Fig S1-S4**).

Primary outcomes

Two different primary outcomes were studied; asthma and lung function. Asthma was defined as a physician-diagnosis of asthma at any time during the course of follow-up (before the age 8 years) derived from medical records using the International Classification of Primary Care (ICPC; R96)[34] or reported by the parents during the study visits. To obtain more statistical power we defined asthma based on medical record and/or parental reported physician diagnosis of asthma.

Lung function was measured in healthy neonates before the age of 2 months during natural sleep. The R_{rs} and C_{rs} of the pulmonary system and time constant of the total respiratory system (TRS) were measured in the absence of respiratory muscle activity using the single occlusion technique (SOT)[35-37]. SOT is a lung function technique for assessment of passive respiratory mechanics (C_{rs} and R_{rs}) suitable for neonates[37]. Airflow was measured using a heated Lilly-type pneumotachometer (series 8300, linear range 0–10 L/min; Hans Rudolph Inc., Kansas City, MO, USA) connected to a face mask (infant mask, size neonate; Hans Rudolph Inc.). To minimize air leakage, the face mask was sealed to the

infant's face using therapeutic silicon putty (Thera flex, resistive hand exerciser; Depco Inc., New York, NY, USA). Pressure changes at the airway opening were measured using a pressure transducer (Honeywell, type 163PC01D75; Morristown, NJ, USA). Volume was measured by electronic integration of the airflow signal. To calibrate flow and volume measurement, before every measurement a 100-mL precision syringe (Viasys Healthcare, Höchberg, Germany) was used. Lung function data was calculated offline using a custom-built software package (Luna 1.6, Utrecht, the Netherlands). Occlusions were accepted or disregarded using the criteria of the European Respiratory Society (ERS)/ American Thoracic Society (ATS) Task Force on Infant Lung Function[37,38]. At least three technically acceptable occlusions were used to calculate mean C_{rs}, R_{rs} and TRS. At the age of 5 years, children were invited for a second visit. Rint was measured, which reflects airway R_{rs} [39] using the MicroRint (Micro Medical Limited, Kent, UK), according to standardized methods[40]. Median Rint was calculated from at least 5 acceptable interruptions within children.

Secondary outcomes

As a secondary outcome allergy was studied. Allergy was defined as a physician-diagnosis of allergic symptoms including allergic conjunctivitis (ICPC: F71), rhinitis (ICPC: R97) or eczema/dermatitis (ICPC: S87, S88) at any time during follow-up.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of patients included. To test whether a GRS for asthma was associated with asthma diagnosis in children, we used Cox proportional hazard models. Time-to-asthma diagnosis was calculated using Kaplan-Meier analyses to test the association with the asthma GRS as a binary variable (comparing GRS≥2 and GRS<2). We also used logistic regression to assess whether there was any association between asthma GRS and risk of allergy among asthmatic children.

For the association between lung function GRS and lung function, we used a linear regression model both unadjusted and adjusted for age and gender. Additionally, we tested whether the lung function GRS was predictive for asthma susceptibility using a Cox proportional hazard model.

A p-value of <0.05 was considered statistically significant. For analysis, we used a combination of PLINK (for the single SNP analyses)[26], R version 3.0.2[41], and SPSS version 23.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) (to prepare, examine and summarize the data).

Power calculation

We used the Genetic Power Calculator for power calculations[42]. For the association between the asthma GRS and the risk of developing asthma, we had 90% power to detect a hazard ratio (HR) of 2.0 in the 1,249 children available with a significance level of 0.05. For the association between lung function GRS and lung function, we had 80% power to detect an association of GRS explaining more than 0.6% of the total phenotypic variation

at a significance level of 0.05. This is lower than the total variance explained by all the 26 SNPs found in previous research[19], in which the reported loci explained 1.5– 3.0% of the additive polygenic variance; we, therefore, had more than 80% power to find these effects. For the children aged 5 years, the sample size was smaller and we had 80% power to detect effects explaining more than 2.0% of the total variation.

RESULTS

For 1,421 infants in the WHISTLER study genotype data was available. In this population, data on physician diagnosis and medical records was available for 1,249 children. Lung function data before age 2 was available for 1,126 infants. In total, 481 children had valid SOT measurements, medical records, and genetic data along with Rint measurement at age 5 years. Overview of the recruitment and inclusion of infants in the WHISTLER study is shown in **Figure 1**.

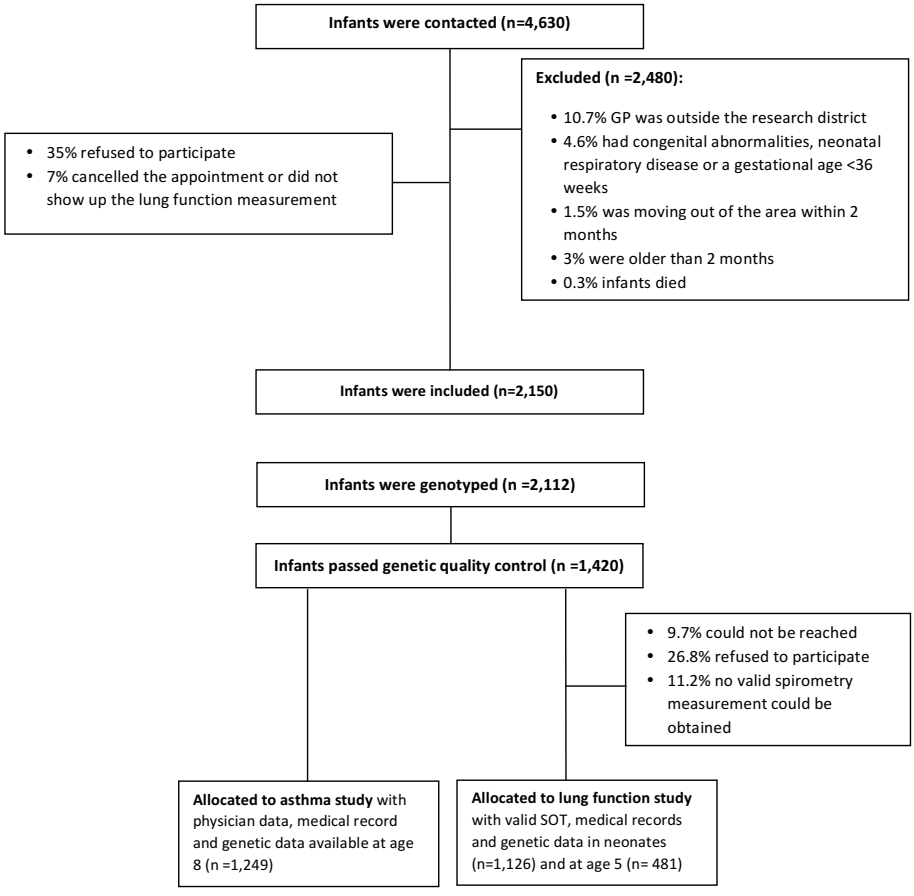

Fig 1. Overview of the recruitment and inclusion of infants in the Wheezing Illnesses Study Leidsche Rijn (WHISTLER) project.
Abbreviations: GP: general practitioner; SOT: single occlusion technique.

Baseline characteristics

Characteristics of children investigated in this study are shown in **Tables 1 and 2**. As shown, boys comprised almost 49% of the population. The total number of asthmatic children (based on medical records and parental report) was 137 (11%) before age of 8 years. The number of asthmatic children solely based on medical records was 126 (10.1%). More than half of children with asthma obtained their diagnosis before the age of 4 years (54.5%). Mean age at measurement of lung function during the first study visit was 35 ± 9.2 days.

Asthma GRS and asthma

The asthma GRS was associated with asthma diagnosis. Children with a higher asthma GRS had a higher risk of an asthma diagnosis during the follow-up; when the asthma GRS increased with one point the risk of asthma increased by 71% (HR: 1.71, 95% CI: 1.16-2.51, p=0.006). When GRS was not analyzed as a continuous variable, but with a cut-off value based on the cohorts median risk score (similar to Belsky et al [8]), there was a statistically significant difference between children with GRS≥2 compared to children with GRS<2. Children with GRS ≥2 had a higher probability of an asthma diagnosis (log-rank p-value=0.03) (**Fig 2**).

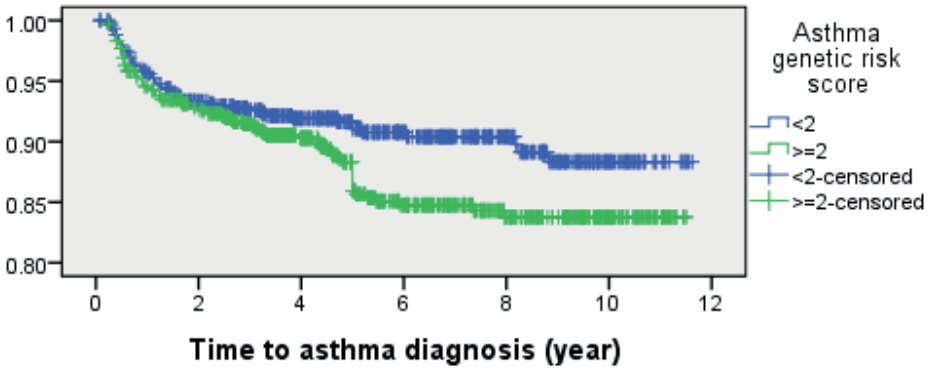

Fig 2. Time to asthma diagnosis comparing high and low asthma genetic risk scores

Asthma GRS and allergy

Of the 137 asthmatic children within our study, 80 (58.4%) reported an allergy. The asthma GRS was not statistically significantly associated with risk of allergy in this population among asthmatic children (OR: 1.44, 95% CI: 0.70-2.95).

Lung function GRS and asthma

There was no association between the lung function GRS and susceptibility of asthma (HR: 0.96, 95% CI: 0.24-3.85).

Table 1. Study characteristics at baseline and during follow-up of the children included for the association analyses between asthma GRS and asthma occurrence

Baseline, neonates, n		1,249
Gender (boys), n (%)		608 (48.7)
Genetic asthma risk score, median (IQR)		2.02 (1.73-2.32)
GRS	<2	600 (48.0)
	≥2	649 (52.0)
Follow-up, n		1,249
Age at asthma diagnosis, n (%)	<1 years	29 (23.0)
	1-2 years	19 (15.1)
	2-3 years	10 (7.9)
	3-4 years	12 (9.5)
	4-8 years	56 (44.4)
Asthmatic children during follow-up*		126 (10.1)
Asthmatic children during follow-up**		137 (11.0)
Physician diagnosed allergic symptoms (ever), n (%)	Allergic conjunctivitis	32 (2.6)
	Rhinitis	70 (5.6)
	Eczema/dermatitis	429 (34.3)
	Allergy***	466 (37.3)
Follow-up time, median (IQR)		5.86 (3.58-8.43)

Abbreviations: GRS: genetic risk score; IQR, interquartile range
*Physician diagnosis asthma based on medical record
** Physician diagnosis asthma either based on medical record or parental reporting
***At least one allergic symptoms including allergic conjunctivitis, rhinitis or eczema/dermatitis

Table 2. Study characteristics at baseline and during follow-up of the children included for the association analyses between lung function GRS and lung function

Baseline, neonates, n	**1,126**
Gender (boys), n (%)	552 (49.0)
Birth weight (g), mean (SD)	3550 (500)
Birth length (cm) , mean (SD)	50.8 (2.5)
Gestational age (days) , mean (SD)	278.5 (9.8)
Age at measurement lung function (days post partum), mean (SD)	35.3 (9.2)
C_{rs} ($mL \cdot kPa^{-1}$)	46.6 (10.7)
R_{rs} ($kPa \cdot L^{-1} \cdot s^{-1}$)	6.65 (2.03)
Follow-up, n	**481**
Gender (boys), n (%)	238 (49.5)
Age (years) , mean (SD)	5.42 (0.42)
Rint ($kPa \cdot L^{-1} \cdot sec^{-1}$)	0.64 (0.17)

Abbreviations: GRS: genetic risk score; SD: standard deviation; C_{rs}: compliance; R_{rs}: resistance; Rint: interrupter resistance

Lung function GRS and lung function

Association results for the lung function GRS are summarized in **Table 3**. A higher lung function GRS, which had a mean of 0.08 and a standard deviation of 0.12 was associated with lower C_{rs} (i.e. lower lung function) at baseline (regression coefficient -6.32 mL·kPa-1/score point, 95% CI: -11.5; -1.10, p=0.02). This inverse association weakened after adjustment for age and gender. The lung function GRS was not associated with R_{rs} at baseline (-0.08 kPa·L^{-1}·s^{-1}/score point, 95% CI: -0.21; 0.06, p=0.25) nor with the Rint at 5 years of age (-0.07 kPa·L^{-1}·s^{-1}/score point, 95% CI: -0.05; 0.19, p=0.26) (**Table 3**).

Asthma GRS and lung function

There was no association between the asthma GRS and C_{rs} (-0.36 mL·kPa-1/score point, 95% CI: -1.66; 0.95, p=0.60), R_{rs} (0.01 kPa·L^{-1}·s^{-1}/score point, 95% CI: -0.03; 0.05, p=0.72) before the age of 2, or Rint at age 5 (-0.01 kPa·L^{-1}·s^{-1}/score point, 95% CI: -0.05; 0.02, p=0.44) (**Table 4**).

Table 3. Association results for the lung function GRS and lung function

	Unadjusted	**Adjusted for age and gender**
Outcome	**Effect /SP (95% CI), P-value**	**Effect/SP, 95% CI, P-value**
C_{rs}	-6.32 (-11.5; -1.10), 0.018	-4.29 (-9.36; 0.78), 0.098
R_{rs}	0.014 (-0.15; 0.13), 0.85	-0.054 (-0.19; 0.085), 0.25
Rint	0.0511 (-0.075; 0.177), 0.43	0.0562 (-0.0697; 0.182), 0.38

Effect/SP: Change in outcome per score point;
Abbreviations: CI: confidence interval; C_{rs}, compliance; R_{rs}, resistance; Rint, interrupter resistance; GRS: genetic risk score

Table 4. Association results for the asthma GRS and lung function

	Unadjusted	Adjusted for age and gender
Outcome	**Effect/SP (95% CI), P-value**	**Effect/SP, 95% CI, P-value**
C_{rs}	-0.6630 (-2.13;0.80), 0.38	-0.77 (-2.19; 0.65), 0.287
R_{rs}	0.013 (-0.026; 0.053), 0.51	0.013 (-0.025; 0.052); 0.50
Rint	-0.0085 (0.044; 0.027), 0.64	-0.0041 (-0.040; 0.031), 0.82

Effect/SP: Change in outcome per score point;
Abbreviations: CI: confidence interval; C_{rs}, compliance; R_{rs}, resistance; Rint, interrupter resistance; GRS: genetic risk score

DISCUSSION

In this study, we assessed whether common genetic variation associated with asthma or lung function development in adults, has a similar effect on the risk of asthma and on lung function development in children. The GRS of SNPs associated with asthma in adults appeared to be also associated with asthma susceptibility in children before the age of 8 years. Moreover, our clustering of patients according to GRS levels showed that children

with a higher GRS (≥2) developed asthma earlier in life. In contrast, the GRS of SNPs associated with lung function in adults was not associated with a lung function measures in neonates or Rint in 5-year old children.

Asthma is a complex heterogeneous disorder with both genetic and environmental risk factors involved[43]. Several distinct asthma phenotypes exist[44], however, common genetic pathways seem to be involved in asthma onset. In our study, we did find an association of the asthma GRS based on GWAS results of asthma in adults with asthma susceptibility in children. Our finding is in line with previous work by Belsky et al. who showed that a GRS based on summing risk alleles across GWAS-identified asthma-associated SNPs was associated with childhood-onset asthma in a population-based birth cohort. They also showed that asthmatic cases with higher asthma GRS had a significantly increased risk of atopy defined as elevated serum IgE level or positive skin-prick test. The results by Belsky et al. suggested that at least some asthma-associated loci are important drivers of atopy[8]. However, in our study we could not find the association with allergic symptoms. A different definition of outcome together with small sample size in our study might explain inconsistent result here.

Children with early persistent asthma are more prone to develop lung function impairment[45]. A recent study has shown that asthma phenotypes were negatively correlated with FEV1 at age 16 years[45]. Hallberg et al. reported that early onset asthma is associated with lung function impairment at age 8 years followed by further reductions in lung function up to age 16 [45]. Previously gene variants related to asthma were shown to be associated with a worse lung function in healthy individuals[46].

Cluster analyses have also shown that lung function is an important factor in the development of asthma[23,47]. Risk factors affecting lung function in early life lead to a higher risk of lung function impairment in adulthood, and to an increased risk of respiratory disorders, particularly asthma, later in life. Recently a lung function GRS has been associated with asthma susceptibility in adults[23,48]; supporting a contribution of lung function impairment to the onset of asthma.

In our study, in contrast with previous studies, we did not find statistically significant associations between asthma GRS and lung function or between lung function GRS and asthma. A part of this might be explained by the small sample size of our study. In a previous study, it was shown that a better parental lung function, as measured by the FEV1 and FVC, was associated with a higher C_{rs} and a lower R_{rs} in children; suggesting genetic mechanisms underlying the lung function development[49]. We hypothesized effects of genetic variation in lung function might even be stronger in neonates, due to fewer environmental exposures compared to adults. For the lung function GRS, this is not supported by our results. Earlier studies have shown that there is a heritable component to the results from the SOT[49], and that the C_{rs} and R_{rs} are associated with wheezing illnesses during infancy and asthma[2,4]. These studies showed that the C_{rs} and R_{rs} in neonates are valuable phenotypes, which are related to outcomes later in childhood. Our research suggests that the heritable component may be different from that found for lung function in adulthood. Supporting our results is a study similar to the present study, published recently[50]. Although the pulmonary phenotypes studied were different (focusing on expiratory volume,

and the provocative dose of methacholine for a certain decrease in lung function), the GRSs used were similar. Kreiner-Møller and colleagues found that the GRSs were not associated with lung function measures soon after birth[50]. Our findings in a larger dataset, with related phenotypes, support this idea: genetic variation associated with lung function at a later age has no, or a small, effect on neonatal lung function. In the unadjusted analyses of the lung function GRS, we found an association with the C_{rs} of the respiratory system in neonates, but in the opposite direction of what was expected. This decrease in C_{rs} with an increase in the GRS was no longer statistically significant after adjusting for age and gender. It is unclear why we found this inverse effect of the unadjusted GRS. In analyses of the C_{rs} and R_{rs}, age was controlled for to prevent possible confounders. Our results show that the expected positive effect of the GRS on the C_{rs} does not exist, but that a negative effect cannot be ruled out. Other explanations for the inversed effect, such as technical errors, seem unlikely, as most of the individual SNPs had a similar inversed effect (as shown in the Supplementary Data), and the direction was identical when SNPs with possible strand issues (A/T and C/G SNPs) were removed from the GRSs (data not shown). Further, there is discussion on the usage of weighted versus unweighted GRSs, as weighted GRS are potentially more precise if effect sizes vary strongly across SNPs[22]. Effect sizes for the individual SNPs were uniform in the lung function GRS, and an unweighted score did not change the association substantially.

While the current study is relatively small when compared to most discovery GWAS, it is the largest reported study into the genetics of neonatal lung function. In our study, we had statistical power to detect small effects (explaining more than 0.6% of the total phenotypic variation). As the lung function SNPs explained between 1.5-3.2% of the variance in a previous study of lung function in adults[19], it is likely we would have found a nominally significant association if its effect was as large in neonates as it is in adults. However, there are some limitations in the current study that have to be discussed. In our association analysis between asthma GRS and asthma we might have introduced confounding bias. We tested the association of genes with asthma phenotype in children in which the majority of asthma diagnosis was after the age of 4 years. It is expected that the influence of environmental factors on the association between asthma GRS and asthma occurrence increases with increasing age in asthma diagnosis; some genes are expressed only in specific environmental contexts[51]. Moreover, SNPs recently found associated with lung function in a GWAS[18] were not available on the genotyping array we have used, as these were published after the design of the exome-chip. Therefore, the associations might have been stronger when we included these additional 4 SNPs as well.

In summary, a GRS based on 12 SNPs associated with asthma risk in GWAS in adults is associated with asthma risk in children. We found no association between a GRS consisting of 20 SNPs associated with better adult lung function and neonatal lung function in the form of C_{rs} and R_{rs} of the respiratory system, or with better lung function at age 5 years. Since, asthma is a common condition with multiple risk factors involved our results provide more insight into the genetic pathway underlying asthma in children.

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SUPPORTING INFORMATION

Histograms of phenotypes

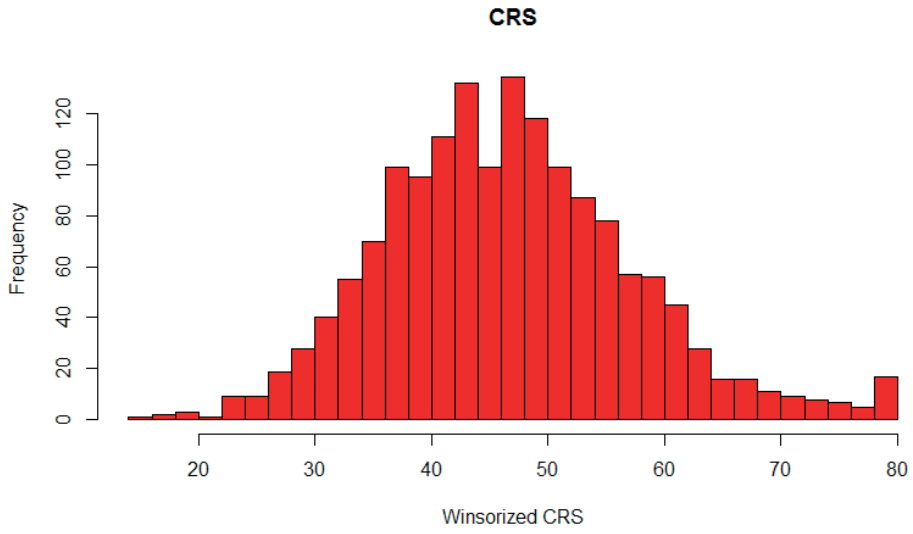

Fig S1. Histogram of winsorized C_{rs} values

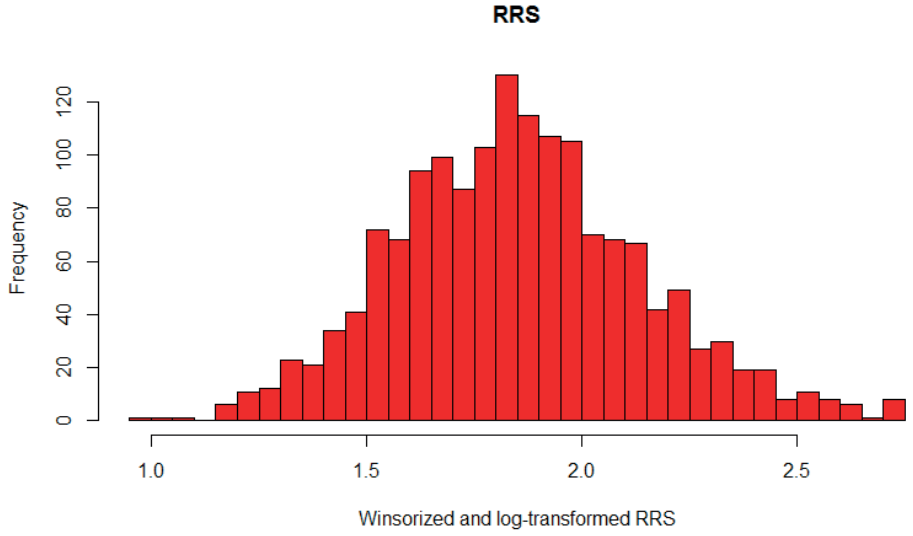

Fig S2. Histogram of log-transformed and winsorized R_{rs} values

Histograms of risk scores

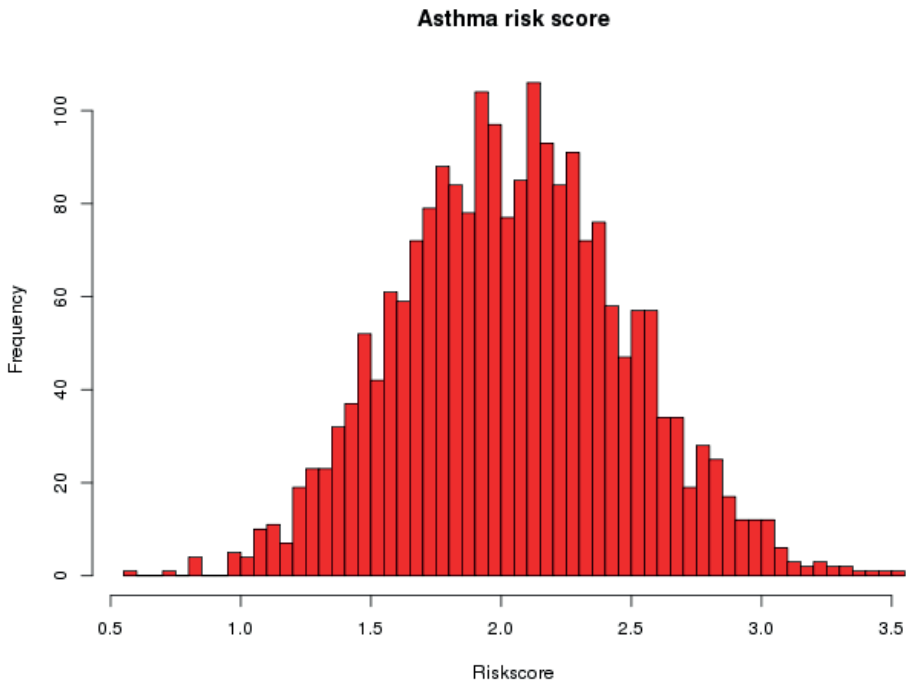

Fig S3. Histogram of the genetic risk score based on SNPs previously associated with asthma.

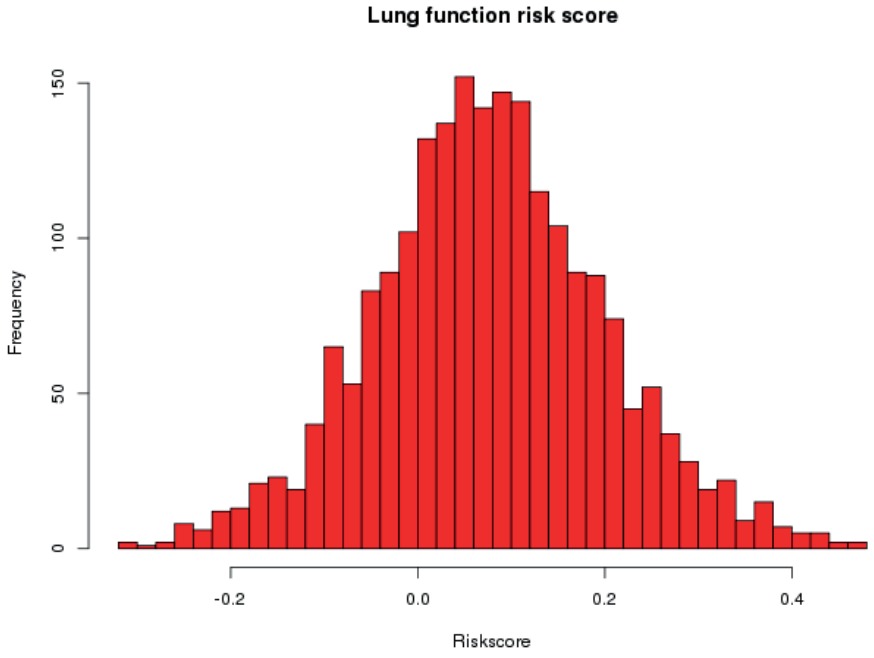

Fig S4. Histogram of the genetic risk score based on SNPs previously associated with lung function.

Table S1. SNPs included in asthma GRS and effect on asthma

Data from GWAS catalog[1]							Results analysis single SNPs in WHISTLER						
CHR	**BP**	**SNP**	**Allele**	**OR**	**P-value**	**PMID**	**ID on exome-chip**	**A1**	**A2**	**Freq. A1**	**HR**	**95%CI**	**P-value**
1	154453788	rs4129267	T	1.09	2E-08	21907864	exm-rs4129267	T	C	0.3692	0.81	0.57-1.13	0.21
5	60073967	rs1588265	C	1.18	3E-08	19426955	exm-rs1588265	C	T	0.3067	1.19	0.85-1.67	0.32
6	32187804	rs204993	A	1.17	2E-15	21804548	exm-rs204993	A	G	0.748	0.82	0.53-1.26	0.36
6	32216568	rs404860	A	1.21	4E-23	21804548	exm-rs404860	A	G	0.8526	1.20	0.80-1.78	0.38
6	32370918	rs3129943	T	1.17	3E-15	21804548	exm-rs3129943	T	C	0.7361	0.85	0.56-1.79	0.45
6	32658092	rs9273349	C	1.18	7E-14	20860503	exm-rs9273349	C	T	0.5637	0.64	0.44-0.92	0.02
6	33075103	rs987870	C	1.4	2E-10	21814517	exm-rs987870	C	T	0.1634	1.02	0.70-1.49	0.91
9	6190076	rs1342326	C	1.2	9E-10	20860503	exm-rs1342326	C	A	0.1524	1.30	0.91-1.85	0.15
12	56018703	rs1701704	G	1.19	2E-13	21804548	exm-rs1701704	G	T	0.3334	1.04	0.74-1.47	0.81
15	67154447	rs744910	G	1.12	4E-09	20860503	exm-rs744910	G	A	0.4936	1.05	0.72-1.54	0.79
17	39913696	**rs7216389**	T	1.45	9E-11	17611496	exm-rs7216389	T	C	0.513	1.20	0.80-1.78	0.38
22	37137994	rs2284033	G	1.12	1E-08	20860503	exm-rs2284033	G	A	0.5666	0.80	0.56-1.14	0.22

Abbreviations: SNPs: single nucleotide polymorphisms; GRS: genetic risk score; CHR: Chromosome, BP: chromosomal location based on hg19, PMID: PubMed ID of initial finding, OR: odds ratio; HR: hazard ratio

Table S2. SNPs included in the lung function GRS and effects on C_{rs}

Data from GWAS catalog[1]							Results analysis single SNPs with C_{rs} in WHISTLER						
CHR	**BP**	**SNP**	**Allele**	**BETA**	**P-value**	**PMID**	**ID on exome-chip**	**A1**	**A2**	**Freq. A1**	**BETA**	**95%CI**	**P-value**
1	17306675	rs2284746	G	-0.04	7.50E-16	21946350	exm-rs2284746	G	C	0.4806	0.005372	-0.74; 0.75	0.989
1	218860068	rs993925	T	0.034	1.16E-08	21946350	exm-rs993925	T	C	0.3353	-0.8038	-1.61; 0.00	0.050
2	239877148	rs12477314	T	0.041	1.68E-12	21946350	exm-rs12477314	T	C	0.1798	0.2603	-0.72; 1.24	0.601
4	89883979	rs7671167	T	-0.042	1.27E-09	20173748	exm-rs7671167	T	C	0.5322	0.4696	-0.27; 1.21	0.215
4	145485738	rs1980057	T	0.063	1.06E-19	20010835	exm-rs1980057	T	C	0.4074	-0.1995	-0.99; 0.59	0.622
5	95036700	rs153916	T	-0.031	2.12E-08	21946350	exm-rs153916	T	C	0.5363	-0.4242	-1.19; 0.34	0.279
5	147842353	rs11168048	T	-0.047	5.97E-11	20010835	exm-rs11168048	T	C	0.5915	0.9496	0.19; 1.71	0.014
5	156932376	rs2277027	C	-0.042	6.65E-09	20010835	exm-rs2277027	C	A	0.3367	-0.5251	-1.32; 0.27	0.195
6	31568469	rs2857595	G	0.037	2.28E-10	21946350	exm-rs2857595	G	A	0.7714	-0.2084	-1.11; 0.69	0.650
6	32151443	rs2070600	T	0.126	9.07E-15	20010834	exm534819	T	C	0.03955	0.5501	-1.49; 2.59	0.596
6	142750516	rs3817928	G	0.059	2.27E-12	20010835	exm-rs3817928	G	A	0.1812	-0.7973	-1.77; 0.17	0.107
9	98231008	rs16909898	G	-0.072	3.94E-09	20010835	exm-rs16909898	G	A	0.09882	0.06927	-1.17; 1.3	0.912
10	12277992	rs7068966	T	0.033	6.13E-13	21946350	exm-rs7068966	T	C	0.4822	0.3341	-0.42; 1.09	0.385
12	57527283	rs11172113	T	-0.032	1.24E-08	21946350	exm-rs11172113	T	C	0.594	0.05361	-0.71; 0.82	0.891
15	71645120	rs12899618	G	0.076	1.86E-15	20010834	exm-rs12899618	G	A	0.8238	-0.8261	-1.8; 0.15	0.097
16	58075282	rs12447804	T	-0.038	3.59E-08	21946350	exm-rs12447804	T	C	0.208	0.8363	-0.09; 1.76	0.077
16	75390316	rs2865531	T	0.031	1.77E-11	21946350	exm-rs2865531	T	A	0.3995	0.01014	-0.73; 0.75	0.979
21	35652239	rs9978142	T	-0.043	2.65E-08	21946350	exm-rs9978142	T	A	0.1641	-0.1791	-1.21; 0.85	0.732

Abbreviations: SNPs: single nucleotide polymorphisms; GRS: genetic risk score; CHR: Chromosome; BP: chromosomal location based on hg19; PMID: PubMed ID of initial finding; BETA, 95%CI, P: results of single SNP analysis without covariates; C_{rs}: compliance

Table S3. SNPs included in the lung function GRS and effects on R_{rs}

Data from GWAS catalog[1]							Results analysis single SNPs with R_{rs} in WHISTLER						
CHR	**BP**	**SNP**	**Allele**	**BETA**	**P-value**	**PMID**	**ID on exome-chip**	**A1**	**A2**	**Freq. A1**	**BETA**	**95%CI**	**P-value**
1	17306675	rs2284746	G	-0.04	7.50E-16	21946350	exm-rs2284746	G	C	0.4806	0.00823	-0.01; 0.03	0.4194
1	218860068	rs993925	T	0.034	1.16E-08	21946350	exm-rs993925	T	C	0.3353	0.02683	0.01; 0.05	0.0145
2	239877148	rs12477314	T	0.041	1.68E-12	21946350	exm-rs12477314	T	C	0.1798	-0.02945	-0.06; 0	0.02697
4	89883979	rs7671167	T	-0.042	1.27E-09	20173748	exm-rs7671167	T	C	0.5322	-0.009007	-0.03; 0.01	0.3741
4	145485738	rs1980057	T	0.063	1.06E-19	20010835	exm-rs1980057	T	C	0.4074	-0.008338	-0.03; 0.01	0.4405
5	95036700	rs153916	T	-0.031	2.12E-08	21946350	exm-rs153916	T	C	0.5363	0.01547	-0.01; 0.04	0.1401
5	147842353	rs11168048	T	-0.047	5.97E-11	20010835	exm-rs11168048	T	C	0.5915	-0.01247	-0.03; 0.01	0.2303
5	156932376	rs2277027	C	-0.042	6.65E-09	20010835	exm-rs2277027	C	A	0.3367	0.009377	-0.01; 0.03	0.3866
6	31568469	rs2857595	G	0.037	2.28E-10	21946350	exm-rs2857595	G	A	0.7714	-0.01153	-0.04; 0.01	0.3481
6	32151443	rs2070600	T	0.126	9.07E-15	20010834	exm534819	T	C	0.03955	-0.005295	-0.06; 0.05	0.8488
6	142750516	rs3817928	G	0.059	2.27E-12	20010835	exm-rs3817928	G	A	0.1812	0.01264	-0.01; 0.04	0.3399
9	98231008	rs16909898	G	-0.072	3.94E-09	20010835	exm-rs16909898	G	A	0.09882	0.01157	-0.02; 0.04	0.492
10	12277992	rs7068966	T	0.033	6.13E-13	21946350	exm-rs7068966	T	C	0.4822	0.01256	-0.01; 0.03	0.2222
12	57527283	rs11172113	T	-0.032	1.24E-08	21946350	exm-rs11172113	T	C	0.594	-0.005938	-0.03; 0.01	0.5712
15	71645120	rs12899618	G	0.076	1.86E-15	20010834	exm-rs12899618	G	A	0.8238	-0.005901	-0.03; 0.02	0.6582
16	58075282	rs12447804	T	-0.038	3.59E-08	21946350	exm-rs12447804	T	C	0.208	-0.01327	-0.04; 0.01	0.2947
16	75390316	rs2865531	T	0.031	1.77E-11	21946350	exm-rs2865531	T	A	0.3995	0.009408	-0.01; 0.03	0.3497
21	35652239	rs9978142	T	-0.043	2.65E-08	21946350	exm-rs9978142	T	A	0.1641	0.02776	0; 0.06	0.04731

Abbreviations: SNPs: single nucleotide polymorphisms; GRS: genetic risk score; CHR: Chromosome; BP: chromosomal location based on hg19; PMID: PubMed ID of initial finding; BETA, 95%CI, P: results of single SNP analysis without covariates; R_{rs}: resistance

SUPPLEMENTAL REFERENCE

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Chapter 3.2

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Early life antibiotic use and the risk of asthma and asthma exacerbations in children

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ABSTRACT

Objectives: Early life antibiotics use might influence the risk of asthma later in life. The research on this topic shows conflicting results. In addition, studies assessing the influence of early life antibiotics use on the risk of asthma exacerbations are limited. In this study we aimed to study the association between use of antibiotics during the first three years of life and the risk of developing childhood asthma and the risk of asthma exacerbations.
Methods: Data from four large childhood cohorts were used; two population-based cohorts: Generation R (n=7,393, the Netherlands) and SEATON (n=924, Scotland, UK), and two asthma cohorts: PACMAN (n=674, the Netherlands) and BREATHE (n=806, Scotland, UK). Odds ratios (ORs) were derived from logistic regression analysis within each database followed by pooling the results using a fixed- or random-effect model.
Results: Antibiotics use in early life was statistically significantly associated with an increased risk of asthma in a meta-analysis of the Generation R and SEATON data (OR: 2.18, 95% CI: 1.04-4.60; I2: 76.3%). However, there was no statistically significant association between antibiotics use in early life and risk of asthma exacerbations later in life in a meta-analysis of the PACMAN and BREATHE data (OR: 0.93, 95% CI: 0.65-1.32; I2: 0.0%).
Conclusions: Children treated with antibiotics in the first three years of life are more likely to develop asthma. However, in asthmatic children early life exposure to antibiotics does not increase the risk of asthma exacerbations.

INTRODUCTION

Asthma and asthma-related complications are global health problems[1]. Asthma is one of the most prevalent chronic disorders worldwide; almost 1–18% of populations (children and adults) in different countries suffer from asthma[2]. Asthma exacerbations may complicate asthma and also occur in patients on asthma medication. Several environmental and genetic factors have been identified as risk factors for asthma and asthma exacerbations[3].

Early life risk factors, such as antibiotic exposure, influence the developing immune system and might also affect the risk of asthma susceptibility and exacerbations[4-6]. Several studies and meta-analyses have addressed this hypothesis but findings are conflicting possibly due to differences in methods applied, study designs, sample sizes, and definitions of exposures and outcome. Furthermore, the association between early life antibiotics use and severity of asthma is not well known[12]. A higher risk of asthma exacerbations in children treated with antibiotics during the first two years of life is suggested by a study from Manchester, UK[12]. Therefore, using four North-European cohorts, the aim of the study was to evaluate the effect of early life antibiotics use on asthma development in the general child population and on the occurrence of asthma exacerbations in asthmatic children.

METHODS

Study setting and population

To study the effect of antibiotics on asthma onset we used data from two population-based cohorts (Generation R[13] and SEATON[14,15]), and to assess the effect of early life antibiotic use on asthma exacerbations we used data from two pediatric asthma cohorts (PACMAN[16] and BREATHE[17,18]).

Generation R is a Dutch prospective population-based cohort study (n=7,585 for current study) which studies children from fetal life until young adulthood. Mothers with a delivery date from 2002 until 2006 were included in this study. This study was designed to identify early environmental and genetic causes of normal and abnormal growth, development and health during fetal life, childhood and adulthood. Detailed information on the design and methods of this study has been described previously[13].

SEATON is a Scottish birth cohort study (n=2,000). Between 1997 and 1999, healthy unselected pregnant women attending an antenatal clinic, at median 11 weeks gestation, were recruited. In this study, singletons born to the recruited women were followed up for 10 years[14,15.]

PACMAN (n=995), is a Dutch cohort of children aged 4-12 years who used asthma medication. They were selected through Dutch community pharmacies. In this cohort, children with at least two years of medication history available and at least three prescriptions for any asthma drug within the last two years and at least one prescription in the last 6 months were selected from pharmacies in different regions in the Netherlands[16].

BREATHE, is a Scottish cohort of children aged 3-19 years (n=1,100) with physician-diagnosed asthma recruited either through primary or secondary clinics. Through a collaboration with the Health Informatics Centre (HIC) in Dundee the Community Health Index (CHI) coding was available for the BREATHE participants and data could be linked to several other databases e.g. community prescribing information and diagnosis of acute hospital admission[17,18].
Within the four cohorts questionnaire-based information was available on general health, allergic symptoms, asthma and respiratory symptoms, healthcare utilization for respiratory symptoms, environmental and socio demographic factors.

Early life antibiotics use:
Early life antibiotics use in the Generation R cohort (first three years of life) and in the SEATON cohort (first 6 months of life) was available from parental-reported data.
In the two asthma cohorts (PACMAN and BREATHE cohorts) pharmacy data were available. In the latter two studies, we only included children with medication data available starting in the 1st year of life. In both the PACMAN and BREATHE cohorts detailed information on dispensed medication is available. In PACMAN, ATC codes (listed in **Table S1)** and in BREATHE, BNF codes (including all antibiotics in Chapter 5.1 of the British National Formulary for children, 2012) are used. Date of dispensing, duration, and amount dispensed and doses were extracted from these databases. In case of missing value, duration of antibiotics was calculated by assuming that a course of antibiotics takes seven days[19].
Depending on the available information in the four databases, antibiotics use was classified as 1) exposed vs. never exposed (in all four databases), 2) the timing of the first antibiotic use: 1st year, the 2nd year or the 3rd year of life (in Generation R, PACMAN and BREATHE), 3) the number of prescriptions for antibiotics during the first three years of life (PACMAN and BREATHE), and 4) the duration of exposure to antibiotics based on the total days of exposure to antibiotics also during the first three years of life (PACMAN and BREATHE).

Respiratory outcomes
Two outcomes were defined in this study: 1)Asthma development: physician-diagnosed asthma was identified if a positive response was given to both of the following questions at age of 9-10 years in Generation R and at age of 10 years in SEATON “has your child ever suffered from asthma?” and “was this confirmed by a doctor?”, and 2) Asthma exacerbations: defined as asthma-related visits to an emergency department (ED) and/or the use of oral corticosteroids (OCS) in the past 12 months in PACMAN and asthma-related hospitalization and/or OCS use in the past 6 months in BREATHE[20].

Statistical analysis
Descriptive statistics using frequencies, means and standard deviations (SDs) were used to describe the four cohorts. Univariate and multivariable logistic regression analyses were used to estimate ORs and 95% confidence intervals (CIs) for the association be-

tween exposure and the two outcomes. Based on available variables in each database, the associations were adjusted by different confounders. In Generation R, the association was adjusted for age, gender, and family history of asthma/allergy. In SEATON, gender, and family history of asthma were included in the adjusted model. In PACMAN and BREATHE, age, gender and family history of asthma/allergy were included in the model. In the studies where pharmacy data was available (PACMAN and BREATHE) we further tested the association of number of courses and duration of antibiotics use with asthma exacerbations. In all analyses children exposed to antibiotics were compared with unexposed children. The estimates for the association between antibiotics use and asthma in the Generation R and SEATON cohorts were pooled using a random-effect model. To reduce heterogeneity in the meta-analysis, in the Generation R cohort we only used the 1st year of life of antibiotics exposure to be more comparable with the SEATON cohort in which the exposure to antibiotics is only known during the first 6 months of life. The same pooled analysis was performed for the association between antibiotic use and asthma exacerbations in PACMAN and BREATHE, using a fixed model. A p-value of 0.05 was used to assess the significance of main effect associations.

All statistical analyses were conducted using statistical package R version 3.2.3 (2015-12-10) and STATA 14/SE (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

RESULTS

Characteristics of children included in the four cohorts are shown in **Table 1**. As shown, in the population-based cohorts, 7,393 out of 7,588 (Generation R) and 924 out of 2,000 (SEATON) children had data available on both exposure and outcome. In Generation R, the rate of early life exposure to antibiotics (1st year) was 55% while in SEATON, this rate (first 6 months) was 28%. Cumulative incidence rates of asthma in Generation R and SEATON were 8% and 10%, respectively.

In the asthma cohorts, 674 out of 995 (PACMAN) and 806 out of 1,100 (BREATHE) children had pharmacy data available starting from the 1st year of life. In these cohorts, the amount of children who had at least one antibiotic prescription during the first three years of life ranged between 78%-84%. Rate of children with at least one asthma exacerbation varied between 11% in PACMAN and 24% in BREATHE.

Early life antibiotics use and asthma susceptibility

In the two population-based cohorts, pooled results showed that children who consumed antibiotics during the 1st year of life were more likely to develop asthma (OR: 2.18, 95% CI: 1.04-4.60; I^2: 76.3%) compared with those who did not (**Fig 1**). In Generation R, antibiotics consumption in the 1st year of life was associated with a higher risk of developing asthma (adjusted (adj)OR: 3.21, 95% CI: 1.89-5.45) compared with antibiotics use in the 2nd year of life (adjOR: 2.25, 95% CI: 1.18-4.30) or in the 3rd year of life (adjOR: 2.21, 95% CI: 0.92-5.33). In SEATON, we found a positive association that was not statistically

significant (p=0.11) between antibiotics use during the first 6 months of life and increased risk of asthma (OR: 1.50, 95% CI: 0.91-2.46) (**Table 2**).

Table 1. Characteristics of the Generation R, SEATON, PACMAN and BREATHE cohorts

		Generation R	SEATON	PACMAN	BREATHE
		N=7,393	N=924	N=960	N=806
Gender (boys), n (%)		3,707 (51.1)	441 (47.7)	598 (62.3)	508 (63.0)
Early life antibiotics exposure, n (%)	Exposed	3,178 (80.3)	260 (29.2)	527 (78.2)	680 (84.4)
	Non-exposed	782 (19.7)	631 (70.8)	147 (21.8)	126 (15.6)
The timing of the first antibiotics use, n (%)	1st year of life	1,820 (55.2)	NA	320 (60.7)	350 (51.5)
	2nd year of life	503 (15.3)	NA	145 (27.5)	201 (29.5)
	3rd year of life	191 (5.8)	NA	62 (11.8)	129 (19.0)
Frequency of antibiotics prescriptions in the first 3 year, n (%)	1	NA	NA	131 (24.8)	142 (20.8)
	2	NA	NA	102 (19.4)	111 (16.4)
	≥3	NA	NA	294 (55.8)	427 (62.8)
Duration of antibiotics used during the first 3 year, n (%)	≤10 days	NA	NA	121 (23.0)	142 (20.8)
	11-30 days	NA	NA	225 (42.7)	270 (39.7)
	>31 days	NA	NA	180 (34.2)	268 (39.4)
Asthma diagnosis, n (%)		448 (9.5)	78 (8.4)	NA	NA
Exacerbations*, n (%)		NA	NA	101 (10.5)	196 (24.3)
Family history of asthma**, n (%)		664 (16.7)	230 (24.9)	440 (45.8)	315 (39.1)
Family history of allergy/atopy***, n (%)		2,739 (60.5)	NA	752 (78.3)	351 (43.5)

Abbreviation: NA, not available
Early life antibiotics use in Generation R (first 3 years) and SEATON (first 6 months) is based on parental-reported data.
Prescribing data for the 1st years of life was available in PACMAN and BREATHE.
*Asthma exacerbations defined as at least one course oral corticosteroids use and/or at least one emergency department visits/hospitalizations due to asthma
**At least one asthmatic parent
***At least one allergic/atopic parent

Early life antibiotic use and asthma exacerbations

In the two asthma cohorts, pooled results showed no associations between early life antibiotic use and asthma exacerbations (OR: 0.93, 95% CI: 0.65-1.32; I^2: 0.0%). In PACMAN, although not statistically significant, children exposed to more courses and longer duration of antibiotics therapy during the first three years of life were more likely to have asthma exacerbations later in life. However, this was not found in the BREATHE study (**Fig 2**).

Table 2. Associations between early life exposure to antibiotics and asthma/asthma exacerbations

		Asthma development		Asthma exacerbations	
		Crude	Adjusted	Crude	Adjusted
Generation R					
Ever exposed vs. never exposed to antibiotics		**3.29 (2.17-5.00)**	**2.84 (1.70-4.75)**	NA	NA
1st year of life		**3.68 (2.40-5.65)**	**3.21 (1.89-5.45)**	NA	NA
2nd year of life		**2.70 (1.61-4.54)**	**2.25 (1.18-4.30)**	NA	NA
3rd year of life		**2.63 (1.35-5.11)**	2.21 (0.92-5.33)	NA	NA
SEATON				NA	NA
Ever exposed vs. never exposed to antibiotics		1.54 (0.94-2.52)	1.50 (0.91-2.46)	NA	NA
PACMAN					
Ever exposed vs. never exposed to antibiotics		NA	NA	1.16 (0.65-2.08)	1.09 (0.60-1.96)
*Frequency of antibiotics use**	1	NA	NA	0.74 (0.33-1.66)	0.68 (0.30-1.56)
	2	NA	NA	1.39 (0.65-2.96)	1.19 (0.54-2.61)
	≥3	NA	NA	1.29 (0.70-2.39)	1.25 (0.67-2.34)
Cumulative duration of antibiotics use	≤ 10 days	NA	NA	0.81 (0.36-1.81)	0.67 (0.28-1.58)
	11-30 days	NA	NA	1.01 (0.52-1.96)	0.95 (0.48-1.87)
	≥30 days	NA	NA	1.66 (0.86-3.18)	1.61 (0.83-3.13)
BREATHE					
Ever exposed vs. never exposed to antibiotics		NA	NA	0.81 (0.53-1.24)	0.85 (0.55-1.32)
*Frequency of antibiotics use**	1	NA	NA	0.88 (0.51-1.52)	0.99 (0.57-1.71)
	2	NA	NA	0.72 (0.40-1.30)	0.78 (0.42-1.44)
	≥3	NA	NA	0.81 (0.51-1.26)	0.82 (0.52-1.31)
Cumulative duration of antibiotics use	≤ 10 days	NA	NA	0.88 (0.51-1.52)	0.99 (0.57-1.71)
	11-30 days	NA	NA	0.82 (0.51-1.33)	0.87 (0.53-1.43)
	≥30 days	NA	NA	0.75 (0.46-1.22)	0.76 (0.46-1.25)

Abbreviation: NA, not available.
Asthma exacerbations defined as at least one course oral corticosteroids use and/or at least one emergency department visits/hospitalizations due to asthma;
* Number of courses of antibiotics

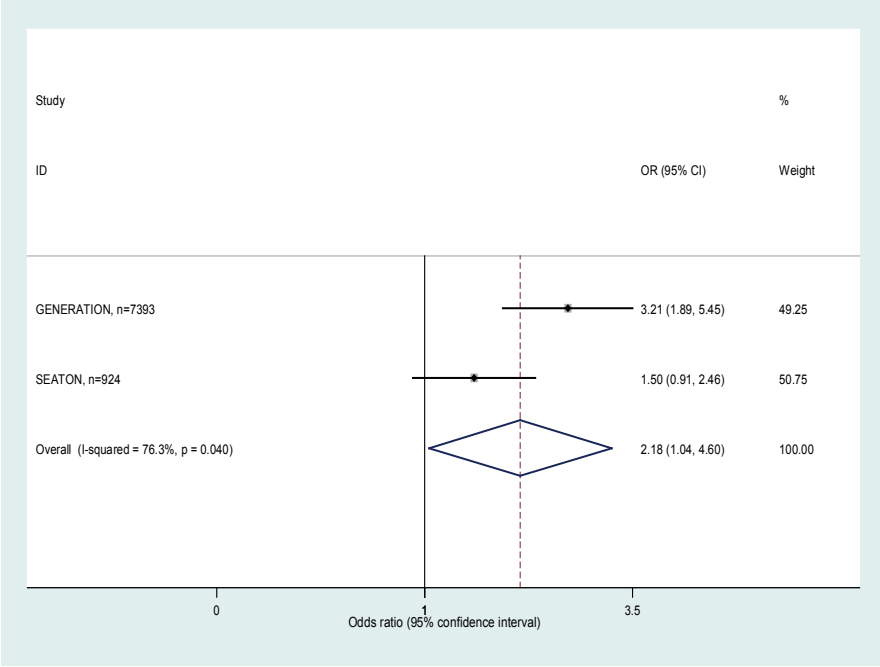

Fig 1. Pooled results of the association between early life exposure to antibiotics and asthma

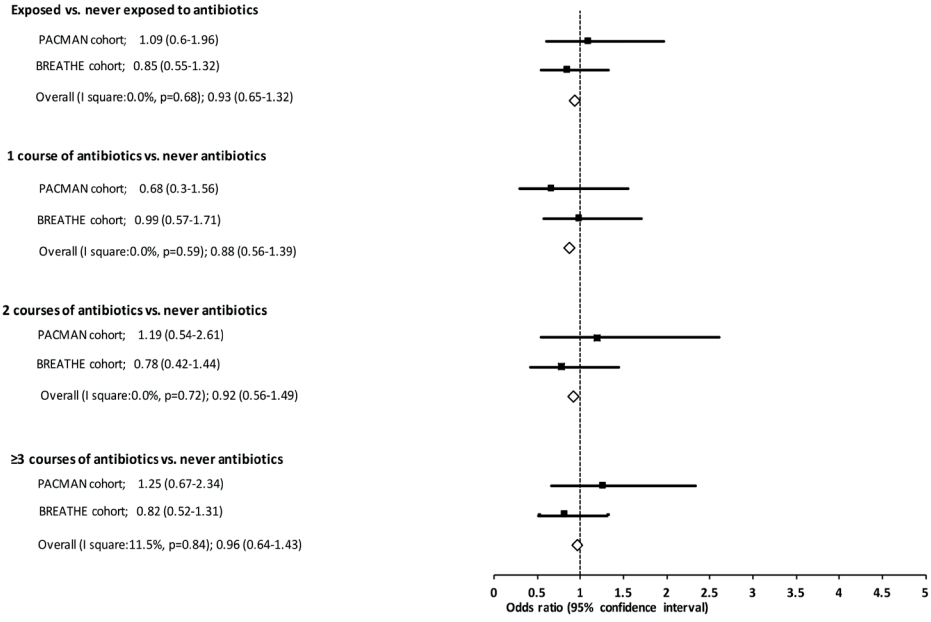

Fig 2. Pooled results of the associations between early life exposure to antibiotics and asthma exacerbations

DISCUSSION

In this study we evaluated the effect of early life antibiotics use on asthma susceptibility and on the risk of asthma exacerbations. We showed an association between early life antibiotics use and the risk of developing asthma in children, but not asthma exacerbations in asthmatic children.

With the worldwide increasing trend in prevalence rates of childhood asthma, it is important to study the impact of potential early life risk factors on this burden[21]. In recent decades, a rise in the proportion of children treated with antibiotics in early childhood has also been reported; the highest rate of antibiotic use (18.7%) was shown in children aged >1 month to 18 years treated for a bacterial lower respiratory tract infection[22]. When antibiotics use in early life indeed increases the risk of the occurrence of asthma, it is important to be more critical about the prescribing of antibiotics. Many studies have shown that antibiotics are often prescribed for indications for which antibiotics are not necessary like common cold and sinusitis caused by viral infections[23,24].

In this study, we used the data from a large population-based birth cohort study the Generation R study from the Netherlands and a Scottish birth-cohort study the SEATON to assess the relationship between antibiotics use in early childhood and asthma later in life. A higher risk of subsequent asthma among children treated with antibiotics during early childhood was shown which is in line with a recent study by Örtqvist et al.[9]. In a large cohort study they showed a higher risk of subsequent asthma in children exposed to antibiotics in early childhood. In this study, the highest risk was observed in children aged 6 months and younger (HR: 3.71, 95% CI: 3.41-4.03) whereas risk decreased with age[9]. A more recent meta-analysis[10] from 2011 including 18 studies also reported a statistically significant association between early life antibiotics exposure and the development of wheeze/asthma (OR: 1.27, 95% CI: 1.12-1.43). However, the association was weak when studies with possible reverse causation and confounding by indication were excluded (n=9)[10].

Moreover, in Generation R, similar to a previous study[12], we showed that the strongest risk of asthma was in children exposed to antibiotics during the first year of life and the effect decreased if they were treated at an older age. Previous studies showed that the increased risk of developing asthma decreased by increasing age; meaning that children exposed to antibiotics in early life developed asthma symptoms shortly after antibiotic use. The results in these previous studies were no longer statistically significant after three years of age[9,12,25]

It has been suggested that the gut microbiome may play a role in modulating the immune system (T helper and regulatory T cell balance) and thereby the risk of asthma and asthma exacerbations[4,26,27]. An experimental animal model of allergic asthma demonstrated that antibiotics in early life shifted the resident gut flora and was associated with asthma-related immune responses in the first three weeks of age[28]. In addition, a previous study in children reported a positive statistically significant association between antibiotics use and asthma exacerbations shortly after treatment with antibiotics (before the age two years), where the association was no longer significant when children were older[12].

Our study could not confirm that early life antibiotics use is associated with an increased risk of asthma exacerbations later in life. However, we were not able to study asthma exacerbations in early childhood.

One of the strengths of the current study is that we used prospectively collected information on antibiotics use and outcomes in the four cohorts. This makes the occurrence of reverse causality bias less likely. In the PACMAN and BREATHE cohorts, we had exact information of the timing of the dispensing of antibiotics and the start of asthma medication using pharmacy data. Some potential limitations in this study should be acknowledged. Importantly, for the association between early life use of antibiotics and asthma diagnosis in the two cohorts (Generation R and SEATON), the information on the use of early life antibiotics was parental reported, therefore the possibility of recall bias should be considered. Also an important limitation is the lack of availability of some important potential confounders such as genetic factors, socioeconomic status and infections/siblings. The risk of confounding by indication is a major concern in which a part of the association between early antibiotics use and later asthma and asthma exacerbations could be explained by this bias.
In the Generation R and SEATON cohorts, we cannot rule out that respiratory symptoms in early life treated with antibiotics might have been the first signs of a developing asthma. If that was the case this might have led to confounding by indication. In none of the cohorts we had information about the indications for the prescribing of antibiotics. Different infections might trigger the onset of asthma symptoms differently[29,30].

In summary, our findings show a higher risk of asthma in children who are treated with antibiotics early in life, but no significant association of early life antibiotics use and asthma exacerbations later in childhood. Current knowledge on the role of the immune system and gut microbiome in the pathogenesis of asthma is limited and therefore more research is needed.

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Table S1. Codes used to identify antibiotic medication for systemic use

Antibiotic medications (ATC code)	Subgroup 1 (ATC code)	Subgroup 2 (ATC code)
Antibacterials (J01)	Tetracyclines (J01A)	
	Amphenicols (J01B)	
	Beta-lactam antibacterials, penicillins (J01C)	Penicillins with extended spectrum (J01CA), beta-lactamase-sensitive penicillins (J01CE), beta-lactamase-resistant penicillins (J01CF), beta-lactamase inhibitors (J01CG), combinations of penicillins, including beta-lactamase inhibitors (J01CR)
	Other beta-lactam antibacterials (J01D)	First-generation cephalosporins (J01DB), second-generation cephalosporins (J01DC), third-generation cephalosporins (J01DD), fourth-generation cephalosporins (J01DE), monobactams (J01DF), carbapenems (J01DH), other cephalosporins and penems (J01DI)
	Sulfonamides and trimethoprim (J01E)	
	Macrolides (J01FA)	
	Aminoglycoside antibacterials (J01G)	
	Quinolone antibacterials (J01M)	
	Other antibacterials (J01X)	

Chapter 3.3

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Early life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis

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ABSTRACT

Objectives: Studies suggested that early life exposure to antibiotics is associated with an increased risk of developing allergies later in life, but results are inconsistent. In this study we aimed to systematically review and quantify the relationship between exposure to antibiotics in early childhood and the risk of allergy/atopy later in life.

Methods: PubMed and Web of Science databases were searched for observational studies published from January 1966 through November 11, 2015. Definition of exposure was antibiotics use during the first 2 years of life. The outcomes were symptoms of hay fever, eczema, food allergy, positive skin prick testing (SPT) or elevated allergen-specific serum/plasma IgE levels later in life. Overall pooled estimates of the odds ratios (ORs) were obtained using fixed or random-effects models.

Results: Statistically significant associations were found for early life exposure to antibiotics and reported symptoms of hay fever, eczema and food allergy. The summary OR for risk of hay fever (21 studies) was 1.23, 95% confidence interval (CI):1.13-1.34; I2: 77.0%. The summary OR for the risk of eczema (22 studies) was 1.26, 95% CI: 1.15-1.37; I2: 74.2% and the summary OR for food allergy (3 studies) was 1.42, 95% CI: 1.08-1.87; I2: 80.8%. However, no association was found for antibiotics exposure early in life and positive SPT or elevated allergen-specific IgE levels.

Conclusions: Early life exposure to antibiotics appears to be related with an increased risk of allergic symptoms later in life, but not to objective atopy measurements including positive SPT or elevated allergen-specific serum/plasma IgE levels.

INTRODUCTION

Antibiotics are among the most commonly prescribed medications in children with acute otitis media (AOM) and upper respiratory tract infections (URIs)[1-5]. A worldwide study of the use of antibiotics showed a prevalence rate of 57.6% among hospitalized neonates and children[6]. A US study by Hick et al. in an outpatient setting also showed that antibiotics were more prescribed in infants and children ≤2 years of age than in other age groups[7].
Early life exposure to antibiotics has been related to some later life morbidities such as obesity, arthritis and allergies[8]. A meta-analysis from 2006 showed a higher risk of asthma among those children exposed to antibiotics in early childhood[9]. However, a more recent meta-analysis from 2011 reported that the association between antibiotics exposure and subsequent development of wheeze/asthma was weak when the analysis was adjusted for reverse causation and confounding by indication[10]. Several studies have suggested that early life exposure to antibiotics is associated with an increased risk of developing allergies and atopies later in life, but results are inconsistent[11-47].
Therefore, the aim of this study was to conduct a systematic review and meta-analysis to assess and quantify the relationship between early life exposure to antibiotics and the risk of developing symptoms of hay fever, eczema, food allergy, positive skin prick testing (SPT) or elevated allergen-specific serum/plasma IgE levels later in life.

METHODS

For this meta-analysis, we followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines[48].

Data source

We conducted a comprehensive literature search of PubMed and the Web of Science using multiple search terms shown in **Table S1**. Additional articles were retrieved through a manual search of references from included articles.

Inclusion and exclusion criteria

Studies were included that a) assessed the association between antibiotics consumption during the first 2 years of life and the risk of developing hay fever, eczema, food allergy, positive SPT or elevated allergen-specific serum/plasma IgE levels, and b) a quantified measurement of association between exposure and outcomes was reported as odds ratio (OR) or hazard ratio (HR) or could be calculated as OR. Our literature search was restricted to studies published in English from January 1966 through November 11, 2015. We excluded those studies that were limited to ever use of antibiotics (not restricted to the first 2 years of life)[45-47].

Data Extraction and clinical end points

The following information was retrieved from the published studies: first author, year of publication, the aim of the study, study design, study size, child's age at time of antibiotics consumption as well as the age at time of outcomes measurement. When available, we extracted the crude or adjusted ORs/HRs for the association of antibiotics and allergy/atopy from the articles. Where direct extraction was not possible, ORs and 95% confidence intervals (CIs) were calculated. When adjusted effect estimates/CIs were not reported, we contacted the authors to provide additional information in order to include these studies in the meta-analysis[35,42].

The outcomes studied in our meta-analysis were: a) hay fever defined as any experience of allergic rhinitis such as sneezing, runny nose, nasal blockage or red and itching eyes, or any medication used for hay fever or a physician diagnosis of hay fever during the last 12 months at the time of outcome measurement, b) eczema defined as any dermatitis e.g. itching skin disease or itchy rash reported by parents or diagnosed by a physician during the last 12 months before outcome measurement, c) food allergy defined as any symptoms to food plus specified criteria e.g. clinical examination and a positive SPT or cow's milk allergy (with ICD-10 codes L27.2 or K52.2), d) a positive response to SPT and e) Increased serum/plasma specific IgE level (≥0.25 IU/mL or ≥0.3 KU/L).

Multiple effect estimates within one study

For studies that reported ≥ 1ORs/HRs for the association between antibiotics and outcomes we selected one effect measurement per study to include in the primary meta-analysis. The choice of the effect measurement was based on:

1 the shortest period of the exposure; e.g. if a study reported two ORs/HRs one for antibiotics exposure within the 1st year of life and one for antibiotics exposure within the 2nd year of life, the 1st year-estimated effect was included in the primary meta-analysis. Moreover, if ORs/HRs were reported for variable amounts of antibiotics (e.g. 1 course, 2 courses, 3 courses etc) the effect measurement of the largest sample size was included in the primary meta-analysis.

2 the longest follow-up period for the outcome e.g. in a study that reported ORs/HRs for the association of early in life antibiotics exposure and the occurrence of outcomes in children at the age of 4 years and children at the age of 8 years, the OR/HR associated to the outcome at 8 years was included in the primary meta-analysis.

Other reported ORs/HRs were used in subgroup meta-analyses.

Quality assessment

Three authors in this study (FA, AHM, SJHV) assessed the quality of the included studies with the checklist of Newcastle-Ottawa Scale (NOS) for cohort, case control studies and cross- sectional[49]. Using this tool, each study was evaluated independently by the authors, on eight items categorized into three groups including the selection of the study group, the comparability of the groups and the assessment of either the exposure or outcome of interest for different studies. In those studies that reported multiple results for the association between exposure and outcomes, the quality of study was assessed based

on the strongest outcome. In cases of disagreement, consensus between the authors was reached after discussion.

Statistical analysis

Overall pooled ORs, together with 95% CIs of the association between early life exposure to antibiotics and allergies/atopies were obtained using either a fixed-effects model or in case of heterogeneity, a random-effects model. Heterogeneity was identified using the I^2 statistic with a significance level of $\alpha = 0.05$, which quantifies inconsistency across studies included; 25% corresponding to low, 50% to moderate and 75% to high heterogeneity. The primary meta-analyses included all studies on the association between exposure and outcomes and were run for hay fever, eczema, food allergy, positive SPT and elevated serum/plasma IgE levels, separately. To assess clinical and methodological heterogeneity in the pooled results, we performed subgroup meta-analyses taking into account the study design, study size, the child's age at the time of antibiotics use, patient's age at the time of diagnosis, the way to collect data for both exposure and outcomes (parental reported or medical/pharmacy record) and the number of courses of antibiotics treatment. Meta-regression analyses were performed on the effect of the child's age at the time of antibiotics consumption and the age at the time of diagnosis. A series of sensitivity analyses was also applied by a) excluding studies with low quality scores (≤5 NOS criteria b) excluding studies which only assessed a high risk population c) excluding multi center studies, separately for each outcome and investigating the effect of these studies on the overall pooled estimate. To test the causal relation between antibiotics and outcomes, the second meta-analysis only included those studies that have explicitly reported that patients diagnosed with allergies/atopies are new incident cases at the time of outcome measurement. Publication bias was evaluated by using funnel plots where the Egger test was applied to measure any asymmetry. In this meta-analysis, for reasons of symmetry, the reported ORs and lower and upper bounds of the 95% CIs were initially log-transformed; the log ORs together with 95% CIs of the log ORs were meta-analyzed using either fixed or random-effects models, then the results were transformed back to the original ORs for reporting. P-values of <0.05 were used to assess the statistical significance of main effect associations. All statistical analyses were conducted using STATA 10/SE (StataCorp. 2007. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP). For meta-analytic procedures, metan, metabias and metareg commands were used.

RESULTS

Systematic search results

A flow chart (**Fig 1)** describes study identification, screening and inclusion. Our literature search yielded 3,839 published articles, and after applying the inclusion and exclusion criteria a total of 34 studies[11-44] were selected for the meta-analysis.

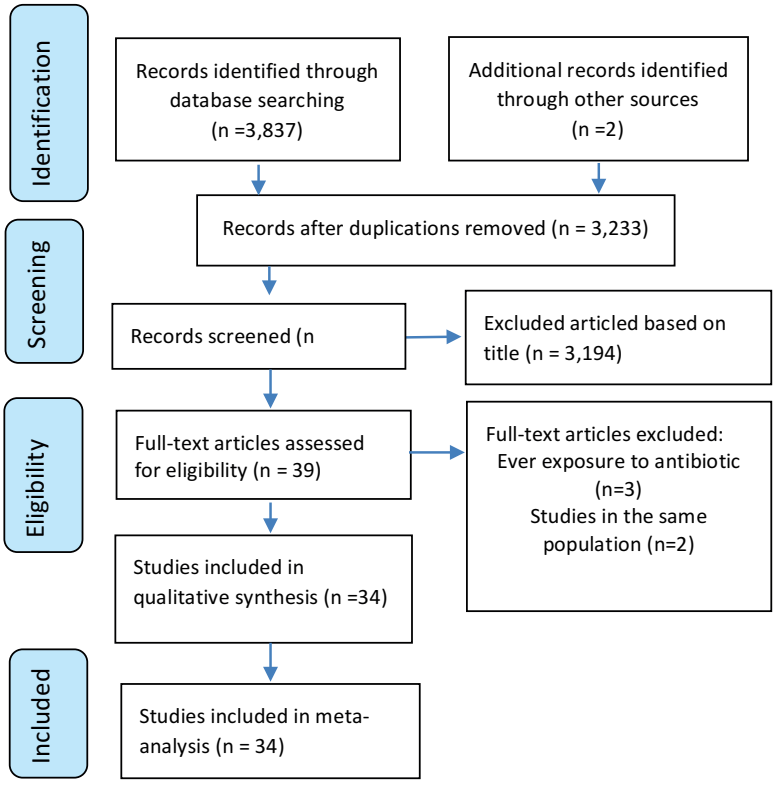


Fig 1. A flow chart diagram showing the selection process of studies

Quality assessment

We investigated the quality of the selected studies with the use of NOS checklist as presented in **Table 1**. Some studies selected were shown to be of high quality (>5 NOS criteria) whereas four studies were scored as ≤5 [12,25,33,40,44]. In a sensitivity analysis, we assessed the effect of those studies with low quality on the association between antibiotics and outcomes. Since the pooled effect estimate for these associations (in different outcomes) were not influenced by low-quality studies (the results are shown later in the sensitivity analysis section), all selected studies were included in the meta-analysis.

Study characteristics

The characteristics of the included studies are summarized in **Table 2** for cohort studies (n=20 studies), **Table 3** for case control studies (n=6 studies) and **Table 4** for cross-sectional studies (n=8 studies); the design of these studies is based on what authors have stated in the manuscript. Twenty-one studies (including 229,080 patients) were selected to study the risk of hay fever, 22 studies (including 394,517 patients) to study the risk of eczema, 3 studies (including 23,878 children) to study the risk of food allergy, 10 studies (including 27,092 children) to study the risk of positive SPT and 8 studies (including 16,043 children) to study the risk of elevated allergen-specific serum/plasma IgE levels.

Table 1. Quality assessment of included studies based on the NOS checklist

	Source	Selection (Maximum of 4 stars)	Comparability (Maximum of 2 stars)	Outcome assessment (Maximum of 3 stars)
1	Alm et al, 2014	***	**	*
2	Bohme et al, 2002	**	**	***
3	Bremner et al, 2003	***	**	**
4	Celedón et al, 2002	***	**	***
5	Cohet et al, 2004	***	**	**
6	Cullinan et al, 2004	****	**	**
7	Dom et al, 2010	***	**	*
8	Droste et al, 2000	***	**	**
9	Farooqi et al, 1998	****	**	***
10	Floistrup et al, 2005	**	**	**
11	Foliaki et al, 2009	***	**	**
12	Harris et al, 2007	****	**	**
13	Hoskin et al, 2013	**	**	**
14	Johnson et al, 2005	***	**	***
15	Kummeling et al, 2007	**	**	*
16	Kusel et al, 2008	***	**	***
17	Mai et al, 2010	***	**	**
18	McKeever et al, 2002	****	**	**
19	Metsälä et al, 2013	****	**	***
20	Muc et al, 2013	**	**	**
21	Mullooly et al, 2007	***	**	***
22	Mutius et al, 1999	**	**	**
23	Ponsonby et al, 1999	**	**	*
24	Purvis et al, 2005	****	-	**
25	Raciborski et al, 2012	***	*	**
26	Risnes et al, 2011	***	**	***
27	Sandini et al, 2011	**	**	***
28	Schmitt et al, 2010	****	**	**
29	Sobko et al, 2010	***	**	**
30	Su et al, 2010	***	*	**
31	Sultész et al, 2010	**	-	**
32	Tamay et al, 2007	***	**	**
33	Thomsen et al, 2006	****	**	**
34	Wickens et al, 2008	****	**	*

Note: Comparability was studied based on the published results. Since in bold studies, the reported effect estimates did not met the inclusion criteria in our study, we had to calculate the crude odds ratios and were not able to adjust for confounders. Therefore, these studies might loss scores in this part.
In this checklist, the highest quality studies are awarded up to 9 stars.

Table 2. Overview of cohort studies included in the meta-analysis

	Source	Country	Study size	Antibiotics measurement	Subgroup of antibiotics assessment	Age range at time of antibiotics use	Outcome (s) measurement	Age range at time of allergy measurement	Extracted effect size (95%CI)
Prospective cohort									
1	Alm et al, 2014	Sweden	4,051	Parental reported	-	First week	Parental reported of hay fever/ physician's diagnosis of allergic rhinitis	School age	Allergic rhinitis OR: 1.75 (1.03-2.97)
2	Bohme et al, 2002	Sweden	4,089	Parental reported	-	First 2 years	Parental reported of physician diagnosis of outcomes	1-2	Eczema calculated OR: 1.35 (1.17-1.57)
3	Celedón et al, 2002	US	448	Parental reported	One course and ≥2 courses antibiotic	First year	Parental reported of outcomes	5	Allergic rhinitis OR: 0.7 (0.3-1.5) Eczema OR: 1.1 (0.4-3.1)
4	Dom et al, 2010	Netherlands	773	Parental reported	-	First year	Parental reported of physician diagnosis of outcomes-Blood test	Up to 4	Eczema OR: 0.61 (0.36-1.01) Elevated IgE OR: 0.38 (0.21-0.71)
5	Harris et al, 2007	UK	642	Prescription data	-	First year	Parental reported of outcomes based on ISAAC questionnaire-SPT	8	Seasonal rhinitis OR: 1.08 (1.00-1.17) Positive SPT OR: 0.96 (0.87-1.07)
6	Hoskin et al, 2013	UK	4,952	Parental reported	≥2 courses antibiotic	First 2 years	Parental reported of physician diagnosis of outcomes-SPT	7.5	Eczema OR: 1.2 (1.02-1.41) Hay fever OR: 1.28 (1.03-1.6) Positive SPT OR: 1.00 (0.80-1.25)
7	Johnson et al, 2005	US	725	Prescription data	Broad spectrum antibiotic	First 6 months	Parental reported of outcomes-SPT-Blood test	6-7	Positive SPT OR: 1.48 (0.94-2.34) Elevated IgE OR: 1.16 (0.72-1.87)

	Source	Country	Study size	Antibiotics measurement	Subgroup of antibiotics assessment	Age range at time of anti-biotics use	Outcome (s) mea-surement	Age range at time of allergy measurement	Extracted effect size (95%CI)
8	Kummeling et al, 2007	Netherlands	2,764	Parental reported	-	First 6 months	Parental reported of physician diagnosis of outcomes-Blood test	First 2 years	Eczema OR: 0.94 (0.75-1.18) Elevated IgE OR: 1.32 (0.86-2.02)
9	Kusel et al, 2008	Australia	198	Daily diary for 5 years	-	First year	Physician diagnosis of outcomes- SPT-Blood test	5	Eczema OR: 1.2 (0.6-2.5) Positive SPT OR: 0.6 (0.3-1.4) Elevated IgE OR: 1.00 (0.50-2.10
10	Mai et al, 2010	Sweden	3,306	Parental reported	-	First year	Parental reported of physician diagnosis of outcomes-Blood test	4 and 8	Eczema OR: 1.3 (1.1-1.5) Allergic rhinitis OR: 1.1 (0.9-1.3) Food allergy OR: 1.4 (1.1-1.7) Elevated IgE OR: 0.9 (0.7-1.1)
11	McKeever et al, 2002	UK	29,238	Prescription data	Types of anti-biotic/ one, 2,3 and ≥4 courses antibiotic	First year	Physician diagnosis of outcomes	2-11	Eczema HR: 1.22 (1.12-1.34) Hay fever HR: 1.14 (0.94-1.38)
12	Ponsonby et al, 1999	Australia	863	Parental reported	-	First month	Parental reported of outcomes based on ISAAC questionnaire	7 years	Hay fever: calculated OR: 1.14 (0.70-1.85)
13	Risnes et al, 2011	US	1,401	Parental reported	Frequency of antibiotic: once, twice, ≥ 3 times/ one and ≥ 2 courses antibiotic	First 6 months	SPT	6	Positive SPT OR: 1.59 (1.10-2.28)

	Source	Country	Study size	Antibiotics measurement	Subgroup of antibiotics assessment	Age range at time of antibiotics use	Outcome (s) measurement	Age range at time of allergy measurement	Extracted effect size (95%CI)
14	Sandini et al, 2011	Finland	1,223	Parental reported	-	First 6 months	Physician diagnosis of outcomes-Blood test	2 and 5 years	Eczema OR: 1.20 (0.79-1.81) Allergic rhinitis OR: 0.92 (0.54-1.56) Elevated IgE OR: 0.82 (0.54-1.25)
15	Schmitt et al, 2010	Germany	370	Health insurance database	Type of antibiotic	First year	Health insurance database which is based on physician diagnosis of outcomes	2	Eczema calculated OR: 1.52 (0.73-3.14)
16	Sobko et al, 2010	Sweden	Cases: 203 controls: 426	Prescription data	-	At birth time	Parental reported of outcomes based on ISAAC questionnaire	7-23	Eczema OR: 0.94 (0.66-1.36) Hay fever OR: 1.28 (0.75-2.17)
17	Su et al, 2010	US	424	Parental reported	-	First 9 months	Parental reported of outcome-Blood test	5	Eczema calculated OR: 1.04 (0.52-2.1) Elevated IgE calculated OR: 1.13 (0.63-2.03)
18	Wickens et al, 2008	New Zealand	1,105	Parental reported	-	First 3 months	Parental reported of outcomes based on ISAAC questionnaire-SPT	4	Eczema OR: 1.52 (0.87-2.65) Positive SPT OR: 1.49 (1.03-2.15)
Retrospective cohort									
19	Cullinan et al, 2004	UK	746	Prescription data	Type of antibiotic	First year	Parental reported of physician diagnosis of hay fever- SPT	Mean age 28	Hay fever OR: 0.95 (0.80-1.13) Positive SPT OR: 0.86 (0.73-1.02)
20	Farooqi et al, 1998	UK	1,934	Prescription data	Type of antibiotic/ one, 2 and 3 courses antibiotic	First 2 years	Physician diagnosis of outcomes	6-12	Eczema OR: 2.04 (1.53-2.73) Hay fever OR: 2.04 (1.59-2.62)

Abbreviation: SPT: skin prick test

Table 3. Overview of case control studies included in the meta-analysis

	Source	Country	Study size	Antibiotics measurement	Subgroup of antibiotics assessment	Age range at time of antibiotics measurement	Outcome (s) measurement	Age range at time of allergy measurement	Extracted effect size (95% CI)
1	Bremner et al, 2003	UK	Cases: 7098 controls: 7,098	Prescription data	Broad spectrum antibiotic/ 1-2 and ≥3 courses antibiotic/type of antibiotics/ duration of antibiotics	First year	Physician diagnosis of outcomes	After 2 years	Hay fever OR GPRD: 1.08 (0.98-1.2) DIN: 1.15 (1.03-1.29)
2	Cohet et al, 2004	New Zealand	Cases: 1,584 controls: 2,539	Parental reported	-	First year	Parental reported of outcomes based on ISAAC questionnaire	6-7	Eczema OR: 1.40 (1.21-1.62) Hay fever OR: 1.52 (1.25-1.85)
3	Metsala et al, 2013	Finland	Cases: 16,237 controls: 16,237	Prescription data	Type of antibiotics	First month	Physician diagnosis of outcome	1 month	Cow's milk allergy OR: 1.71 (1.59-1.84)
4	Mullooly et al, 2007	US	Cases: 844 controls: 230	Dispensing data	-	First 2 years	Parental reported of outcomes- SPT	6-16	Eczema OR: 1.02 (0.95-1.10) Allergic rhinitis OR: 1.03 (0.99-1.08) Positive SPT OR: 0.95 (0.89-1.01)
5	Purvis et al, 2005	New Zealand	Cases: 87 controls: 463	Parental reported	-	First year	Dermatitis by trained investigator	3.5	Eczema OR: 1.18 (0.61-2.26)
6	Thomsen et al, 2006*	Denmark	480	Parental reported	-	First 2 years	Parental reported of outcomes	7-17	Hay fever OR: 0.73 (0.38-1.40)

Abbreviation: SPT: skin prick test
***Case cohort study**

Table 4. Overview of cross-sectional studies included in the meta-analysis

	Source	Country	Study size	Antibiotics measurement	Subgroup of antibiotics assessment	Age range at time of antibiotics measurement	Outcome (s) measurement	Age range at time of allergy measurement	Extracted effect size (95% CI)
1	Droste et al, 2000	Belgium	1,206	Parental reported	-	First year	Parental reported of outcomes- SPT	7-8	Eczema OR: 1.3 (1.00-1.80) Hay fever OR: 2.3 (1.3-3.8) Positive SPT OR: 1.1 (0.7-1.7)
2	Muc et al, 2013	Portugal	1,063	Parental reported	-	First 12 months	Parental reported of outcomes based on ISAAC questionnaire	6-9	Rhinitis OR: 1.81 (1.26-2.60)
3	Mutius et al, 1999	Germany	15,043	Parental reported	1-2, 3-5 and ≥courses antibiotic	First 2 years	Parental reported of physician diagnosis of outcomes based on ISAAC questionnaire- SPT	5-11	Eczema OR: 1.26 (1.05-1.52) Hay fever OR: 1.12 (0.84-1.50) Positive SPT OR: 0.96 (0.80-1.17)
4	Raciborski et al, 2012	Poland	1,461	Parental reported	Frequency of antibiotic: once, 1-2 times, 2-3 times, 3-4 times and >4 times	First year	Parental reported of outcomes based on ISAAC questionnaire	6-8	Eczema OR: 1.43 (1.08-1.91) Hay fever OR: 1.28 (0.88-1.85) Food allergy OR: 1.30 (0.89-1.89)
5	Sultész et al, 2010	Hungary	6,335	Parental reported	-	First year	Parental reported of physician diagnosis of outcomes	6-12	Allergic rhinitis OR: 1.60 (1.37-1.87)
6	Tamay et al, 2007	Turkey	2,500	Parental reported	-	First year	Parental reported of physician diagnosis of outcomes based on ISAAC questionnaire	6-12	Allergic rhinitis OR: 1.26 (1.01-1.57)
Multi-center studies									
7	Foliaki et al, 2009	71 centers in 29 countries	193,412	Parental reported	-	First year	Parental reported of outcomes	6-7	Eczema OR: 1.42 (1.33-1.51)
8	Floistrup et al, 2005	Netherlands, Austria, Germany, Sweden, Switzerland	6,630	Parental reported	-	First year	Parental reported of physician diagnosis of outcomes- Blood test	5-13	Eczema OR: 1.63 (1.22-2.17) Elevated IgE OR: 0.91 (0.60-1.37)

Abbreviation: SPT: skin prick test

Note: in cross-sectional studies both exposure and outcome were questioned at one point of time, however the period over which exposure and outcome were requested was different and therefore these studies have been accepted in our meta-analyses.

The studies assessing hay fever, eczema and positive SPT as outcomes, applied an exposure window of the first 2 years of life. The studies assessing food allergy and increased serum/plasma IgE levels, applied a shorter exposure window, namely the first year of life.

For the association between early life antibiotics use and eczema two studies were multi center studies consisting of 193,412[21] and 6,630[20] children. None of studies that reported a positive significant association between antibiotics and hay fever or eczema had a positive significant association with atopies including positive SPT or elevated allergen-specific serum/plasma IgE levels.

Antibiotics exposure and hay fever

Our primary meta-analysis (including all studies) showed a statistically significant higher risk of developing hay fever among children exposed to antibiotics during the first 2 years of life compared to never exposed children in the same period of time; OR: 1.23 (95% CI: 1.13-1.34; I^2:77.0%) (**Fig 2**).

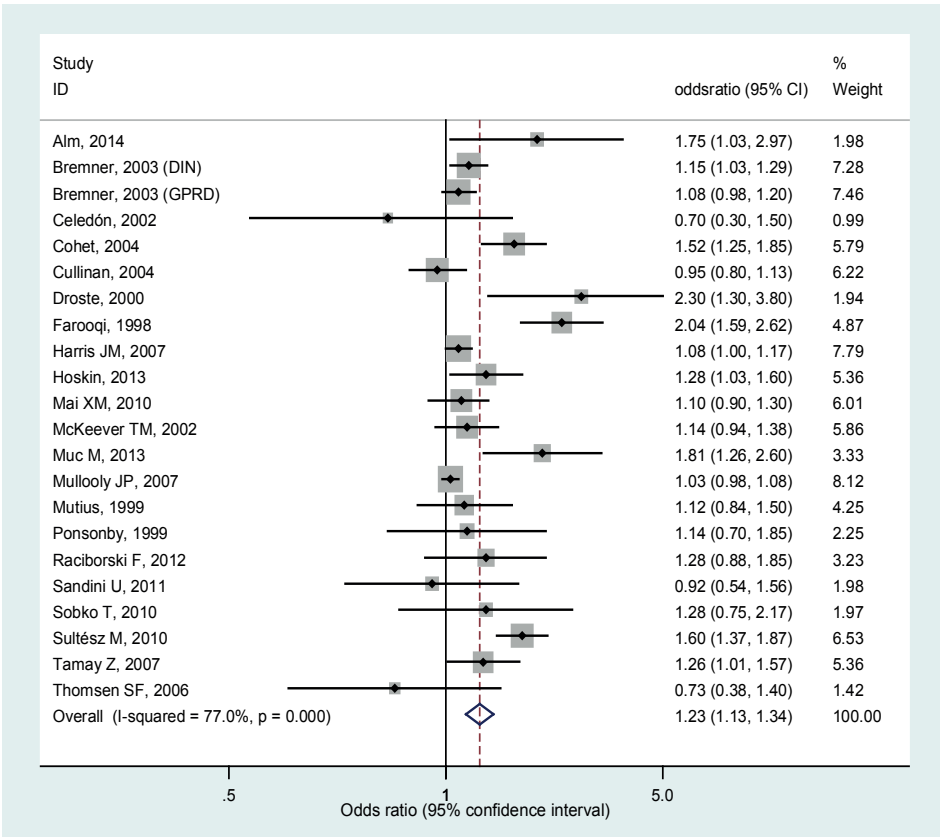


Fig 2. Association early life exposure to antibiotics and risk of hay fever later in life

A subgroup analysis stratified by study design showed the summary ORs for risk of hay fever as 1.19 (95% CI: 1.03-1.37; I^2: 72.3%) in the meta-analysis of the cohort studies (n=47,403), 1.14 (95% CI: 1.02-1.27; I^2: 72.3%) in the meta-analysis of case control studies (n=154,069) and 1.56 (95% CI: 1.29-1.90; I^2: 63.6%) in the meta-analysis of cross-sectional studies (n=27,608).

When meta-analysis stratified by timing of antibiotics exposure, the pooled ORs for risk of hay fever were 1.23 (95% CI: 1.12-1.35; I^2: 69.2%) in the 1st year (n=205,597) and 1.23 (95% CI: 1.13-1.34; I^2: 77.0%) in the first 2 years of life (n=229,080).

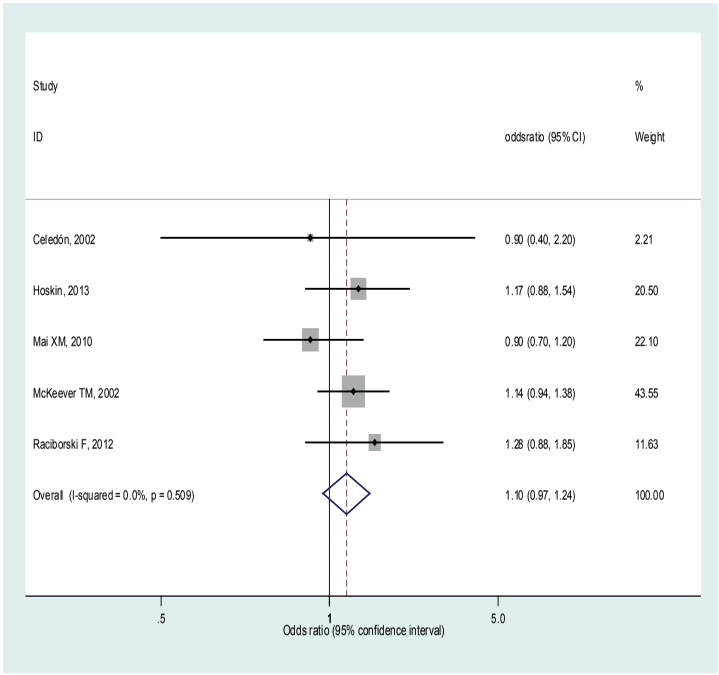
The results also revealed that there was a trend towards a stronger association if patients had been treated with ≥2 courses of antibiotics (n=54,448) compared with those that had been treated with one course (n=39,405), OR: 1.21 (95% CI: 0.98-1.50; I^2: 58.0%) versus 1.10 (95% CI: 0.97-1.24; I^2: 0.0%), respectively (**Fig 3**).

For the association between antibiotics and different age at the time of hay fever measurement the pooled ORs were 1.11 (95% CI: 1.03-1.19; I^2: 0.0%) in children age younger than 5 years (n=29,615) and 1.28 (95% CI: 1.14-1.43; I^2: 81.1%) in children ≥5 years (n=170,227). **Table 5** presents the pooled effect estimates of the association between antibiotics and hay fever for different characteristics of studies included. The results in all different strata were similar; all showed a positive association that was statistically significant except in a subset of studies with sample size less than 500 (n= 2 studies).

Table 5. Pooled effect estimates for the association between early life exposure to antibiotics and risk of hay fever

Early life antibiotics		No. of studies	Sample size	OR (95% CI)	I^2
Study design	Cohort	10	47,403	1.19 (1.03-1.37)	72.3
	Case control	6	154,069	1.14 (1.02-1.27)	72.3
	Cross-sectional	6	27,608	1.56 (1.29-1.90)	63.6
Sample size	<500	2	928	0.72 (0.43-1.19)	0.0
	≥500	20	228,152	1.25 (1.14-1.36)	78.5
Antibiotics measurement	Parental reported	13	46,191	1.14 (1.05-1.24)	73.7
	Medical or pharmacy data	9	182,889	1.13 (1.03-1.24)	75.3
Hay fever measurement	Parental reported	16	205,597	1.23 (1.12-1.35)	69.2
	Medical data	6	61,650	1.22 (1.03-1.45)	77.6
Child's age at antibiotics consumption	First year	16	205,597	1.23 (1.12-1.35)	69.2
	First 2 year	21	229,080	1.23 (1.13-1.34)	77.0
Courses of antibiotics	One course	5	39,405	1.10 (0.97-1.24)	0.0
	≥ 2 courses	6	54,448	1.21 (0.98-1.50)	58.0
Child's age at hay fever diagnosis	<5 years	3	29,615	1.11 (1.03-1.19)	0.0
	≥5 years	18	170,227	1.28 (1.14-1.43)	81.1
Study region	Europe	18	159,285	1.25 (1.13-1.38)	75.3
	Non-Europe	5	9,008	1.18 (0.95-1.47)	77.5

Abbreviations: OR: odds ratio; CI: confidence interval



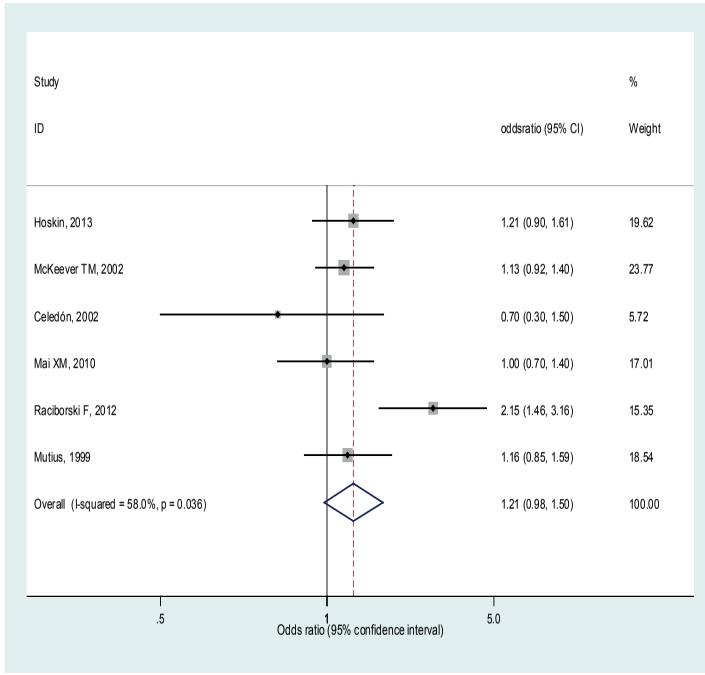


Fig 3. Association early life exposure to a) 1 course of antibiotics and b) ≥2 courses of antibiotics and risk of hay fever later in life

Antibiotics exposure and eczema

Children who were exposed to antibiotics in early life (first 2 years of life) had a statistically significantly increased risk of eczema later in life compared with those who were never exposed during the same time period, OR: 1.26 (95% CI: 1.15-1.37; I^2:74.2%) (**Fig 4**). In subgroup analyses, the effect estimates were consistent across the different study designs: in cohort studies (n=170,824) OR: 1.22 (95% CI: 1.09-1.36; I^2: 56.0%), in case control studies (n=5,941), 1.19 (95% CI: 0.90-1.56; I^2: 86.2%) and in cross-sectional studies (n=217,752) 1.41 (95% CI: 1.33-1.49; I^2: 0.0%).

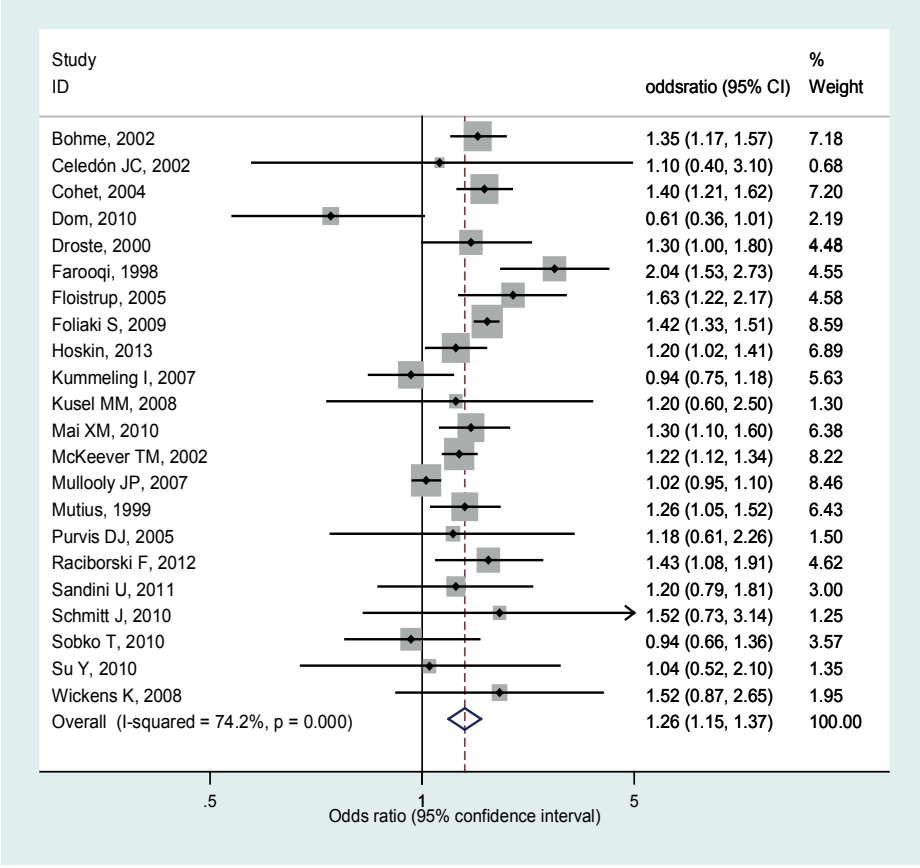


Fig 4. Association early life exposure to antibiotics and risk of eczema later in life

When stratified by timing of antibiotics exposure, the pooled ORs for risk of eczema were 1.26 (95% CI: 1.15-1.38; I^2: 51.6%) in the 1st year (n=367,425) and 1.26 (95% CI: 1.15-1.37; I^2: 74.2%) in the first 2 years of life (n=394,517). **Figure 5** shows the ORs for the association between number of antibiotics courses and risk of eczema. Again there was a trend towards a stronger association if patients had been treated with two or more courses of antibiotics (n=54,818) during their childhood compared with one course (n=39,775):

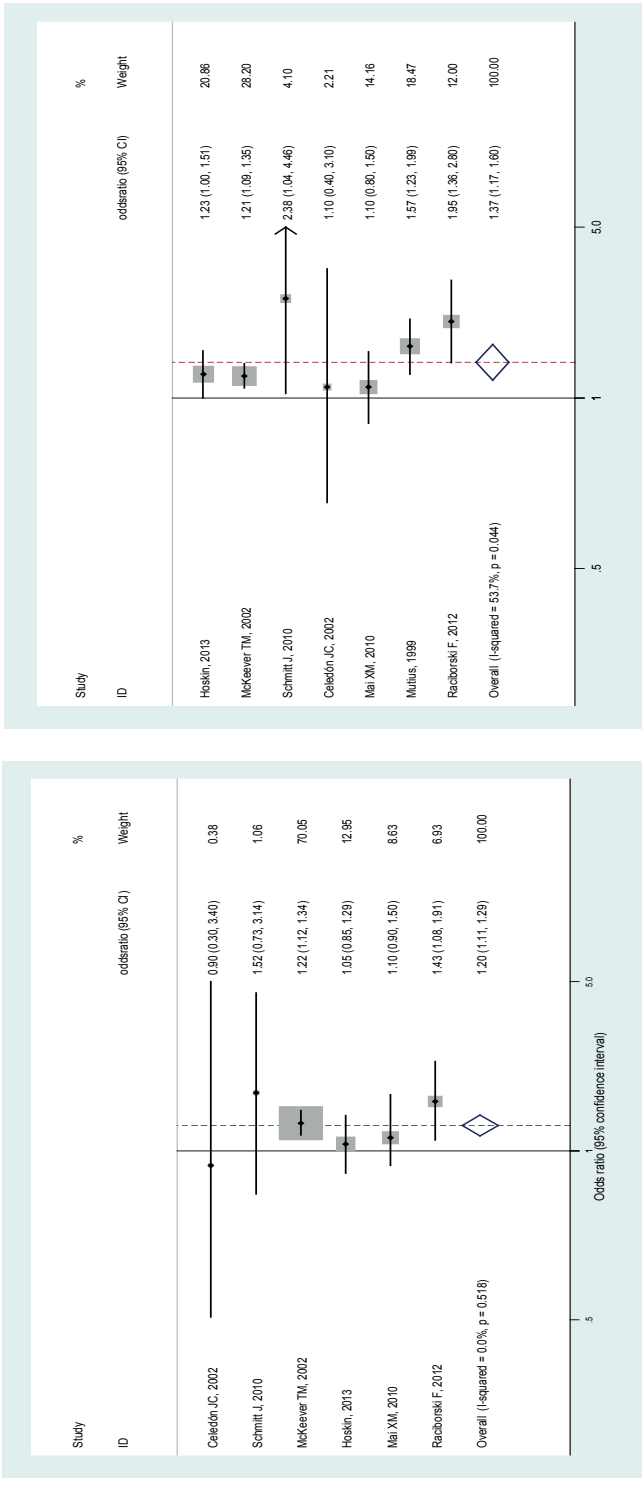


Fig 5. Association early life exposure to a) 1 course of antibiotics and b) ≥2 courses of antibiotics and risk of eczema later in life

OR: 1.37 (95% CI: 1.17-1.60; I^2: 53.7%) and OR: 1.20 (95% CI: 1.11-1.29; I^2: 0.0%), respectively.

When stratified by different age at the time of eczema measurement the pooled ORs were 1.12 (95% CI: 0.86-1.45; I^2: 65.8%) in children age younger than 5 years (n=9,845) and 1.31 (95% CI: 1.17-1.47; I^2: 80.0%) in children ≥5 years (n=355,434). No considerable differences were observed for other study characteristics in subgroup analyses (**Table 6**).

Table 6. Pooled effect estimates for the association between early life exposure to antibiotics and risk of eczema

Early life antibiotics		No. of studies	Sample size	OR (95% CI)	I^2 (%)
Study design	Cohort	14	170,824	1.22 (1.09-1.36)	56.0
	Case control	3	5,941	1.19 (0.90-1.56)	86.2
	Cross-sectional	5	217,752	1.41 (1.33-1.49)	0.0
Sample size	<500	4	1,440	1.44 (1.02-2.03)	0.0
	≥500	22	394,077	1.25 (1.14-1.37)	79.9
Antibiotics measurement	Parental reported	16	241,703	1.28 (1.17-1.39)	51.0
	Medical or pharmacy data	4	152,246	1.23 (1.00-1.51)	88.7
Eczema measurement	Parental reported	16	360,810	1.23 (1.10-1.36)	79.6
	Medical data	4	32,593	1.44 (1.06-1.95)	73.5
Child's age at antibiotics consumption	First year	17	367,425	1.26 (1.15-1.38)	51.6
	First 2 years	22	394,517	1.26 (1.15-1.37)	74.2
Courses of antibiotics	One course	6	39,775	1.20 (1.11-1.29)	0.0
	≥ 2 courses	7	54,818	1.37 (1.17-1.60)	53.7
Child's age at eczema diagnosis	< 5 years	6	9,845	1.12 (0.86-1.45)	65.8
	≥ 5 years	15	355,434	1.31 (1.17-1.47)	80.0
Study region	Europe	13	186,359	1.22 (1.10-1.36)	62.6
	Non-Europe	7	8,116	1.26 (1.03-1.54)	65.3

Abbreviations: OR: odds ratio; CI: confidence interval

Antibiotics exposure and food allergy

Our meta-analysis showed a statistically significant relationship between early life antibiotics consumption and the risk of food allergy later in life. The risk of food allergy was higher in children exposed to antibiotics during the 1st year of life compared to those in non-exposed group, OR: 1.42 (95% CI, 1.08-1.87; I^2:80.8%) (**Fig 6**). We were not able to perform subgroup analyses because the number of studies (n=3) included in meta-analysis for this outcome was insufficient.

Antibiotics exposure and positive SPT

Early life antibiotics consumption (first 2 years) was not statistically significantly related to the risk of positive SPT later in life; OR: 1.01 (95% CI: 0.92-1.11; I^2:54.8%) (**Fig 7**). The pooled ORs in different study design were 1.08 (95% CI: 0.91-1.29; I^2:68.3%) in the meta-analyses of the cohort studies (n=9,769); 0.95 (95% CI: 0.89-1.01; I^2: 0.0%) in the case

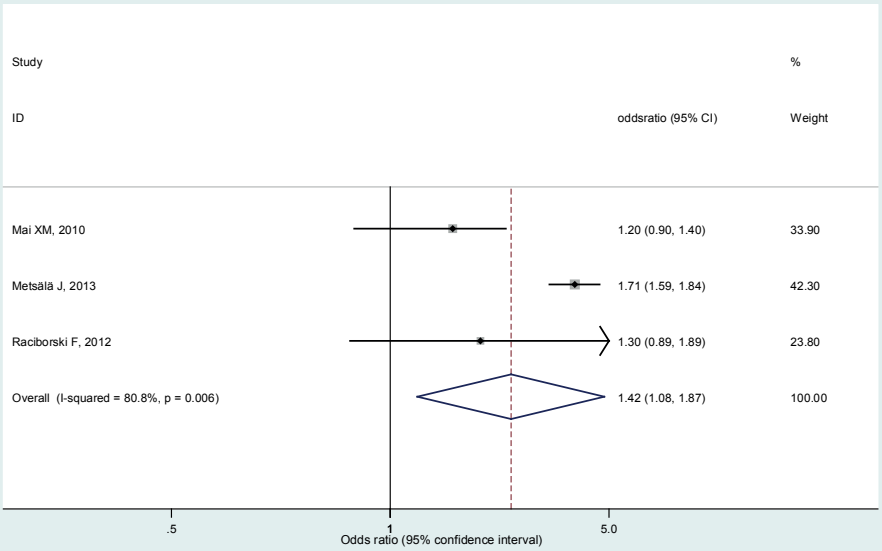


Fig 6. Association early life exposure to antibiotics and risk of food allergy later in life

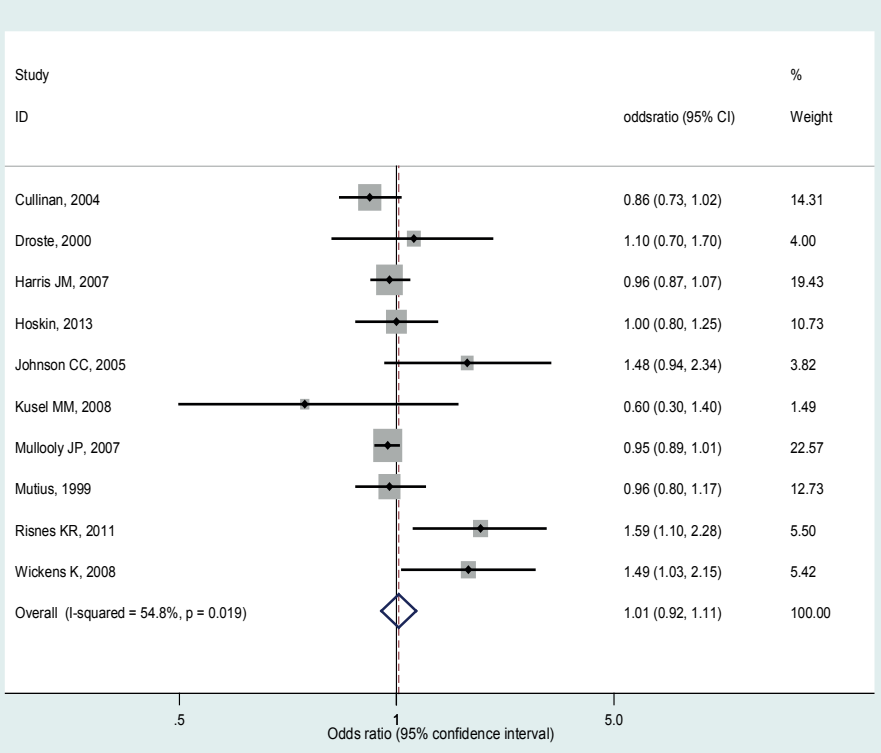


Fig 7. Association early life exposure to antibiotics and risk of positive skin prick test (SPT) later in life

control studies (n=1,074) and 0.98 (95% CI: 0.82-1.17; I^2: 0.0%) in the cross-sectional studies (n=16,249). The summary ORs for risk of positive SPT stratified by different time periods of exposure to antibiotics were 1.11 (95% CI: 0.91-1.34; I^2:68.6%) in the 1st year (n=6,023) and 1.01 (95% CI: 0.92-1.11; I^2: 54.8%) in the first 2 years of life (n=27,092).
In stratified meta-analysis for the association exposure to antibiotics and different age groups of positive SPT, the summary ORs were 1.49 (95% CI: 1.03-2.15; I^2: 0.0%) in children aged younger than 5 years (n=1,105) and 0.98 (95% CI: 0.90-1.07; I^2: 44.9%) in children ≥5 years (n=25,987). Subgroup analyses showed that there was no effect of other study characteristics on this association (**Table 7**).

Table 7. Pooled effect estimates for the association between early life exposure to antibiotics and risk of positive SPT

Early life antibiotics		No. of studies	Sample size	OR (95% CI)	I^2 (%)
Study design	Cohort	7	9,769	1.08 (0.91-1.29)	68.3
	Case control	1	1,074	0.95 (0.89-1.01)	-
	Cross-sectional	2	16,249	0.98 (0.82-1.17)	0
Sample size	<500	1	198	0.6 (0.3-1.4)	-
	≥500	9	26,894	1.02 (0.93-1.12)	56.6
Antibiotics measurement	Parental reported	5	23,707	1.16 (0.95-1.41)	5605
	Medical or pharmacy data	4	3,187	0.95 (0.87-1.03)	40.3
Child's age at antibiotics consumption	First year	7	6,023	1.11 (0.91-1.34)	68.6
	First 2 years	10	27,092	1.01 (0.92-1.11)	54.8
Child's age at positive SPT	<5 years	1	1,105	1.49 (1.03-2.15)	-
	≥5 years	9	25,987	0.98 (0.90-1.07)	44.9
Study region	Europe	5	22,589	0.95 (0.88-1.02)	0
	Non-Europe	5	4,503	1.20 (0.88-1.64)	77.0

Abbreviations: SPT: skin prick test; OR: odds ratio; CI: confidence interval

Antibiotics exposure and elevated serum/plasma IgE levels:

The risk of elevated allergen-specific serum/plasma IgE levels later in life was not significantly associated with antibiotics consumption during the 1st year of life, OR: 0.95 (95% CI: 0.77-1.16; I^2:44.9%) (**Fig 8**). The summary ORs for the association antibiotics and different age groups of elevated allergen-specific IgE were 0.72 (95% CI: 0.21-2.45; I^2: 90.7%) in children age younger than 5 years (n=3,537) and 0.99 (95% CI: 0.85-1.14; I^2: 0.0%) in children ≥5 years (n=12,506). The pooled ORs for risk of elevated IgE levels were 0.95 (95% CI: 0.74-1.21; I^2: 52.4%) in the cohort studies (n=9,413) and 0.91 (95% CI: 0.60-1.37; I^2: 0.0%) in cross-sectional studies (n=6,630). No statistically significant differences were observed for any of the subgroup analyses (**Table 8**).

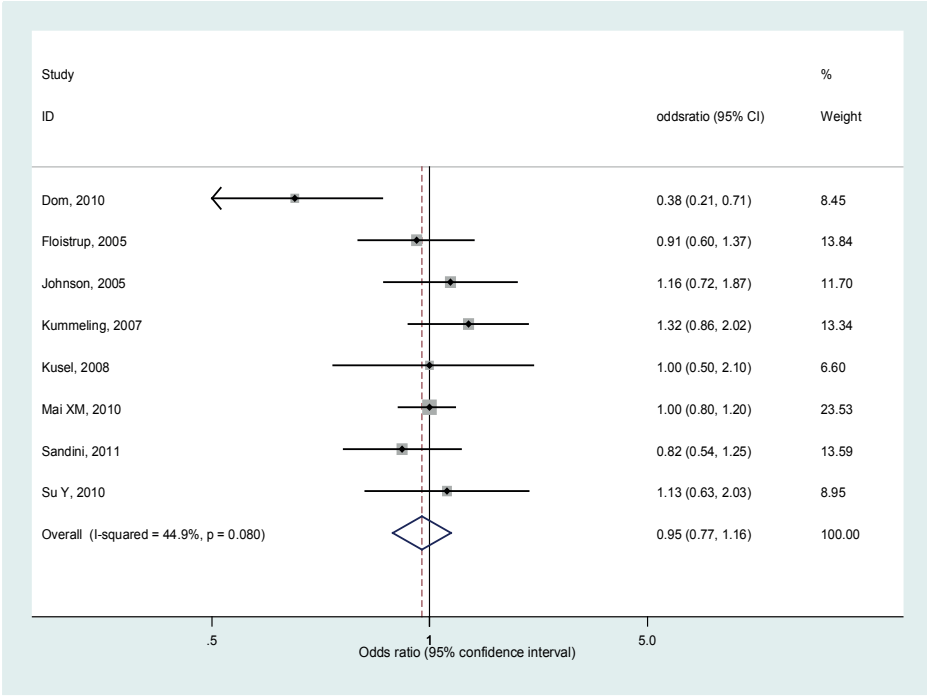


Fig 8. Association early life exposure to antibiotics and risk of elevated serum/plasma IgE level later in life

Table 8. Pooled effect estimates for the association between early life exposure to antibiotics and risk of elevated serum/plasma IgE level

Early life antibiotics		No. of studies	Sample size	OR (95% CI)	I^2 (%)
Study design	Cohort	7	9,413	0.95 (0.74-1.21)	52.4
	Cross-sectional	1	6,630	0.91 (0.60-1.37)	-
Sample size	<500	2	622	1.08 (0.68-1.69)	0
	≥500	6	15,421	0.92 (0.71-1.18)	59.7
Antibiotics measurement	Parental reported	6	15,120	0.91 (0.70-1.17)	58.6
	Medical or pharmacy data	1	725	1.16 (0.72-1.87)	-
Child's age at elevated IgE level	<5 years	2	3,537	0.72 (0.21-2.45)	90.7
	≥5 years	6	12,506	0.99 (0.85-1.14)	0
Study region	Europe	5	14,696	0.87 (0.65-1.17)	65.8
	Non-Europe	3	1,347	1.12 (0.80-1.55)	0

Abbreviations: OR: odds ratio; CI: confidence interval

Sensitivity analyses

The sensitivity analyses showed that the magnitude of associations were similar and remained statistically significant; a) when studies with low quality scores[12,25,33,40,44] were omitted: OR hay fever:1.20 (95% CI: 1.11-1.31; I^2: 72.7%) and OR eczema:1.28 (95% CI: 1.16-1.41; I^2: 75.7%) b) when studies conducted among high risk children[14,18,26,37] (paren-

tal history of allergies/atopies) were excluded: OR hay fever:1.25 (95% CI: 1.14-1.36; I^2: 78.8%) and OR eczema:1.26 (95% CI: 1.15-1.38; I^2: 77.9%) c) when multi center studies[20,21] were excluded: OR eczema:1.23 (95% CI: 1.12-1.34; I^2: 65.3%).

Causal inference

To evaluate the causality and avoid confounding by reverse causation, a second meta-analysis was run by including only those studies that explicitly reported that the exposure to antibiotics preceded the occurrence of hay fever (6 studies including 184,257 patients)[11,13,19,22,28,39] and eczema (8 studies including 156,924 patient)[17,19,26-28,38,39,43]. The results showed that early life antibiotics consumption (first 2 years) was statistically significantly related to the risk of hay fever (OR: 1.23 (95% CI: 1.08-1.41; I^2: 77.3%)) and risk of eczema (OR: 1.25 (95% CI: 1.03-1.52; I^2: 68.2%)) later in life.

Publication bias

As shown in **Figures 9**, there was no evidence of asymmetry to show potential publication bias in the primary meta-analyses for eczema (p=0.52), food allergy (p=0.43), positive SPT (p=0.14) and elevated allergen-specific serum/plasma IgE levels (p=0.38). Asymmetry appeared in the funnel plot for the association between antibiotics and hay fever (p=0.01). This means that small studies showing a strong association probably have not been published yet.

Meta-regression

We conducted a meta-regression analysis to identify the independent effect of variables (child's age at the time of antibiotics consumption and patient's age at the time of diagnosis of allergies/atopies) on the ORs. The results showed that none of the distinct times of exposure and the time of outcomes measurement statistically significantly influenced the risk estimated separately for most outcomes. However, the age at which elevated allergen-specific IgE levels were observed, were found to be a statistically significant related to the estimated OR (p-value: 0.05) (**Table 9**)**.**

Table 9. Meta-regression analyses

	Predictors p-value	
	Time of antibiotics consumption	Time of outcome diagnosed/reported
Hay fever	0.41	0.28
Eczema	0.36	0.57
Food allergy	**-**	-
Positive SPT	0.37	0.08
Elevated serum/plasma IgE level	0.06	**0.05**

Abbreviation: SPT: skin prick test

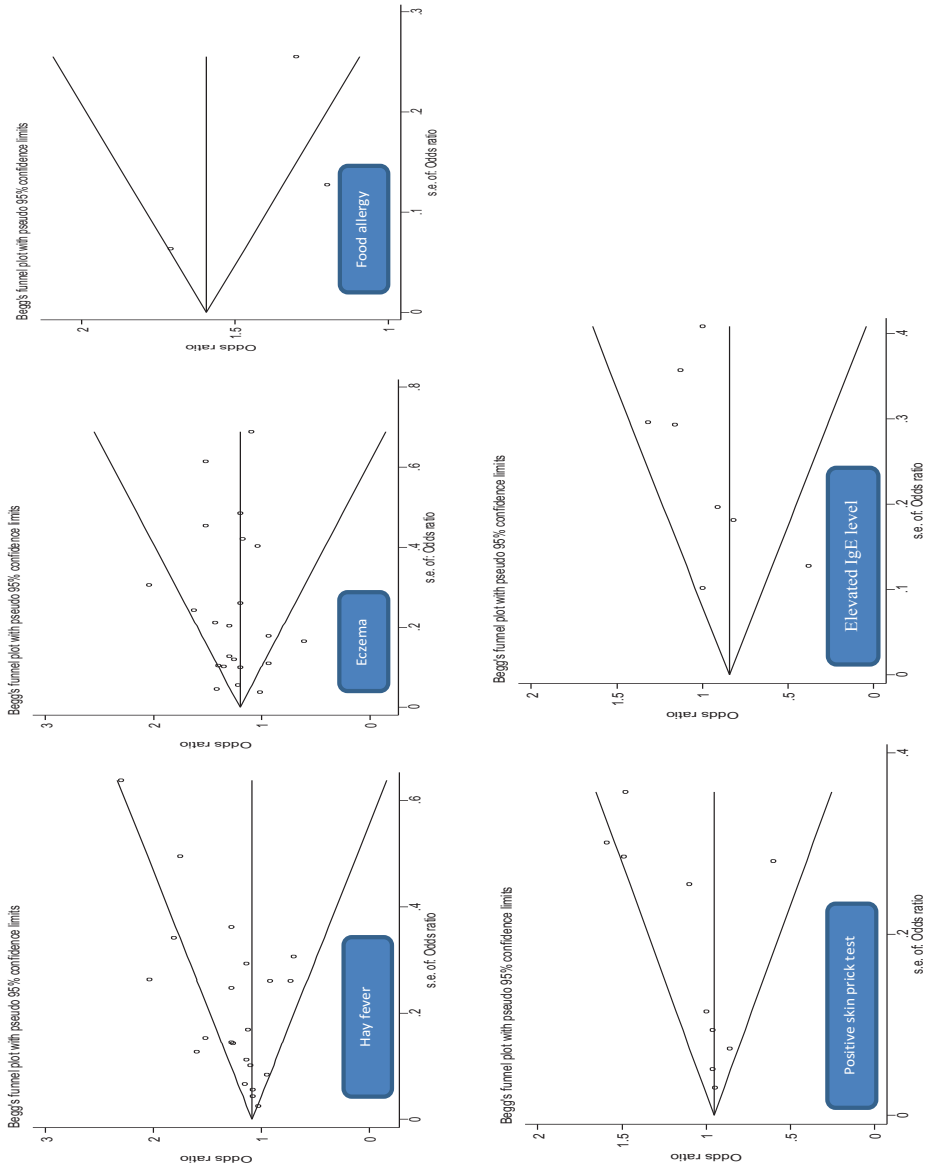


Fig 9. Publication bias in the association of early life exposure to antibiotics and risk of allergies/atopies

DISCUSSION

To the best of our knowledge this systematic review and meta-analysis provides the first large quantitative summary estimates of the association between early life exposure to antibiotics and the risk of developing allergy and atopy later in life. Our main meta-analysis (including 34 studies and 340,428 patients) showed that exposure to antibiotics during the first 2 years of life is associated with an increased risk of hay fever, eczema and food allergy later in life. However, remarkably, there was no association between exposure to antibiotics early in life and a positive SPT or elevated allergen-specific serum/plasma IgE levels later in life.

The increased risk of hay fever, eczema and food allergy among children exposed to antibiotics in early childhood might be explained by the immunomodulatory effect of these compounds. Gut microbiota are thought to play an important role in the development of the immune system early in life[50,51]. At the time of birth, the immunologic response to novel antigens is strongly Th2 skewed since during gestation the T helper type 1 (Th1) immune response of fetus has been suppressed in order to inhibit immune rejection between mother and child[52-54]. Exposure to infections in infancy helps the development of a Th1 immune response leading to a proper balance between Th1 and Th2[55,56]. The protective effect of exposure to infection in early life against allergic disorders has been confirmed by previous studies[32,57,58]. In contrast, reduced gut microbial diversity by exposure to antibiotics in early infancy leads to an imbalanced Th1/Th2 response[51,59-61], and have been related with increased risk of allergies and even other immune related disorders[62-66]. Since several factors can influence the gut microbiome[8,67,68] and as a consequence an imbalanced Th1/Th2 response[69], further research is needed to find the impact of antibiotics by itself on this association in early childhood. On the other hand, our positive results might also be explained by confounding by indication that is the association between antibiotics and allergies might be confounded by a third factor e.g. childhood otitis may lead to the use of antibiotics and also may increase the subsequent risk of eczema [70] later in life.

Our finding that there was no significant evidence for the association between antibiotics and objectively measured atopies although there is positive association with allergic symptoms is in line with previous studies[18,71-73]. Therefore, the clinical relevance of measuring allergen-specific serum/plasma IgE and positive SPT compared with symptoms histories should be reconsidered[71,74-76]. The risk association with clinical allergies is real and objective markers of sensitization do not closely correlate with allergies. There are many individuals without allergen-specific serum IgE that do develop allergic symptoms and vice versa. Previous studies have rigorously discussed how IgE sensitization translates into clinical allergy and that many different factors e.g. family history of atopy have been associated with the clinical presentation of allergic symptoms in children with a positive (specific) serum IgE test[74-76].

There are several potential limitations in the current study that should be addressed. Firstly, study design, the methods to analyze the data, and confounders involved in the adjusted models are not consistent across the studies included. Cross-sectional studies are limited by the fact that they are carried out at one point in time to obtain information on

all factors (exposure, outcome and confounders). Therefore no conclusions about causal relationships can be drawn from studies with this design. However, in the self-defined cross-sectional studies that we have included in our meta-analysis (Table 4) the exposure to antibiotics was requested from a period in the past.

There was a high degree of heterogeneity in patient's characteristics e.g. child's age at time of antibiotics consumption and patient's age at the time of outcome measurement. These heterogeneities might have contributed to the discrepant results reported by previous epidemiological studies. However, our results were robust and consistent across different subset of studies e.g. distinct study designs and different patient's characteristics. In pediatric allergic or atopic related disorders, the timing of antibiotics consumption might be of importance as well, in which the first 6 months of life has been suggested to be the critical period[77]. The association of exposure to antibiotics in 3-week-old mice and increased risk of atopies measured by IgE serum levels have been already shown[78]. In addition, Wickens et al. have shown the significant increased risk of positive SPT in children exposed to antibiotics during the first 3 months[43]. Although, in our meta-analysis we were limited to test this association in the first 3 months (low number of studies included), we found very similar results for this association in the first 6 months exposure to antibiotics (data not shown).

Dealing with heterogeneity was limited in the association between antibiotics and risk of food allergy. Studies selected for this association were extremely heterogeneous, most importantly, in the definition of the outcome. Food allergy is phenotypically a very wide-ranging group of diseases which is often confused with other disorders e.g. celiac disease[79]. To confirm the significant positive association reported by our study further research is needed.

Another important limitation consists of poor reporting of the types and the amount of antibiotics courses taken; this was often not mentioned in the studies. Previous studies reported that early use of broad-spectrum antibiotics was more strongly related to an altered gut microbiome, and therefore the increased risk of allergies, compared with narrow-spectrum antibiotics[13,16,19,24,28,29,38]. Unfortunately, we could not study the difference between broad and narrow-spectrum antibiotics because the number of studies that reported the association stratified by type of antibiotics was not sufficient. The present study was also limited by including studies that used the parental-reported exposure to antibiotics (n=23), which might be prone to recall bias. Parents may not always be able to remember and give accurate information on their child's medication use especially when the time of exposure is long ago. However, the results of our subgroup meta-analyses, separately for each outcome, showed no significant difference between the pooled effect estimates in studies with parental-reported measurement of antibiotics compared with medical/pharmacy-record based studies.

Moreover, hay fever, eczema and food allergy were not objectively measured in a number of studies (n=25), therefore, there is a risk of misclassification. Nevertheless, results of our study were similar for studies that used questionnaire-based outcomes compared with studies that used medical record-based outcomes.

The putative association between antibiotics and allergies could be caused by reverse causation in which children were prescribed antibiotics to treat symptoms related to allergies. Only 11 out of 34 studies included[11,13,17,19,22,26-28,38,39,43] explicitly reported that the use of antibiotics preceded the diagnosis of allergies. In our meta-analysis, we tested the association between antibiotics and risk of hay fever and eczema only in these 11 studies to infer causality and the results still showed significant association.

We tested the robustness of our findings using multiple subgroup and sensitivity analyses and consistently found an association between antibiotics exposure early in life and hay fever, eczema and food allergy later in life. However, it remains unclear what causes these associations. Further research into the impact of antibiotics on gut microbiome and related immune fitness is warranted. Since inappropriate use of antibiotics in children is a major public health issue[80], we strongly suggest more attention to improve health care quality by treating only those indications in children with antibiotics for which evidence of efficacy is available. For instance the use of antibiotics in children with influenza should be prevented [81]. Additionally, it is important to focus on educational programs in both individual patients and clinicians regarding judicious use of antibiotics[82]. For instance, intervention studies including both patient and clinician awareness have shown that inappropriate use of antibiotics was significantly reduced by displaying poster-sized commitment letters in examination rooms[80].

In summary, early life exposure to antibiotics appears to be related with an increased risk of allergic symptoms later in life, but not to objective atopy measurements including positive SPT or elevated allergen-specific serum/plasma IgE levels.

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Table S1. Search strategy for the systematic review

Domain		Determinant				Outcomes		
Children		Anti-Bacterial		Eczema		Hay fever		Food allergy
Childhood		Anti-Bacterial Agents		Eczemas		Seasonal Allergic Rhinitis		
Pediatrics		Antibiotics		Dermatitis, Eczematous		Allergic Rhinitides, Seasonal		
		Bacteriocidal Agents		Dermatitides, Eczematous		Allergic Rhinitis, Seasonal		
		Bacteriocides		Eczematous Dermatitides		Rhinitides, Seasonal Allergic		
	AND		AND		OR		OR	
		Anti-Mycobacterial Agents		Eczematous Dermatitis		Rhinitis, Seasonal Allergic		
		Anti-Mycobacterial Agents				Seasonal Allergic Rhinitides		
						Pollen Allergy		
						Allergies, Pollen		
						Pollen Allergies		
						Pollinosis		

Chapter 3.4

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Breast-feeding is associated with a decreased risk of asthma exacerbations later in life

Submitted for publication

SHORT REPORT

Asthma severity is a major health problem and accounts for significant morbidity in the asthmatic population[1]. Severe asthma consists of a rapid progression of asthma symptoms and airflow obstruction[2]. The biological processes underlying severe asthma phenotypes are not completely understood. In addition to genetic factors, various environmental factors have been thought to be associated with asthma outcome parameters[3].

Studies in breastfed children show that breast-feeding is associated with prevention of immune-mediated disorders such as asthma and allergies, implying a possible positive influence on maturation of the neonatal immune system[4]. The effect of breast-feeding on development of infants' immune system could be explained by two mechanisms. Breast milk contains high levels of factors that can help the development of a healthy immune system in the offspring such as immunoglobulines, cytokines, prebiotic fibers and even low levels of specific microbes. It is very well known that breastmilk can influence the microbiome in the infants thanks to the unique prebiotic structures and microbes present in human milk. For example the Bifidobacteria might passively affect the immune response via changing the gut microbiome at a young age[4]. The composition of healthy gut microbiome is highly diverse whereas reduced gut microbial diversity has been associated with a higher risk of asthma and allergies[5].

However, epidemiological studies that assessed the relation between breast-feeding and risk of asthma and allergies show conflicting results[3]. Moreover, the association between breast-feeding and asthma severity has not been studied. In this study, using a large cohort of pediatric asthma medication users, we report that breast-feeding was statistically significantly associated with a decreased risk of asthma exacerbations later in childhood.

We used data from the Pharmacogenetics of Asthma medication in Children: Medication with Anti-inflammatory effects (PACMAN)[6] study. In this observational cohort, paediatric users of asthma medications were included through Dutch community pharmacies. Children aged 4-12 years with at least two years of medication history available and at least three prescriptions for any asthma drug within the last two years and at least one prescription in the last 6 months were selected from pharmacies in different regions in the Netherlands. Data on general health, allergic symptoms, asthma and respiratory symptoms, healthcare utilization for respiratory symptoms, medication uses and compliance, environmental and sociodemographic factors were collected with questionnaires.

Exposure to breast-feeding was defined as ever versus never breastfed. Furthermore, we subdivided breastfed children by duration of breast feeding (≥6 versus <6 months), based on a previous meta-analysis[7].

Outcome included in this study was asthma severity with two definitions 1) poor asthma control defined based on the Asthma Control Questionnaire (ACQ-6≥0.75) in the past 1 week, 2) asthma exacerbations defined as asthma-related visits to an emergency department (ED) and/or prescribed courses of oral corticosteroids (OCS) in the past 12 months. Univariate and multivariable logistic regression analyses were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Additionally, logistic analyses were stratified by family history of asthma.

Data were available for 960 children (mean age was 8.4 years). The majority of children in this study were boys (62%). Almost three-quarter of the population was breastfed (74%). Data on duration of breast-feeding was available for 684 children. In 366 out of 684 (53.5%) children, the duration of breast-feeding was 6 months or longer. Less than half of the population reported poor asthma control (42%), and 11% reported asthma exacerbations in the past 12 months.

Breastfed children in our population had a statistically significantly lower risk of asthma exacerbations; adjusted OR (adj. OR): 0.59, 95% CI: 0.38-0.93. After stratification for duration of breast-feeding the adj. ORs were 0.53, 95% CI: 0.31-0.91 and 0.68, 95% CI: 0.41-1.12 for shorter and longer than 6 months feeding, respectively (**Table 1**).

To evaluate the effect of family history of asthma on this association, we further stratified our analyses. Our findings showed that the protective effect of breast-feeding on asthma exacerbations was even stronger (adj. OR: 0.34, 95% CI: 0.18-0.67) in children with a positive family history of asthma, while the association was no longer statistically significant in children with no family history of asthma (adj. OR: 0.86, 95% CI: 0.44-1.68). Furthermore, stratified analyses showed a statistically significant protective effect of breast-feeding on asthma exacerbations in children with both less than 6 months (adj. OR: 0.30, 95% CI: 0.13-0.68) and more than 6 months of breastfeeding (adj. OR: 0.38, 95% CI: 0.18-0.81) in children with a family history of asthma. No statistically significant association with poor asthma control was observed between ever breastfed compared with never breastfed children (adj. OR: 0.99, 95% CI: 0.72-1.36) with no effect of different durations of breast-feeding (**Table 1**).

This is the first study evaluating the effect of breast-feeding on asthma severity later in life. Breast-feeding was associated with a lower risk of asthma exacerbations. Remarkably, stratified analyses showed that the beneficial effect of breast-feeding on asthma exacerbations was no longer significant in a subset of children with no family history of asthma. The protective effect of breast-feeding on asthma exacerbations in our study might be explained by the influence of breast feeding on the immune system[4,8]. It has been hypothesized that factors influencing the immune system via changing the composition of the gut microbiome might contribute to improve asthma and allergies susceptibility in later childhood[5].

Table 1. Associations between breast-feeding and asthma outcomes

		Asthma exacerbations		**Poor asthma control (ACQ≥0.75)**	
		Crude OR (95% CIs)	Adj. OR* (95% CIs)	Crude OR (95% CIs)	Adj. OR* (95% CIs)
Breast-feeding (ever vs. never)		**0.56 (0.36-0.86)**	**0.59 (0.38-0.93)**	1.06 (0.79-1.43)	0.99 (0.72-1.36)
Breast-feeding (duration)	*< 6 months*	**0.50 (0.30-0.86)**	**0.53 (0.31-0.91)**	1.14 (0.81-1.60)	1.03 (0.72-1.48)
	≥6months	0.64 (0.39-1.03)	0.68 (0.41-1.12)	1.02 (0.73-1.41)	0.97 (0.68-1.38)

Abbreviations: OR: odds ratio; CI: confidence interval; Adj: adjusted
Reference is never exposed group in all analyses.
Exacerbations: either oral corticosteroids use or emergency department visits due to asthma
*Adjusted for age, gender, ethnicity, eczema, hay fever, food allergy and family history of asthma/allergy

The role of duration of breast-feeding on the functioning of the immune system is not clear. Although, in our study the association between breast-feeding and asthma exacerbations was no longer significant in children with more than 6 months of breast feeding this is probably a power problem. A recent meta-analysis of 117 studies showed that children aged 3-6 years who had been breastfed for more than 6 months had a lower risk of developing asthma compared to those with less than 6 months[7].

Our finding showed no statistically significant association of breast-feeding with asthma exacerbations in children without a positive family history of asthma that might be explained by different asthma phenotype in this population. We think that children without a positive family history of asthma might have non-allergic asthma with non-immunologic etiology independent of breast-feeding. Further studies into the role of genetic factors might elucidate a potential mechanism.

No significant association of breast-feeding and poor asthma control in this study might be due to the way poor asthma control was defined. Our assessment of asthma control in this study has been limited to 1-week using the ACQ questionnaire which does not necessarily translate to long-term asthma control; agreement between current and long-term asthma control has been reported to be limited[9].

Some potential limitations of the present study should be acknowledged. Importantly, our study was limited by the use of questionnaire-based data that might be prone to recall bias and therefore lead to misclassification bias in both exposure and outcomes. However, studies have shown that mothers can remember breast-feeding very well after many years[10]. Also, several potential confounders were not available such as genetic susceptibility, dietary habits during pregnancy and infancy, socioeconomic status and exposure to infection/siblings. It is thought that early and regular introduction of solid foods prevents the development of allergy by promoting tolerance[11]. Another important limitation could be a possibility of misclassified asthmatic children especially in young children. Asthma-like symptoms are common in children younger than 5 years due to the smaller airways.

Apart from all established beneficial effects of breast-feeding on health status in both mother and her child, our findings suggest a long-term protective effect of breast-feeding on asthma exacerbations.

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Chapter 3.5

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Childhood obesity in relation to poor asthma control and exacerbations- A meta-analysis

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ABSTRACT

Objectives: To estimate the association between obesity and poor asthma control or risk of exacerbations in asthmatic children and adolescents, and to assess whether these associations are different by gender.

Methods: A meta-analysis was performed on unpublished data from three North-European pediatric asthma cohorts (BREATHE, PACMAN and PAGES) and 11 previously published studies (cross-sectional and longitudinal studies). Outcomes were poor asthma control (based on asthma symptoms) and exacerbations rates (asthma-related visits to the emergency department, asthma- related hospitalizations or use of oral corticosteroids). Overall pooled estimates of the odds ratios (ORs) were obtained using fixed or random-effects models.

Results: In a meta-analysis of 46,070 asthmatic children and adolescents, obese children (BMI≥95th percentile) compared with non-obese peers had a small but significant increased risk of asthma exacerbations (OR: 1.17, 95% CI: 1.03-1.34; I^2:54.7%). However, there was no statistically significant association between obesity and poor asthma control (n=4,973, OR: 1.23, 95% CI: 0.99-1.53; I^2: 0.0%). After stratification for gender, the differences in ORs for girls and boys were similar, yet no longer statistically significant.

Conclusions: In asthmatic children, obesity is associated with a minor increased risk of asthma exacerbations but not with poor asthma control. Gender does not appear to modify this risk.

INTRODUCTION

Studies have shown that overweight and obesity are associated with an increased risk of asthma in children[1-3]. Mechanisms which might explain how obesity could lead to asthma include increased weight on the chest wall leading to breathing at lower lung volumes[4] and/or pro-inflammatory mediators released by adipocytes[5]. These mechanisms might also lead to children with asthma and who are obese having either more symptoms or worse disease compared with children who are not obese[6]. It has been reported that obese boys have a significantly higher risk of asthma than obese girls[7], although some other studies have found the opposite[8-10].

In addition to the risk of developing asthma, there has been an inconclusive debate about whether obesity is associated with an increased risk of poor asthma control[11-18] and exacerbations[19-24]. Studies reporting on gender differences for the association of obesity and poor asthma control also show conflicting results[14,17]. Luisa et al. reported that obese boys are more at risk of poor asthma control compared to obese girls[14]. In contrast, Kattan and colleagues showed that obese girls had a higher risk of poor asthma control compared to obese boys[17].

Therefore, the purpose of this study was to perform a meta-analysis including unpublished results (from three Northern European asthma cohorts) and all previously published studies on overweight/obesity and the risk of poor asthma control or exacerbations in asthmatic children and adolescents. Additionally, we intended to assess whether this association is different for boys and girls.

METHODS

In this study, we followed the guideline reported by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) statement[25] for presenting systematic reviews.

Data source

Studies were identified by conducting a literature search in PubMed and Web of Science with the keywords strategy shown in **Table S1**. Additional articles were retrieved through a manual search of references from articles identified in the initial search. We also included unpublished results of the analysis of three North-European asthma cohorts (BREATHE, PACMAN and PAGES) (all information regarding methods and the results of these studies are presented as supplementary information).

Inclusion and exclusion criteria

Targeted studies were those in which the association of overweight and obesity (body mass index (BMI)≥85th percentile) or obesity (BMI≥95th percentile) with poor asthma control and/or exacerbations rate in children and adolescents was evaluated as, or could be calculated as odds ratios (ORs). Studies on this association were included in this meta-analysis if they met following criteria:

1 Data on overweight and/or obesity was available (based on BMI percentile).
2 Data on asthma control was available as ACQ[26], ACT[27], NHLBI[28] or GINA guidelines[29] OR
3 Data on severe asthma exacerbations was available either as a) asthma emergency department (ED) visits/ unscheduled health care visits or b) asthma- related hospitalization or c) prescribed courses of oral corticosteroids (OCS).
4 Only publications in English language available in PubMed and Web of Science before17th of Feb 2015 were considered.

Low-quality studies (criteria for this exclusion are explained later in the quality assessment section) were excluded from this meta-analysis. Studies that evaluated adolescents and adults without showing separate results[30,31] or studies that used other measurements of outcomes (e.g. missing schools due to wheezing and wheezing with exercise)[13,18,32-37] were also excluded. We also excluded studies in which the association of overweight/obesity and uncontrolled asthma was evaluated only in children with an asthma-related ED visit[38-40].

Data extraction

The following data were extracted: first author, year of publication, study design, patient characteristics (gender, age and number of patients). When available, the crude or adjusted ORs for the association of overweight/obesity and outcomes were extracted from the articles. For the remaining studies, the numbers of exposed/non-exposed subjects were selected to calculate the unadjusted ORs and 95% confidence intervals (CIs). In case the reported association was not obtained from a regression analysis or ORs were not reported, we contacted the authors to provide additional information in order to be included in the meta-analysis.

Quality assessment and publication bias

Quality assessment of included published studies was assessed independently by three authors (FA, AHM, SJHV) using the checklist of Newcastle-Ottawa Scale (NOS) for cohort-studies or adapted for cross-sectional studies. Using this tool, each study was evaluated on eight items categorized into three groups including the selection of the study group, the comparability of the groups and the assessment of either the exposure or outcome of interest for cross-sectional and cohort studies. When a study met ≥5 NOS criteria, the study was considered to be of high quality. Studies with a NOS score <5 were excluded from the meta-analysis[41]. Publication bias was evaluated by using funnel plots and the Egger test was applied to measure any asymmetry.

Meta-analysis

Overall pooled ORs, together with 95% CIs of the association between obesity and outcomes were obtained using either a fixed-effects model or a random-effects model. In association BMI and risk of asthma exacerbations we performed separate meta-analyses in those studies that reported ED visits, hospitalizations due to asthma or OCS

use. Heterogeneity of the studies was tested by the I^2 measure of inconsistency with 25% corresponding to low heterogeneity, 50% to moderate and 75% to high. If significant moderate or high heterogeneities existed, we used a random-effects model instead of a fixed-effects model for the meta-analysis.
In this meta-analysis, for reasons of symmetry, the reported/calculated ORs and lower and upper bounds of the 95% CI were initially log-transformed; the log ORs together with 95% CIs of the log ORs were meta-analyzed using either fixed or random-effects models, then the results were transformed back to the original ORs for reporting.

Sensitivity analyses
A series of sensitivity analyses was applied to find:

a The impact of unpublished results on these associations; separate meta-analyses were performed for unpublished and published studies.
b The effect of different asthma control measurements on the association between obesity and poor asthma control; a separate meta-analysis was performed in those studies that used the ACQ or ACT for asthma control measurement.
c The effect of different asthma definition on this association; separate meta-analysis for studies with physician-diagnosed asthma and those with self/parental-reported asthma.
d The effect of severity of asthma on this association; the meta-analysis was stratified based on the source of recruitment, primary versus secondary health care system.
e The effect of study design on the association between obesity and poor asthma control/exacerbations; separate meta-analyses were performed in cross sectional and longitudinal studies.

P-values of 0.05 were used to assess the statistical significance of main effect associations. We used STATA 12/SE (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

RESULTS

Search results
As shown in **Figure S1**, our literature search yielded 1,060 published articles on overweight/obesity and childhood poor asthma control/exacerbations. After applying the inclusion and exclusion criteria 11 studies remained eligible, and were included in the meta-analysis together with the analyses of the BREATHE, PACMAN and PAGES studies.

Study characteristics
Features of the included studies are presented in **Table 1**. A total of 52,147 patients from 14 studies were included in this meta-analysis. Sample sizes ranged from 56[12] to 32,321[20] patients. The design of the studies was cross-sectional (8 studies), retrospective or prospective cohort (3 studies) or a randomized clinical trial (3 studies). The studies were

Table 1. Baseline characteristics of studies included in the meta-analysis

Source	Study design	Region of study	Study size	Age at follow-up time	BMI percentile	Asthma control in 12 months/6 months	Asthma exacerbations in 12 months/6 months	Overweight/ Obesity, %	Well controlled asthma, %	Hospitalization, %	ED visits, %	OCS use, %
BREATHE	Cross-sectional	UK, Scotland	1,318	4-18	Obesity		OCS use Hospitalization due to asthma	13.4		15.2		25.9
PACMAN	Cross-sectional	Netherlands	648	4-12	Obesity	ACQ	OCS use ED visits	10.3	59.0		6.2	5.1
PAGES	Cross-sectional	UK, Scotland	422	4-17	Obesity	ACT	OCS use Hospitalization due to asthma	15.4	36.2	14.3		39.4
Sasaki M, et al. 2015	Cross-sectional	Japan	3,066	6-11	Obesity	ACT		11.8	85.4			
Lang J, et al. 2015	Cross-sectional	US	56	10-17	Overweight Obesity	ACQ		62.5 41.1	37.5			
Lang J, et al. 2013	Multicenter clinical trial*	US	306		Obesity	ACQ		31	33.7			
Lang J, et al. 2011	Multicenter clinical trial*	US	107	6-17	Obesity	ACQ		23.4	71			
Kattan M, et al. 2010	Randomized clinical trial*	US	368	12-20	Obesity	ACT		35.1	89.1			
Borrell LN, et al. 2013	Cross-sectional	US	2,174	8-19	Overweight Obesity	Asthma control [a]		35.6	17.6			
Sah PK, et al. 2013	Cross-sectional	US	269		Obesity		Hospitalization due to asthma ED visits [b]	24.9		32.7	15.6	
Schatz M, et al. 2013	Cohort	US	10,700	5-17	Obesity		OCS use [c]	28.1				11.7

Source	Study design	Region of study	Study size	Age at follow-up time	BMI percentile	Asthma control in 12 months/6 months	Asthma exacerbations in 12 months/6 months	Overweight/ Obesity, %	Well controlled asthma, %	Hospitalization, %	ED visits, %	OCS use, %
Quinto KB, et al. 2011	Cohort	US					Hospitalization & ED visits due to asthma	19.3		NA	NA	NA
					Overweight		OCS use					
			32,321	5-17			Hospitalization & ED visits due to asthma	30.0		NA	NA	NA
					Obesity		OCS use					
Luder E, et al. 1998	Cross-sectional	US					Hospitalization due to asthma [d]	39.7		23.9	72.2	
			209	2-18	Overweight		ED visits [e]					
Hom j, et al. 2009	Cohort	US					Hospitalization due to asthma	59.0		36.1	30.1	
			183	6-18	Overweight		ED visits [f]					

Abbreviations: ED: emergency department; OCS: oral corticosteroid

[a] Asthma control based on NHLBI guidelines (meeting at least three criteria has been defined as poor asthma control); [b] One time emergency department (ED) visit over the preceding 12 months; [C] Within last 7 days of asthma exacerbations diagnosis; [d] ≥3 times hospitalization per year, [e] ≥10 ED visits during the past year; [f] ≥1 ED visits during the past 30 days

* Findings in these 3 studies are the results of post hoc analyses of data from randomized clinical trials not specifically designed to assess the effect of obesity on asthma outcomes.

performed in the United State (US) (10 studies), the United Kingdom (UK) (2 studies), Japan (1 study) and the Netherlands (1 study). 12 studies evaluated the association of obesity (BMI ≥95th percentile) with the outcomes while in 2 other studies overweight and obesity were combined (BMI ≥85th percentile). Overall, the highest proportion of obese children was observed in the studies conducted in the US (ranging between 23-41%) and the lowest proportion in the study conducted in the Netherlands (10%).

Meta-analysis of combined unpublished and published studies

Poor asthma control was studied in 8 studies; three studies used the ACT questionnaire, 4 studies the ACQ questionnaire and one study NHLBI guidelines. The association of obesity with asthma exacerbations was studied in 8 studies by ED visits (4 studies), hospitalization due to asthma (5 studies), OCS use (6 studies), both ED visits and hospitalization (5 studies) and both ED visits/hospitalization and OCS use (2 studies).

All studies recruited both girls and boys in their studies however the ORs for the association of exposure and poor asthma control were stratified by gender in 7 studies and only in 3 studies for asthma exacerbations.

The quality of the studies was scored according to the three sections of the NOS checklist (selection, comparability and assessment of outcome). The results showed a high quality for all studies included but one scored below the threshold of 5 [22] and was excluded from the meta-analysis (**Table 2**).

The funnel plot and Egger's test showed no evidence of any asymmetry for the association of overweight/obesity with poor asthma control (p=0.81) and exacerbations (p=0.80), suggesting no publication bias in our meta-analysis (**Fig S2**).

Table 2. Quality assessment of included studies based on NOS checklist

Studies	Selection (Maximum of 4 stars)	Comparability (Maximum of 2 stars)	Outcome assessment (Maximum of 3 stars)
Lang J, et al. 2015	***	**	**
Sasaki M, et al. 2015	***	-	**
Borrell LN, et al. 2013	***	**	**
Lang J, et al. 2013	***	**	**
Schatz M, et al. 2013	***	-	***
Sah PK, et al. 2012	***	-	***
Quinto KB, et al. 2011	****	**	***
Lang J, et al. 2011	****	**	**
Kattan M, et al. 2010	****	**	*
Hom j, et al. 2009	****	-	*
Vargas PA, et al. 2007	***	-	*
Luder E, et al. 1998	***	-	***

In this checklist, the highest quality studies are awarded up to 9 stars.

Association BMI and poor asthma control

The association of obesity and poor asthma control in the total population has been reported by 7 studies [PACMAN, PAGES, 11,12,15-17]. Estimated heterogeneity in these studies was low (p= 0.71). The pooled OR for this association of obesity and poor asthma control in the total population was 1.23, 95 % CI: 0.99-1.53; I^2: 0.0%, p-value: 0.06 (**Fig 1**). Gender effect on this association is shown in **Figure 2**; in girls the OR was 0.96 (95% CI: 0.72-1.29; I^2:7.8%, p-value: 0.79) and in boys the OR was: 1.30 (95% CI: 0.92-1.83; I^2:22.9%, p-value: 0.15).

Association BMI and asthma exacerbations

An estimation of the association between obesity (BMI≥95th percentile) and overweight (BMI> 85th percentile) in asthmatic children and the risk of exacerbations was reported in 8 studies [BREATHE, PACMAN, PAGES, 19-21,23,24]. We performed meta-analysis in those studies that reported ED visits, hospitalizations due to asthma or OCS use, separately. The results showed that heterogeneity was moderate in the three associations and by applying a random effects model the overall pooled estimate in the association overweight/obesity and OCS use was shown to be statistically significant; OR: 1.17, 95% CI: 1.03-1.34; I^2:54.7%, p-value: 0.02 when boys and girls combined (**Fig 3**). For the association between overweight/obesity and ED visits (1.04, 95% CI: 0.98-1.11; I^2:0.0%, p-value: 0.21) and between overweight/obesity and asthma-related hospitalizations (1.18, 95% CI: 0.91-1.53; I^2:0.0%, p-value: 0.22), there were no statistically significant associations, however it seemed that there was a trend towards a higher risk of asthma exacerbations in obese compared with non-obese children (**Fig 4 and 5**).

The summarized ORs for the association of obesity (BMI≥95th percentile) with asthma exacerbations showed that obese children were statistically significantly at higher risk of asthma exacerbations measured by OCS use (1.17, 95% CI: 1.03-1.34; I^2:54.7%, p-value: 0.02). Obese children were also more likely to have ED visits (1.03, 95% CI: 0.65-1.62; I^2:44.3%, p-value: 0.90) and hospitalizations due to asthma (1.23, 95% CI: 0.89-1.69; I^2:0.0%, p-value: 0.21). After stratification by gender, the effect size of the ORs in the association obesity and OCS use appeared to be similar to the non-stratified ORs although the differences were not statistically significant anymore; OR, 1.30, 95% CI: 0.42-4.07; I^2:76.0%, p-value: 0.65 in girls and OR, 1.19, 95% CI: 0.81-1.74; I^2: 0.0%, p-value: 0.37 in boys. For the association between obesity and ED visits, there were no statistically significant associations for boys (1.27, 95% CI: 0.78-2.09; I^2:0.0%; p-value: 0.34) or girls (0.91, 95% CI: 0.48-1.72; I^2:0.0%, p-value: 0.77).

Sensitivity analysis

We evaluated the impact of unpublished studies on the association between obesity and poor asthma control and showed that the 95% CIs of the pooled results in this association in published studies **(**OR: 1.26, 95 % CI: 0.99-1.61; I^2: 0.0%) and in unpublished studies (OR: 1.14, 95 % CI: 0.68-1.89; I^2: 11.0%) were the same and overlapping. The associations between obesity and OCS use in published (OR: 1.20, 95% CI: 1.05-1.38; I^2: 79.4%) and unpublished (OR: 1.03, 95% CI: 0.73-1.46; I^2: 14.1%) studies were similar.

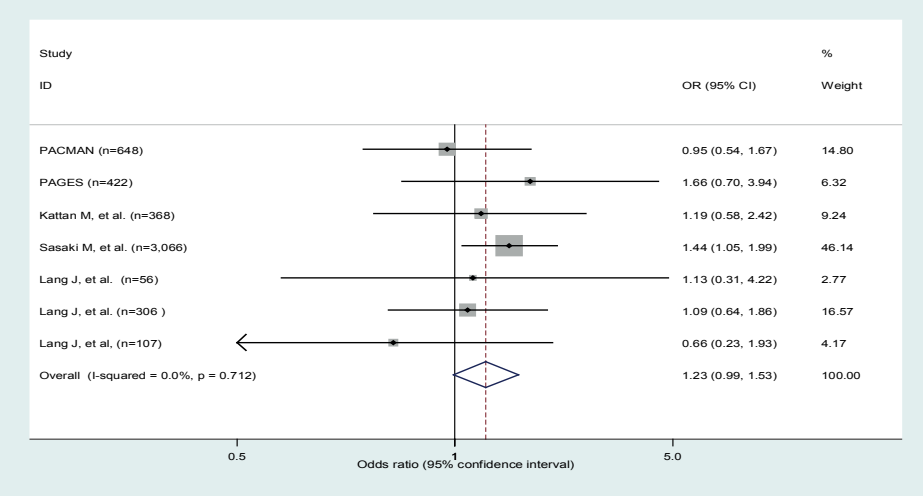
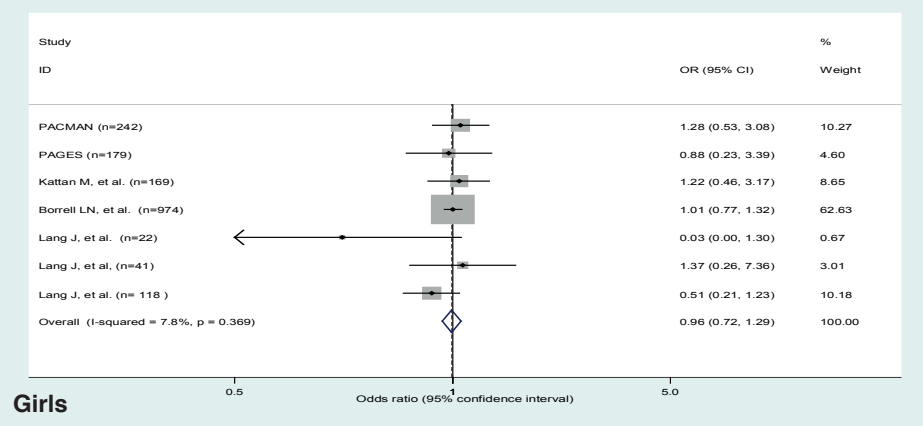


Fig 1. Pooled odds ratio of the association obesity and poor asthma control in obese compared with non-obese children



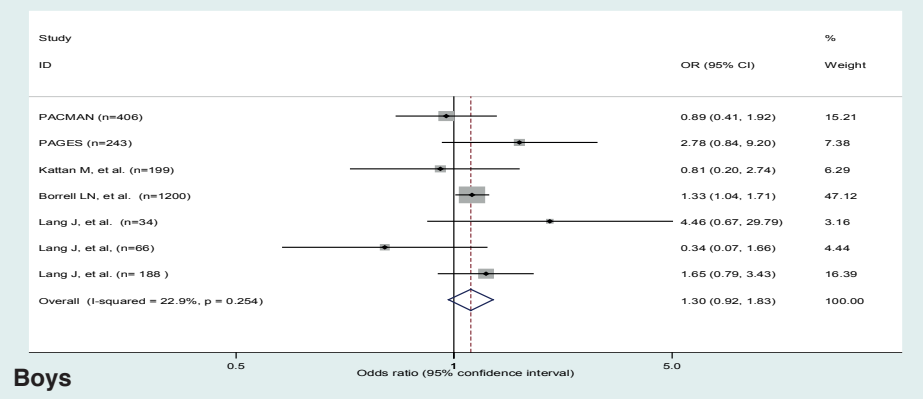


Fig 2. Pooled odds ratio of the association obesity and poor asthma control in obese compared with non-obese children, stratified by gender

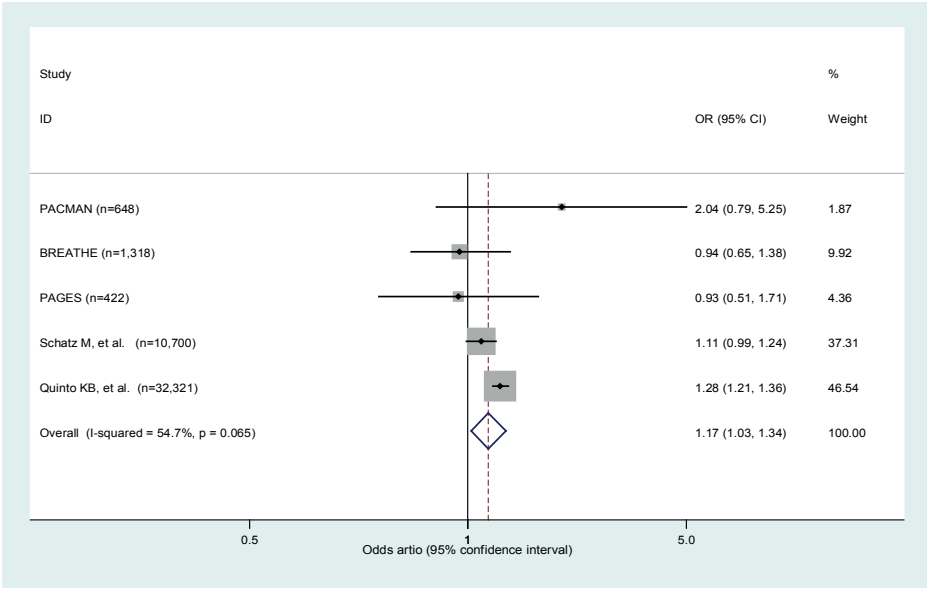


Fig 3. Pooled odds ratio of the association combined overweight and obesity with oral corticosteroids (OCS) use

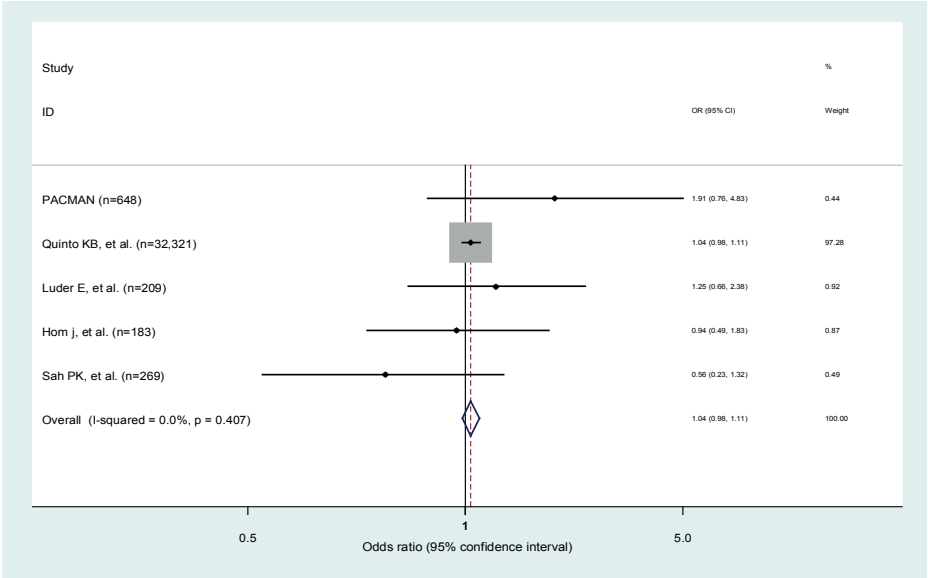


Fig 4. Pooled odds ratio of the association combined overweight and obesity with emergency department (ED) visits

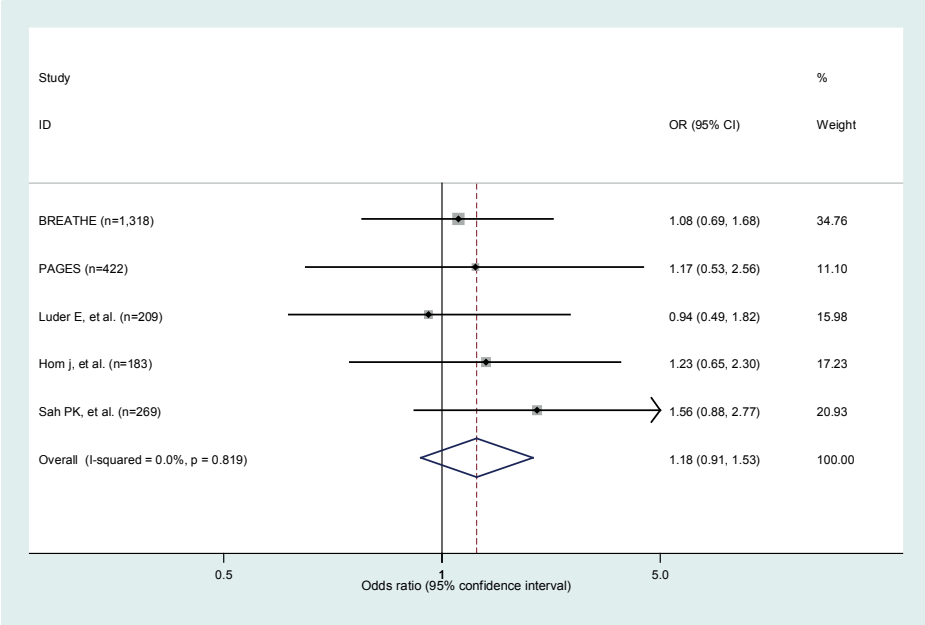


Fig 5. Pooled odds ratio of the association combined overweight and obesity with hospitalization due to asthma

The associations of obesity with ED visits and asthma-related hospitalization separately in published versus unpublished studies were also evaluated. The results illustrated that there was no difference between the results of these associations in published (OR: 1.04, 95% CI: 0.98-1.10; I^2: 0.0% and OR: 1.25, 95 % CI: 0.87-1.78; I^2: 0.0%, respectively) and unpublished studies (OR: 1.91, 95% CI: 0.76-4.83; I^2: 0.0% and OR: 1.10, 95% CI: 0.75-1.62; I^2: 0.0%, respectively).

The effect of different measurements of asthma control on the association between BMI and asthma control was evaluated by a sensitivity analysis; the results showed that obesity was significantly associated with poor asthma control measured by ACT (OR: 1.42, 95% CI: 1.08-1.87; I^2:0.0%) but not with ACQ (OR: 0.98, 95% CI: 0.69-1.39; I^2: 0.0%) and the point estimates were in the opposite direction. Obese children were also more likely to have asthma exacerbations measured by ED visits, hospitalizations due to asthma or OCS use compared with non-obese peers in both combined studies with self/parental reported asthma (OR: 1.24, 95% CI: 0.63-2.44; I^2: 33.2%, OR: 1.23, 95% CI: 0.65-2.30; I^2: 0.0% and OR: 2.04, 95% CI: 0.79-5.25; I^2: 0.0%, respectively) and studies with asthmatic children diagnosed by physician (OR: 1.03, 95% CI: 0.86-1.23; I^2: 10.8%, OR: 1.17, 95% CI: 0.87-1.56; I^2: 0.0%, and OR: 1.16, 95% CI: 1.01-1.32; I^2: 61.3%, respectively). We further stratified the meta-analysis based on recruitment of the patients in the studies. Based on our results obesity was related to increase asthma exacerbations either ED visits or OCS use in those studies with children recruited from primary care (OR: 1.18, 95% CI: 0.73-1.89; I^2:39.5% and OR: 1.22, 95% CI: 1.06-1.39; I^2:66.1%, respectively) but not in children from secondary care (OR: 0.89, 95% CI: 0.41-1.93; I^2:52.6% and OR: 0.94, 95% CI: 0.68-1.29; I^2:0.0%, respectively).

The effect of study design on these associations was also assessed and the results showed statistically significant association between obesity and poor asthma control in cross sectional studies (OR: 1.32, 95% CI: 1.02-1.72; I^2:0.0%) however obese children in longitudinal studies also were more likely to have poor asthma control compared with non-obese peers (OR: 1.04, 95% CI: 0.70-1.55; I^2:0.0%). The same results were also shown in the associations between obesity and ED visits (OR: 1.06, 95% CI: 0.68-1.63; I^2: 24.8%; OR: 1.04, 95% CI: 0.98-1.11; I^2: 0.0%) and OCS use (OR: 1.03, 95% CI: 0.73-1.46; I^2: 14.1%; OR: 1.20, 95% CI: 1.05-1.38; I^2: 79.4%) in the cross-sectional and cohort studies, respectively.

DISCUSSION

To the best of our knowledge, this systematic review and meta-analysis provides the first quantitative summary estimates of the relation between BMI and poor asthma control/ exacerbations. Our analysis in 14 studies included (52,147 asthmatic children and adolescents) shows that obese and overweight children have a slightly higher risk for severe asthma exacerbations, yet not for poor asthma control (based on asthma symptoms). Furthermore, we showed that gender does not influence these risks.

Childhood obesity has become a global public health issue especially in developed nations. Although data from many countries including US, Netherlands and UK have shown stabilization of obesity levels in children in 1995-2008[42], the results of most recent national estimates of obesity in children aged 2-9 years in US reported that obesity prevalence remains high, almost 17% between 2003 and 2012[43].

Several studies have proposed biological mechanisms, which may underlie the association between obesity and the risk of asthma exacerbations. An increased BMI might cause increased weight on the chest wall leading to breathing at lower lung volumes[4]. A recent meta-analysis suggested that children with higher infant weight gain were associated with asthma outcomes reflecting a direct mechanical effect on lung function[44]. In addition, obesity is associated with a chronic inflammatory state. Adipose tissue macrophages produce pro-inflammatory mediators, and these cells are abundantly present in obese individuals[5]. It is an ongoing debate whether obesity is associated with a distinct inflammatory asthma phenotype[45,46]. It has been suggested that pediatric obesity-associated asthma is characterized by Th1 polarization[47], in contrast to the more common Th2-driven atopic childhood asthma phenotype. Moreover, obesity is associated with a decreased response to bronchodilator medications in children and adolescents with asthma[48,49].

There is increasing evidence that some potential confounders e.g. age, gender, and race do play an important role in the association between obesity and asthma severity. Results of data-analyses in our three pediatric asthma cohorts highlighted the confounding effects of factors including age, eczema, rhinitis, breast feeding, family history of asthma and allergy in the association between obesity and the risk of asthma severity.

Race might also be an important confounder for the relationship between obesity and asthma severity. In PACMAN study, race/ethnicity was an actual confounder in the asso-

ciation obesity and asthma exacerbations. While in previous studies, obesity has been associated with poor asthma control and increased risk for exacerbations independent of race[20] or with a little effect[14,15].

Given the concern that obesity has been implicated in the onset of asthma, it is important to focus on prevention approaches for the individual patient. The positive effects of weight loss on asthma-related health outcomes have been already reported in overweight and obese adults with asthma[50,51]. However, an intervention study aimed at reducing asthma exacerbations by weight reduction strategies would be the only way to answer the question to what extent there is an association between weight reduction and asthma severity in children. Furthermore, it could help in answering the question if "obesity associated asthma" is a distinct asthma phenotype in children.

There are several limitations in the current study that should be addressed. Importantly, heterogeneity in sample size and type of characteristics e.g. geographic regions should be addressed in this study, and that the exposure and outcomes are not uniform across the combined studies. The present study was limited by the use of parental-reported questionnaires based data in some studies, which might be prone to recall bias. Self-reported BMI-data (e.g. in PACMAN) might be less accurate than standardized way using weight and height. Parents may not always be able to give an accurate estimate of their child's medication use. However, there is a reasonable agreement between parental-reported OCS use data and pharmacy prescription data within the PACMAN cohort (Cohen's kappa coefficient is 0.51; results not published). Additionally, definition of asthma control slightly differed between the separate studies; poor asthma control was defined by ACQ, ACT or NHLBI guidelines. Results from our sensitivity analyses showed statistically significant association between obesity and asthma control measured by ACT but not with ACQ. In a meta-analysis of Jia et al. including 21 studies, the ACT and ACQ had also significant differences in the assessment of controlled and not well-controlled asthma[52]. The assessment of asthma control has been limited to 1-week in the ACQ questionnaire but 4 weeks in the ACT questionnaire and NHLBI guidelines, which may have underestimated or overestimated long asthma control for participants[53]. Moreover, seasonal variation has been shown to have a substantial impact on asthma control[53], which might lead to differences in asthma control reported by different studies.

Studies included in our meta-analysis did not all have the same definition of asthma diagnosis however, most studies used physician-diagnosed asthma. Children younger than 5 years can have asthma-like symptoms[54,55] that could be explained by the smaller airways. Therefore, there is a likelihood of misclassified asthmatic children especially in young children. Children in some studies such as PACMAN were recruited through community pharmacies based on regular asthma medication use, while participants of some other studies e.g. BREATHE and PAGES were recruited through primary and secondary asthma clinics, and might, therefore, reflect a more severe population of asthmatics. It is possible that for patients with mild asthma on intermittent bronchodilators alone, being obese might not be associated with severity of asthma, but, while in more severe disease, use of systemic corticosteroids or physical inactivity might lead to a stronger relation of BMI with asthma severity. Another important limitation is about missing values for weight

and height in the three cohorts (BREATHE 11%, PACMAN 35% and PAGES 45%) that may have existed in other studies included as well. In most of the other studies included in the meta-analysis there is no information about missing values for BMI. Therefore, our estimates of overweight and obesity should not be interpreted as prevalence rates nor extrapolated to the general pediatric asthma population. Although in the three cohorts we have adjusted for the most important potential confounders such as age, eczema and family history of asthma the possibility remains that some factors which we have not measured still caused confounding, e.g. birth weight, gestational age, puberty, socioeconomic status and genetics. Furthermore, there is a lack of relevant adjustment for the association of BMI and asthma severity in some of the studies included in the present meta-analysis, which might influence this association differentially.

We were unable to check the onset of obesity and the subsequent development of asthma complications in which the time of obesity must be preceded. Therefore, reverse causality might affect these studies for which in subgroup of children especially those with early asthma onset, asthma might precede obesity.

We excluded studies that had different measurement of exposure and outcomes because we intended to reduce heterogeneity as much as possible. A statistically significant higher risk of asthma severity in obese compared with non-obese children was reported by five excluded studies[31,32,34,36,37]. The other four excluded studies[13,33,35,56] showed no significant association between obesity and asthma control. Since the pattern of these results is similar to our main findings we assume that the impact of excluding these studies on the pooled effect estimates of our study probably would be very small.

Multiple sensitivity analyses were used to test the robustness of the findings. Even though the point estimates were a bit different in some of these analyses, the 95% confidence intervals largely overlapped and that these differences were mainly caused by chance findings related to relatively low patient numbers. In our meta-analyses, we have pooled data from primary, secondary and tertiary care. Furthermore, we included studies from different parts of the world (Europe, USA, and Japan). Multiple sensitivity analyses showed the robustness of our findings. Therefore we conclude that our findings are generalizable to most children with asthma.

In summary, we have related asthma severity to BMI in a population of children with asthma and our findings suggest that both overweight and obesity have a small, but statistically significant deleterious effect on the risk of OCS use (as a marker for asthma exacerbations) but not on poor asthma control. Though a study where an intervention leads to weight reduction in asthmatic children with high BMI is needed to determine the true nature of the relationship between asthma and increasing BMI in children, weight loss is by far the best recommendation.

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SUPPORTING INFORMATION

Table S1. Search strategy for the systematic review

Domain		Determinant (s)				Outcome (s)
Children OR		BMI OR		Asthma control		Asthma exacerbation OR
Childhood AND	AND	Body mass index	AND		OR	Asthma severity AND
Adolescents OR		Obesity OR				Hospitalization OR
Pediatrics		Overweight				Oral corticosteroid use

Abbreviation: BMI: body mass index

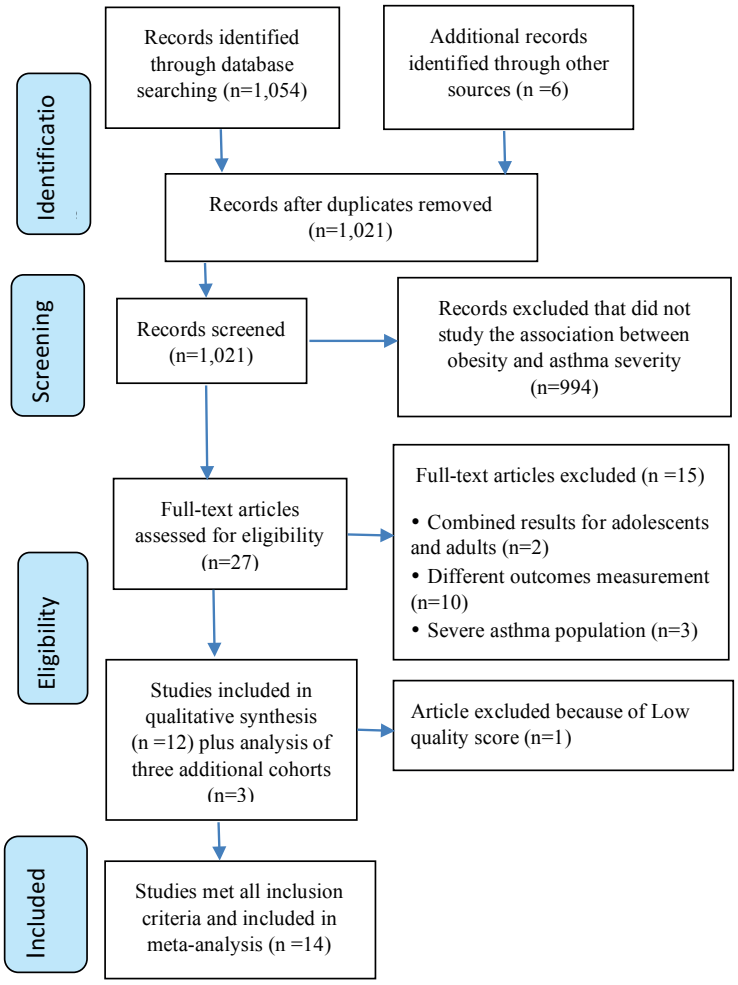

Fig S1. Flow diagram of study selection

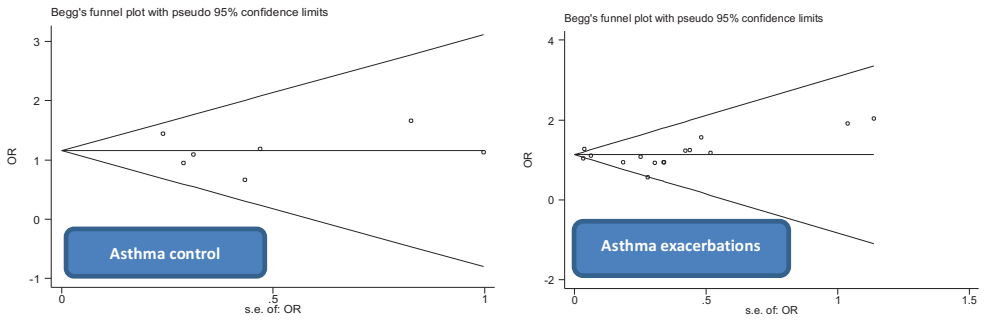

Fig S2. Funnel plots for publication bias in the association of overweight/obesity with poor asthma control (p=0.81) and asthma exacerbations (p=0.80)

Association of body mass index (BMI) and poor asthma control/exacerbations in BREATHE, PACMAN and PAGES

METHODS AND STATISTICAL ANALYSIS

Association of BMI and poor asthma control/exacerbations was tested in BREATHE, PACMAN and PAGES databases. For this cross-sectional analysis, the study sample consisted of asthmatic children and adolescents who participated in three North-European pediatric cohorts including the BREATHE cohort (Scotland, UK, age: 4–18 years) [1,2], the Pharmacogenetics of Asthma Medication in Children study (PACMAN) cohort (The Netherlands, age: 4–12 years) [3] and the Pediatric Asthma Gene Environment Study (PAGES) cohort (Scotland, UK, age: 4–17 years) [4]. All three studies are retrospective cohort studies. In BREATHE, children and adolescents with physician-diagnosed asthma were recruited either through primary or secondary clinics. In PACMAN paediatric users of asthma medication were selected through Dutch community pharmacies. In this cohort, children aged 4-12 years with at least 2 years of medication history available and at least 3 prescriptions for any asthma drug within the last 2 years and at least 1 prescription in the last 6 months were selected from pharmacies in different regions in the Netherlands. In PAGES physician-diagnosed asthmatic children were recruited through primary and secondary care. A detailed clinical history including e.g. information on asthma symptoms, treatment, asthma control and exacerbations was obtained from the parents and children. In PACMAN, BMI at the time of recruitment was calculated using weight and height measures for each child at the time of recruitment in pharmacy or the values were obtained from parental questionnaire. In BREATHE and PAGES weight and height were measured using the calibrated equipment in each hospital's clinic and BMI calculated. Data on asthma control were available in PACMAN and PAGES. In PACMAN the Asthma Control Questionnaire score (ACQ) was used to measure asthma control. An ACQ≥0.75 was considered as poor asthma control [5]. For children and adolescents in PAGES,

asthma control was assessed using the 7-item childhood Asthma Control Test scores (ACT). An ACT score of ≤19 was considered poor asthma control [6]. Two measures of asthma exacerbations were applied [7]; 1) asthma-related visits to an emergency department (ED) in the past 12 months from the date of completion of the questionnaire (PACMAN) and asthma-related hospitalization in the past 6 months (BREATHE and PAGES) 2) prescribed courses of oral corticosteroids (OCS) in the past 12 months (PACMAN) and in the past 6 months (BREATHE and PAGES). We used SAS version 9.1 for Windows to calculate age- and gender-adjusted BMI percentiles for children using height and weight measures as defined by the CDC standardized sex- and age-specific growth charts (**http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm**). Children were classified as follows: a) non-obese: either normal weight (BMI≥5th to <85th percentile) or overweight (BMI≥85th to <95th percentile) and b) obese (BMI≥95th percentile) [8]. Underweight children were not included in the logistic regression analyses. The differences in baseline characteristics of children with and without missing values in the three cohorts were compared. The frequency of baseline characteristics in the different BMI percentile categories were stratified by gender. The associations of obesity with poor asthma control and/or risk of exacerbations among obese girls and boys versus non-obese peers was estimated using binary logistic regression in univariate and multivariate ways to calculate crude and adjusted odd ratios (ORs) with 95% confidence intervals (CIs). In subgroup analysis, the effect of age on this association was tested in stratified analyses in three different age categories 4-6, 6.01-12.99 and 13-18 years. In a sensitivity analysis, the associations between obesity versus normal weight and poor asthma control and/or risk of exacerbations among obese girls and boys were also evaluated. Age, eczema, hay fever, pet's exposure, breast feeding, family history of asthma and family history of allergy, asthma in sibling and race/ethnicity were considered as potential confounders in these associations.

P-values of 0.05 were used to assess the statistical significance of main effect associations. We used SPSS 23.0 to analysis the data.

RESULTS

Data were available for 1,318 children and adolescents ages 4-18 years of the BREATHE cohort, 648 children ages 4-12 year of the PACMAN cohort and 422 children and adolescents ages 4-17 years of the PAGES cohort. The baseline characteristics of the three study populations are presented in **Table S2**. The incidence of obesity was 13% in BREATHE, 11% in PACMAN and 15% in PAGES. Poor asthma control was higher in PAGES (64%) compared with PACMAN (40%). Asthma exacerbations (either ED visits/ asthma related hospitalization or OCS dispensing) rates were higher in BREATHE (27%) and PAGES (40%) compared with PACMAN (10%).

We found statistically significant differences in baseline characteristics of the patients with and without data on BMI which was more pronounced in BREATHE and PAGES. Children in BREATHE and PAGES cohorts with missing data on BMI have remarkably less asthma

exacerbations (both hospitalization due to asthma and OCS use) compared with those that have data on BMI (**Table S3**).

The frequency of the baseline characteristics in the different BMI percentile categories was shown in **Table S4**. As shown, no significant differences between obese vs. non-obese children in different baseline characteristics were observed neither in girls nor in boys in the BREATHE cohort. In the PACMAN cohort, there were statistically significant differences between obese and non-obese peers in different baseline characteristics e.g. age ($p=0.003$), race ($p=0.03$) and exacerbations ($p<0.001$) in girls and family history of asthma in boys ($p=0.01$). In the PAGES cohort, significant difference between obese and non-obese children was shown only in girls with eczema ($p=0.05$) and in boys with poor asthma control ($p=0.04$).

As shown in **Table S5**, when boys and girls were combined, there was no association between obesity and poor asthma control in either population with an opposite direction in PACMAN and PAGES cohorts (OR: 0.95, 95% CI: 0.54-1.67 and OR: 1.66, 95% CI: 0.70-3.94, respectively). Obese girls in the PACMAN cohort were more likely to have poor asthma control compared to non-obese girls while in the PAGES cohort obese boys were at increased risk of poor asthma control than non-obese boys. No asthma control data was available for the BREATHE cohort.

There was an increased risk for exacerbations (both ED visits and OCS use) among obese girls compared to non-obese girls in the PACMAN population (OR: 4.03, 95% CI: 1.06-15.38 and OR: 5.66, 95% CI: 1.37-23.31, respectively) but not in the other populations. When boys only and when boys and girls were combined, there was no significant association between obesity and risk for exacerbations (**Table S5**).

When stratifying logistic regression analysis by age, the results showed no difference between different age categories (**Table S6**).

A sensitivity analysis was conducted to evaluate the association between obesity and outcomes in obese versus normal-weight children and the results showed no significant differences compared with the results for these associations in obese versus non-obese peers (data not shown).

Actual confounders in the association between obesity and asthma severity were the following: in BREATHE, age, rhinitis, and family history of asthma, in PACMAN, age, eczema, breast feeding, ethnicity/race, family history of asthma and allergy, and in PAGES, age, eczema, rhinitis and family history of allergy.

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Table S2. Baseline characteristics of children with asthma (with available BMI)

		BREATHE (n=1,318)	PACMAN (n=648)	PAGES (n=422)
Gender (girls), n (%)		534 (40.5)	242 (37.3)	179 (42.4)
Age, mean (SD), years		10.1 (3.7)	8.4 (2.4)	9.8 (3.3)
Age groups, years	4-6	230 (17.5)	145 (22.4)	71 (16.8)
	6.01-12.99	777 (59.0)	503 (77.6)	271 (64.2
	13-18	311 (23.6)	-	80 (19.0)
Race/ethnicity	White in UK Caucasian* in Netherlands	-	564 (87.0)	313 (74.2)
	Others	-	68 (10.5)	15 (3.6)
BMI (kg/m^2), mean (SD)		19.0 (4.1)	16.9 (2.7)	19.1 (4.8)
BMI percentile category, n (%)	Underweight (<5th percentile)	43 (3.3)	63 (9.7)	14 (3.3)
	Normal weight ((≥5th to 85th percentile)	877 (66.5)	438 (67.6)	280 (66.4)
	Overweight (≥85th to 95th percentile)	221 (16.8)	80 (12.3)	63 (14.9)
	Obese (≥95th percentile)	177 (13.4)	67 (10.3)	65 (15.4)
Asthma control, n (%)	Well-controlled	-	368 (56.8)	93 (36.2)
	Poor-controlled	-	258 (39.8)	164 (63.8)
ED visits/hospitalization due to asthma, n (%)		200 (15.2)	40 (6.2)	57 (14.3)
OCS use, n (%)		342 (25.9)	33 (5.1)	157 (39.4)
Asthma exacerbations[ψ], n (%)		357 (27.1)	62 (9.8)	170 (40.3)
Medication used based on BTS[a] treatment step, n (%)	Step 1	249 (18.9)	62 (9.7)	33 (7.9)
	Step 2	706 (53.6)	383 (59.8)	93 (22.2)
	Step 3	198 (15.0)	107 (16.7)	237 (56.6)
	Step 4	165 (12.5)	31 (4.8)	56 (13.4)
Atopy, n (%)	Eczema	706 (53.6)	387 (59.7)	167 (39.6)
	Hay fever	348 (26.7)	256 (39.5)	165 (39.1)
	Food allergy	-	309 (47.7)	148 (35.1)
Environmental factors, n (%)	Breast feeding	-	462 (71.3)	-
	Pets exposure	829 (64.5)	273 (42.1)	230 (54.5)
Family history of asthma [b], n (%)		541 (41.0)	287 (44.3)	315 (74.6)
Family history of allergy [c], n (%)		643 (48.8)	497 (76.7)	148 (35.1)
Asthma in sibling [d], n (%)		398 (30.2)	184 (28.4)	-

Abbreviations: BMI: body mass index; SD: standard deviation; ED: emergency department; OCS: oral corticosteroids; BTS: British Thoracic Society.

*Caucasian including Dutch, Turkish and Moroccan.

[a] The treatment step was modified from BTS guidelines as follows: step 1 is use of short-acting beta agonists (SABAs) as needed; step 2 is the step 1 plus regular inhaler corticosteroids (ICSs); step 3 is the step 2 plus regular long-acting beta agonists (LABAs); and step 4 is the step 3 plus oral leukotriene receptor antagonists (LTRAs).

[b] At least one asthmatic parent.

[c] At least one allergic parent.

[d] At least on asthmatic sibling.

[ψ] Asthma exacerbations defined as either ED visits/hospitalization due to asthma or OCS use

Table S3. Differences in baseline characteristics of children with and without data on BMI

		Missing BMI	**Non-missing BMI**		**Missing BMI**	**Non-missing BMI**		**Missing BMI**	**Non-missing BMI**	
		BREATHE (n=162)	BREATHE (n=1,318)	P-value	PACMAN (n=347)	PACMAN (n=648)	P-value	PAGES (n=340)	PAGES (n=422)	P-value
Gender, n (%)	Girls	69 (11.4)	534 (86.6)	0.61	137(36.1)	242 (63.9)	0.51	145 (**44.8**)	179 (**55.2**)	0.95
	Boys	93 (10.6)	784 (89.4)		210 (34.1)	406 (65.9)		195 (44.5)	243 (55.5)	
Age groups, years	4-6	56 (19.6)	230 (80.4)		68 (31.9)	145 (68.1)	0.31	19 (21.1)	71 (78.9)	
	6.01-12.99	25 (7.5)	310 (92.4)	**<0.001**	279 (35.7)	503 (64.3)		222 (45.0)	271 (55.0)	**<0.001**
	13-18	25 (7.4)	311 (92.6)		-	-		99 (**55.3**)	80 (**44.7**)	
Race/ethnicity	White in UK Caucasian* in Netherlands	-	-	-	317 (36.0)	564 (64.0)	**0.01**	63 (1**6.8**)	313 (**83**.2)	**0.07**
Asthma control, n (%)	Poor controlled	-	-	-	148 (44.6)	258 (41.2)	0.32	88 (62.4)	164 (63.8)	0.78
ED visits/hospitalization due to asthma, n (%)		67 (41.4)	200 (15.2)	**<0.001**	21 (6.2)	40 (6.5)	0.87	14 (4.6)	57 (14.3)	**<0.001**
OCS use, n (%)		78 (48.1)	342 (25.9)	**<0.001**	27 (7.9)	33 (5.2)	0.09	45 (14.8)	157 (39.4)	**<0.001**
Asthma exacerbations, n (%)		87 (53.7)	357 (27.1)	**<0.001**	39 (11.4)	62 (9.7)	0.39	46 (15.1)	170 (42.6)	**<0.001**
Atopy, n (%)	Eczema	96 (59.3)	706 (53.6)	0.17	232 (69.3)	387 (60.9)	**0.01**	116 (37.7)	167 (39.6)	**0.60**
	Hay fever	67 (41.4)	348 (26.7)	**<0.001**	155 (47.1)	256 (40.8)	0.06	111 (36.0)	165 (39.1)	**0.40**
	Food allergy	-	-	-	177 (53.0)	309 (48.8)	0.22	74 (24.0)	148 (35.1)	**0.001**
Environmental factors, n (%)	Pets exposure	92 (57.1)	829 (64.5)	0.07	112 (33.1)	273 (42.9)	**0.003**	196 (57.6)	230 (54.5)	0.39
Family history of asthma¨, n (%)		82 (50.6)	541 (41.0)	**0.02**	161 (50.5)	287 (45.9)	0.19	247 (80.2)	315 (74.6)	**0.08**
Family history of allergy¨¨, n (%)		112 (69.1)	643 (48.8)	**<0.001**	271 (83.9)	497 (79.1)	**0.08**	153 (45.0)	148 (35.1)	0.005

Abbreviations: BMI: body mass index; ED: emergency department; OCS: oral corticosteroids;
*Caucasian including Dutch, Turkish and Moroccan.
¨At least one asthmatic parent.
¨¨At least one allergic parent.

Table S4. Frequency of baseline characteristics in different BMI percentile categories stratified by gender

		BREATHE						PACMAN						PAGES					
		Girls (n=534)			Boys (n=784)			Girls (n=242)			Boys (n=406)			Girls (n=179)			Boys (n=243)		
BMI percentile categories		Obese	Non obese	P-value	Obese	Non obese	P-value	Obese	Non obese	P-value	Obese	Non obese	P-value	Obese	Non obese	P-value	Obese	Non obese	P-value
N (%)		68 (12.7)	443 (83.0)		109 (13.9)	655 (83.5)		31 (12.98)	191 (78.9)		36 (8.9)	327 (80.5)		30 (16.8)	143 (79.9)		35 (14.4)	200 (82.3)	
Age groups, years	4-6	16 (18.0)	73 (82.,0)		25 (17.9)	115 (82.1)		14 (26.4)	39 (73.6)	**0.003**	11 (14.9)	63 (85.1)	0.11	5 (17.2)	24 (82.8)		6 (14.3)	36 (85.7)	
	6.01-12.99	35 (12.5)	245 (87.5)	0.36	61 (13.0)	408 (87.0)	0.35	17 (10.1)	152 (89.9)		25 (8.7)	264 (91.3)		18 (16.5)	91 (83.5)	0.89	19 (12.8)	130 (87.2)	0.26
	13-18	17 (12.0)	125 (88.0)		23 (14.8)	132 (85.2)		-	-		-	-		7 (20.0)	28 (80.0)		10 (22.7)	34 (77.3)	
Race / ethnicity	White in UK Caucasian˚ in Netherlands	-	-		-	-		22 (11.4)	171 (88.6)	**0.03**	28 (8.8)	290 (91.2)	0.21	23 (17.3)	110 (82.7)	0.32	27 (15.5)	147 (84.5)	0.72
Asthma control, n (%)	Not-well controlled	-	-		-	-		13 (44.8)	79 (42.7)	0.83	14 (9.9)	127 (90.1)	0.89	12 (63.2)	66 (70.2)	0.54	17 (81.0)	68 (56.7)	**0.04**
ED visits/ hospitalization due to asthma, n (%)		9 (13.2)	67 (15.1)	0.68	19 (17.4)	97 (14.8)	0.48	5 (17.2)	8 (4.4)	**0.008**	2 (5.9)	20 (6.9)	0.91	4 (14.3)	19 (13.8)	0.94	5 (15.2)	28 (14.8)	0.96
OCS use, n (%)		12 (17.6)	113 (25.5)	0.16	33 (30.3)	175 (26.7)	0.44	5 (16.1)	7 (3.7)	**0.005**	3 (8.3)	18 (5.6)	0.50	9 (32.1)	45 (32.6)	0.96	15 (45.5)	87 (46.3)	0.93
Exacerbations, n (%)		12 (17.6)	116 (26.2)	0.13	35 (32.1)	183 (27.9)	0.37	9 (29.0)	12 (6.4)	**<0.001**	4 (11.1)	32 (9.8)	0.81	11 (18.3)	49 (81.7)	0.70	16 (14.7)	93 (85.3)	0.94

		BREATHE						PACMAN						PAGES					
	Eczema	34 (50.0)	233 (52.6)	0.69	52 (47.7)	365 (55.5)	0.12	17 (56.7)	124 (66.7)	0.29	16 (44.4)	189 (58.5)	0.11	7 (23.3)	61 (42.7)	**0.05**	12 (34.3)	84 (42.0)	0.39
Atopy, n (%)	Hay fever	19 (28.4)	107 (24.3)	0.48	37 (34.3)	171 (26.4)	0.09	7 (24.1)	81 (43.8)	**0.046**	10 (28.6)	138 (43.1)	0.10	7 (23.3)	57 (39.9)	0.09	15 (42.9)	78 (39.0)	0.67
	Food allergy	-	-		-	-		15 (51.7)	85 (46.4)	0.60	14 (38.9)	160 (49.2)	0.24	6 (20.0)	51 (35.7)	0.10	10 (28.6)	78 (39.0)	0.24
Environ-mental factors, n (%)	Breast feeding	-	-		-	-		18 (62.1)	135 (72.6)	0.25	26 (72.2)	231 (72.0)	0.97	-	-		-	-	
	Pets expo-sure	45 (66.2)	295 (68.9)	0.65	65 (60.2)	394 (61.7)	0.77	9 (30.0)	93 (49.7)	**0.04**	12 (33.3)	136 (42.1)	0.31	16 (53.3)	81 (56.6)	0.74	21 (60.0)	106 (53.0)	0.44
Family history of asthma, n (%)**		29 (43.3)	189 (43.1)	0.90	40 (37.0)	270 (41.8)	0.37	17 (56.7)	101 (54.9)	0.86	7 (20.0)	133 (42.1)	**0.01**	23 (76.7)	116 (81.1)	0.58	27 (77.1)	142 (71.0)	0.46
Family history of atopy*, n (%)**		35 (52.2)	219 (50.0)	0.76	48 (44.4)	311 (48.1)	0.51	23 (76.7)	149 (81.0)	0.58	20 (57.1)	255 (80.2)	**0.002**	9 (30.0)	58 (40.6)	0.28	12 (34.3)	65 (32.5)	0.84
Asthma in sibling**, n (%)**		24 (35.8)	142 (32.3)	0.57	34 (31.5)	189 (29.3)	0.65	8 (40.0)	57 (36.3)	0.75	11 (34.4)	91 (34.2)	0.99	-	-		-	-	

Abbreviations: BMI: body mass index; ED: emergency department; OCS: oral corticosteroids;
* Caucasian including Dutch, Turkish and Moroccan, **At least one asthmatic parent ***At least one atopic parent, **** At least one asthmatic sibling.

Table S5. Association of obesity and poor asthma control/exacerbations stratified by gender; obese vs. non-obese children

	BREATHE			PACMAN			PAGES		
		OR (95%CI)			OR (95%CI)			OR (95%CI)	
	Subjects included, n	Crude	Adjusted*	Subjects included, n	Crude	Adjusted*	Subjects included, n	Crude	Adjusted*
Poor asthma control									
Girls	-	-	-	213	1.08 (0.49-2.37)	1.28 (0.53-3.08)	113	0.73 (0.26-2.04)	0.88 (0.23-3.39)
Boys	-	-	-	351	0.96 (0.47-1.94)	0.89 (0.41-1.92)	141	3.25 (1.03-10.24)	2.78 (0.84-9.20)
Total	-	-	-	564	1.02 (0.60-1.72)	0.95 (0.54-1.67)	254	1.57 (0.75-3.32)	1.66 (0.70-3.94)
ED visits/asthma-related hospitalization									
Girls	511	0.86 (0.41-1.81)	0.80 (0.38-1.71)	210	4.51 (1.36-14.90)	**4.03 (1.06-15.38)**	166	1.04 (0.33-3.34)	1.26 (0.38-4.17)
Boys	764	1.21 (0.71-2.08)	1.27 (0.73-2.21)	344	0.91 (0.20-4.06)	0.70 (0.15-3.24)	222	1.03 (0.37-2.88)	1.29 (0.44-3.81)
Total	1275	1.07 (0.69-1.66)	1.08 (0.69-1.68)	554	2.07 (0.86-4.95)	1.91 (0.76-4.83)	388	1.03 (0.48-2.23)	1.17 (0.53-2.56)
OCS use									
Girls	511	0.63 (0.32-1.21)	0.57 (0.29-1.12)	221	5.03 (1.49-17.01)	**5.66 (1.37-23.31)**	166	0.98 (0.41-2.34)	1.06 (0.42-2.69)
Boys	764	1.19 (0.76-1.86)	1.27 (0.80-2.00)	361	1.55 (0.43-5.54)	1.47 (0.39-5.56)	221	0.97 (0.46-2.03)	0.90 (0.40-2.02)
Total	1275	0.96 (0.67-1.38)	0.94 (0.65-1.38)	582	2.66 (1.15-6.16)	2.04 (0.79-5.25)	387	0.95 (0.55-1.67)	0.93 (0.51-1.71)

Abbreviations: OR: odds ratio; CI: confidence interval; ED: emergency department; OCS: oral corticosteroids

*Adjusted for age, hay fever, eczema, family history of asthma and allergy, breast-feeding, pet's exposure and race/ethnicity by stepwise logistic regression model.

Data on breast-feeding was not available in BREATHE and PAGES cohorts.

Data on race/ethnicity was not available in BREATHE cohort.

Table S6. Association of obesity and poor asthma control/exacerbations stratified by age; obese versus non-obese children

	BREATHE		PACMAN		PAGES	
	Subjects included, n	OR (95% CI)	Subjects included, n	OR (95% CI)	Subjects included, n	OR (95% CI)
Poor asthma control						
4-6 year	-	-	124	0.83 (0.30-2.31)	21	-
6.01-12.99 year	-	-	440	0.98 (0.49-1.95)	158	1.32 (0.45-3.88)
13-18 year	-	-	-		75	1.35 (0.34-5.42)
ED visits/asthma-related hospitalization						
4-6 year	229	0.84 (0.35-1.97)	124	3.13 (0.90-10.92)	69	1.69 (0.26-11.16)
6.01-12.99 year	749	1.05 (0.59-1.88)	430	1.40 (0.31-6.35)	243	1.42 (0.53-3.81)
13-18 year	297	1.46 (0.45-4.70)	-	-	76	0.40 (0.05-3.43)
OCS use						
4-6 year	229	0.89 (0.43-1.87)	127	2.72 (0.61-12.12)	69	1.45 (0.29-7.11)
6.01-12.99 year	749	0.90 (0.54-1.49)	455	2.04 (0.55-7.56)	242	0.91 (0.40-2.07)
13-18 year	297	1.17 (0.49-2.80)	-	-	76	0.86 (0.27-2.72)

Abbreviations: OR: odds ratio; CI: confidence interval; ED: emergency department; OCS: oral corticosteroids
*Adjusted for gender, hay fever, eczema, family history of asthma and allergy, breast feeding, pet's exposure and race/ethnicity by stepwise logistic regression model.
Data on breast feeding was not available in BREATHE and PAGES cohorts.
Data on race/ethnicity was not available in BREATHE cohort.

Chapter 4 General discussion

SCOPE OF THESIS

Chronic illnesses like cancer, diabetes, asthma and allergy are defined as physical or mental conditions that need frequent hospitalizations and are present for more than three months per year[1]. Prevalence rates of childhood chronic diseases have increased substantially during the past decades[2]. Epidemiological studies have shown that the number of chronically ill children approximates 7 to 20 percent of all children; that is almost 500,000 children in the Netherlands and roughly 7 million in the United States[1,2]. This number is expected to increase further due to lifestyle and environmental changes[2].
Chronic disorders have complex etiologies involving both genetic and environmental factors. Factors such as birth weight, breast-feeding, diet, change in immune response, exercise and altered gut microbiome are common factors that might contribute to childhood chronic disorders[2]. Recent evidence shows that for example early exposure to factors that can influence the gut microbiome is associated with the development of childhood diseases such as asthma, allergic disorders (atopic dermatitis, rhinitis), chronic immune-mediated inflammatory diseases, type 1 diabetes mellitus (T1DM) and obesity[3,4].
However, which specific genetic and environmental risk factors cause these diseases and to what extend risk factors can influence the onset and course of childhood chronic diseases remains an issue of debate. Therefore, epidemiologic studies concerning risk factors and complications related to chronic diseases in children are needed.
The aims of the studies in this thesis were to explore:
1) Comorbidities and co-medications use in childhood T1DM including cardiovascular disease (CVD) risk factors, and asthma
2) Risk factors (genetic and environmental) associated with the occurrence of childhood asthma/asthma exacerbations/allergy
This general discussion chapter critically discusses the key findings described in this thesis in comparison with literature and the methodological challenges together with strengths and limitations of our studies. Finally, in the section on future perspectives we will provide possible implications for clinical practice and research recommendations.

MAIN FINDINGS

Trends in comorbidities and co-medications use related to T1DM in children

Children with T1DM are at increased risk of co-morbidities compared with children in the general population. Poor glycemic control defined as HbA1c≥58 mmol/mol is a determinant for CVD risk factors e.g. hypertension[5] as well as micro and macrovascular complications[6]. Despite higher rates of CVD risk factors in children with T1DM[7-10] data on prophylactic cardiovascular (CV) medication use in pediatric T1DM patients and long-term follow-up investigating the development of CVD risk factors and the risk of CVD later in life is limited. In ***Chapter 2***, we performed two studies with respect to the epidemiology of CVD risk factors, CVD and patterns of CV medication use in children with T1DM.

We used the PHARMO linkage database (***Chapter 2.1***) and the CPRD database (***Chapter 2.2***) to compose two cohorts of children aged<19 years with incident T1DM; defined as at least two insulin prescriptions. Our findings in the two datasets showed that children with T1DM compared with age and gender matched reference cohorts without T1DM were more likely to have hypertension (2-fold) and hypercholesterolemia (25-fold), and they were also more likely to use CV medication (3-fold) in the period after the onset of diabetes. However, the percentage of children diagnosed with CVD events, as expected, was in the T1DM cohorts as low as in the reference cohorts. We also studied the years before the onset of T1DM to see whether the asymptomatic period of beta cell destruction prior to the clinical presentation of T1DM is associated with a higher occurrence of CVD risk factors.

The statistically significantly higher rates of hypertension and hypercholesterolemia followed by CV medication use already started one year before the onset of diabetes and further increased during the 20-year follow-up. Gender did not modify these associations. Children aged 15-18 years at index date (the date of diagnosis of T1DM) were more likely to have hypertension and hypercholesterolemia compared with the index date of younger peers in both cohorts. As shown in ***Chapter 2.2***, antihypertensive medication including diuretics, beta blockers, angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs) and calcium channel blockers were statistically significantly ($p<0.001$) more often used (defined as at least one prescription of one of these groups during follow-up) in children with T1DM (16.0%) compared with the reference group (8.0%) over the 20 years after the onset of diabetes. The same trend was observed for lipid-lowering drugs including statins, nicotinic acid, fibrates and bile acid sequestrants (9.0% vs. 0.3%, respectively). Moreover, ACEIs (9.0% vs. 0.4%) and statins (8.0% vs. 0.2%) were the most commonly used CV medications in children with T1DM after the onset of diabetes.

An important finding in this chapter was the undertreatment of hypertension and hypercholesterolemia in children with T1DM during the years after the onset of diabetes. Our data indicated that a substantial number of children with hypertension (70%) and hypercholesterolemia (98%) were undertreated at least for one year during the follow-up.

The association of T1DM and asthma in children has been studied with controversial results. Some studies found a significant reduction in the prevalence rate of asthma in children with T1DM[11,12], while other studies found an effect in the opposite direction[13-15]. Therefore, in ***Chapter 2.3*** we studied asthma related medication use and asthma exacerbations in children with T1DM. Our findings showed that a T1DM diagnosis was associated with more asthma medication use compared with a group of age- and gender-matched diabetes-free children in the 1st year after the onset of diabetes. However, this difference disappeared afterwards. In our study, we found that children aged 4 years and younger in both cohorts were more likely to have asthma medication prescriptions compared to the other age groups. No statistically significant difference between the two cohorts was observed in the use of specific types of asthma medication (short acting beta agonists (SABAs), inhaled corticosteroids (ICSs) and long acting beta agonists (LABAs)) except

for short acting muscarinic antagonists (SAMAs) that were significantly more used in the T1DM cohort (5.5%) compared with the reference cohort (0.62%) after the onset of diabetes. The observed higher prevalence rate of asthma medication use in the T1DM cohort might be because of the link between glucose regulation and lung function as suggested by previous studies[14,16]. We, therefore, speculated that when diabetes is well regulated the asthma-like complaints might decrease in these children. However, we cannot exclude that T1DM children visit doctors more frequently and therefore have a higher chance to be diagnosed with lung problems and being treated for that.

Risk factors associated with asthma/allergy in children

Despite the fact that only a small proportion of asthma occurrence can be explained by genetic factors[17], the most recent genome-wide association studies (GWASs) reported several high-risk alleles that are strongly associated with childhood asthma[18]. Molecular genetic studies show that there is a strong genetic component in asthma, but they have suffered from variability in asthma phenotype definitions and study populations. Because of this, current knowledge is not necessarily applicable to childhood asthma. For this, in ***Chapter 3.1***, using data collected in the Wheezing illnesses study (WHISTLER cohort), we used two genetic risk scores (GRSs) based on GWAS-identified lung function and asthma-associated single nucleotide polymorphisms (SNPs)[18-22] in adults and tested whether these GRSs were associated with lung function and asthma in children, respectively. Asthma GRS as continuous variable was used for Cox regression analysis. Our findings showed that the asthma GRS developed in adults was associated with the occurrence of asthma in children; when the asthma GRS increased with one point the risk of asthma increased by 71% (HR: 1.71, 95% CI: 1.16-2.51, p=0.006).
Children with GRS≥2 (median GRS is 2) developed asthma earlier in life compared with those with GRS below 2 (log-rank p=0.03). Genetic variation associated with better adult lung function was not associated with better lung function in neonates and young children.

Global increasing rates in prevalence of childhood asthma and allergy suggest a substantial role of environmental factors[23,24] including factors occurring early in life. An example of environmental influence can be found in the microbiome. The gut microbiome plays an important role in health and disease[25]. Changes in the intestinal bacterial diversity during the first months of life influence the immune system[4,25-27]. Evidence showed that modifiable factors such as gut microbiome and diet have a key role in the maturation of the neonatal immune system[27]. The healthy gut microbiome is highly diverse and can be disturbed by environmental factors e.g. bacterial infections, antibiotic therapy and lifestyle in early infancy [4,26-28]. In infants, at birth, the immunologic response to novel antigens is mainly from the T helper type 2 (Th2) since at the time of pregnancy the T helper type 1 (Th1) immune response of fetus has been suppressed[29]. For instance, use of antibiotics decreases the development of a Th1 immune response leading to a disbalance between Th1 and Th2[30].

Therefore, factors influencing the immune system early in life such as antibiotics and breast-feeding might also have an effect on asthma and allergy susceptibilities later in life[31].
However, the conclusions from epidemiological studies are inconsistent[30]. Prior studies assessing the association between early life environmental factors and asthma severity are limited[32]. We hypothesized that there is association between early life factors and asthma complications in asthmatic children. Therefore, in ***Chapter 3.2***, we assessed the effect of early life exposed vs. non-exposed to antibiotics on the onset of childhood asthma and asthma exacerbations. Using different databases including Generation R and SEATON for the association of antibiotics and asthma and PACMAN and BREATHE for the association of antibiotics and asthma exacerbations, we did find an association between early life exposure to antibiotics and asthma susceptibility later in life (OR: 2.18, 95% CI: 1.04-4.60; I^2: 76.3%). However, there was no statistically significant association between exposure to antibiotics and asthma exacerbations within asthmatic children (OR: 0.93, 95% CI: 0.65-1.32; I^2: 0.0%).

We also conducted a systematic review and meta-analysis including 34 published studies on the association between early life antibiotics usage and the risk of long-term allergy and atopy (***Chapter 3.3***). Our findings suggested that exposure to antibiotics during the first two years of life is associated with an increased risk of allergic symptoms including hay fever (OR: 1.23, 95% CI: 1.13-1.34; I^2: 77.0%), eczema (OR: 1.26, 95% CI: 1.15-1.37; I^2: 74.2%)) and food allergy (OR: 1.42, 95% CI: 1.08-1.87; I^2: 80.8%) later in life, but there was no relation of antibiotics use with a positive skin prick test (SPT) (OR: 1.01, 95% CI: 0.92-1.11; I^2:54.8%)) or elevated allergen-specific serum/plasma IgE levels (OR: 0.95, 95% CI: 0.77-1.16; I^2:44.9%)).

Furthermore, we evaluated the association of breast-feeding with asthma exacerbations in a pediatric population with asthma (***Chapter 3.4***); where we found a lower risk of asthma exacerbations in breastfed children (OR: 0.59, 95% CI: 0.38-0.93). Breastfed infants with a short duration of breast-feeding (<6 months) were protected most from asthma exacerbations.

The findings of the association between early life factors and risk of asthma and allergies later in life are inconsistent. These differences might be caused by varieties in methods, study design, population (it is likely that the effect has being influenced by race/ethnicity or environmental differences due to different geography (place of birth[33]), studies' sample size, and definitions of exposure, outcome and confounders. Additionally, different ages of children at the time of the outcome(s) measurement could be the most important reason for heterogeneities in the results of these studies. With increasing age the possibility of effects from other exposures influencing the association is increasing. This makes it difficult to distinguish the impact of the separate risk factors.

Childhood asthma and obesity are common chronic conditions in many countries and obesity has been implicated in asthma causation[34]. Previous studies have shown conflicting results for the association between childhood obesity and asthma severity while the effect of gender on this association was also inconsistent. Therefore, to further elucidate whether there is an association between obesity and asthma severity (poor asthma control and exacerbations) we conducted a meta-analysis including 14 studies in asthmatic children (***Chapter 3.5***). In this study, we included all published studies on this association applying a systematic review and then pooled the results together with the results of PACMAN, BREATHE and PAGES studies in which this association was not studied before. We found a slightly enhanced risk of asthma exacerbations among overweight and obese asthmatic children (OR: 1.17, 95% CI: 1.03-1.34; I^2: 54.7%), however, there was no statistically significant association with poor asthma control (OR: 1.23, 95% CI: 0.99-1.53; I^2: 0.0%). Furthermore, gender did not appear to modify this risk. Mechanisms which might explain how obesity could lead to asthma include increased weight on the chest wall leading to breathing at lower lung volumes and/or pro-inflammatory mediators released by adipocytes[35-37].

METHODOLOGICAL CONSIDERATIONS, STRENGTHS, AND LIMITATIONS

Data sources

For the studies in this thesis different databases across Europe were used including PHARMO (Netherlands), CPRD (UK), PACMAN (Netherlands), BREATHE (UK), PAGES (UK), Generation R (Netherlands), SEATON (UK) and WHISTLER (Netherlands). Different designs, objectives, study populations, and variability in the way of collecting data allowed us to study different exposures, confounders and outcomes related to childhood chronic disorders. All studies have their own strengths and limitations. The studies that were used were all observational studies using electronic patient records. This kind of data is broadly used in clinical research but might be limited by the accuracy of the data; for instance errors resulting from the data entry process or the use of different definitions to classify diseases[38]. Lack of information on the indication for dispensed/prescribed medications is another important issue, which might lead to misclassification bias (***Chapter 2 & 3***). In two chapters of this thesis (***Chapter 2.1 and 2.3***), we used medication-dispensing data using the PHARMO database to compare children with T1DM with a reference cohort. An important issue in this kind of databases is related to the reference cohort. Since the reference cohort was captured from the same database as the children with health-related problems, they might not be truly representative for the general population. For example in the PHARMO database healthy children might never visit a pharmacy and are therefore not registered. This means that our estimation for the difference between the two cohorts in terms of medication use and comorbidities might be underestimated. Moreover, studies concerning medication utilization (in both dispensing and prescribing databases) are limited for the lack of information on over the counter (OTC) medication.

In some studies the main variables of interest (exposure and/or outcomes) were obtained using questionnaire-based data (***Chapter 3.2 & 3.4 & 3.5***). Self-reporting is one of the most widely used methods of collecting information. Despite widespread use, there might be problems with accuracy and validity of self-reported data. Accuracy and validity in this kind of data has been affected by situational factors including time frame (time required for recall, for example, three months compared to one year), utilization frequency especially in quantitative questions (the frequency of the event for example number of courses of antibiotics), mode of data collection (surveys, in-person interviews, internet surveys) and questionnaire design (location, structure and wording of items in a questionnaire; self-report questionnaires might include questions that might lead to different conceptions)[39].

Study design & internal validity

The study design is an important factor that influences the validity and precision of pharmacoepidemiological studies. Main study designs used in this thesis were the retrospective cohort and cross-sectional design.

The identifying feature of a cohort study design is that the subjects are followed over time. Cohort studies begin with exposure to a certain factor and studies whether this influences the development of disease during follow-up. In several chapters (***Chapters 2.1, 2.2, 2.3, 3.1, 3.2 and 3.4***) in this thesis we chose the cohort design since cohort studies are an appropriate study design 1) to measure incidence rates, absolute and relative risks, 2) to evaluate trends in disease progression, and 3) to assess causality due to the temporal nature of the study design. The data in our cohort studies were prospectively collected however, for specific research questions thought of later the data collection might be insufficient. Existing data might be limited by missing values, inaccurate data, or inconsistent measurements[40]. There was for instance a substantial number of missing data of body mass index (BMI) and smoking status in ***Chapter 2.3***. Cross-sectional studies when exposure, medication use and outcomes are measured at one moment (or period) in time are most appropriate for evaluating the prevalence rates of diseases, risk factors and treatments. However, when they are used for etiological research the risk of ***reverse causality*** is a major problem. Because from the data it cannot be known whether the exposure preceded the outcome. In the meta-analyses in which we studied the association between antibiotics exposure in early life and risk of allergies/atopies later in life (***Chapter 3.3***) and the association between childhood obesity and risk of asthma severity (***Chapter 3.5***), cross-sectional studies were included. In some of these studies using questionnaires it was explicitly stated that the exposure information (e.g. antibiotics during the first two years of life) preceded the outcome (allergic symptoms e.g. eczema) but in some of them not. However, we tried to address this problem by performing sensitivity analyses to assess the impact of including these studies on the pooled effect estimates and found out that our findings did not change. Cohort studies or case control studies are more optimal to prevent the problem of reverse causation. However, we decided to also include the cross-sectional studies and exclude them in a sensitivity analysis because of limited power of the remaining studies.

Several other biases might have jeopardized the internal validity of our studies. ***Selection bias*** might have occurred by the way the populations used in our studies were collected; for instance there can be a problem when the children were not representative of the population they were collected from. Asthma, the most common chronic illness of childhood, is a very heterogeneous disease in which diagnosis and grades of progress might differ between and within individual patients[24]. In ***Chapter 3***, where we assessed the association between exposures and asthma/asthma severity we might have introduced selection bias by the fact that there is no "gold standard' for the definition and assessment of asthma in young children. Asthma should be measured objectively with a validated instrument, differentiating between phenotypes, such as atopic and non-atopic asthma. Literature shows that children younger than 5 years can have asthma-like symptoms originating from smaller airways without asthma being present[41,42]. ***Misclassification bias***, also known as observation, recall or information bias results from incorrect determination of exposure, potential confounders and/or outcome. Misclassification bias is a potential concern in ***Chapter 2*** since we only used dispensing and prescription data and did not have information on indications for treatment. In ***Chapter 3***, where the asthma or allergic symptoms were physician-based or parental-reported misclassification can also be a problem. ***Confounding bias*** is one of the major concerns in epidemiological research, as it is one of the most difficult biases to detect and also to control for. Confounding by indication may arise when the reason for prescription e.g. antibiotics is also a risk factor for the development of the outcome of interest. As an example the risk of confounding by indication is a concern in ***Chapter 3.2 & 3.3*** in which the association between early antibiotics consumption and later asthma and allergies could be explained by this bias. For example childhood otitis increases the risk of receiving antibiotic prescriptions early in life and it may also increase the subsequent risk of eczema later in life[43].

Meta-analysis

Sometimes observational studies represent the best available evidence in epidemiologic research when randomized controlled trials (RCTs) are infeasible or unethical, they report long-term or less common serious outcomes, or when they reflect associations in real-world settings in terms of populations included and comparisons made. Despite many advantages in observational studies they are prone to overestimate the treatment response[44]. A systematic review of observational data, therefore, could help to reduce sources of bias by deciding on explicit inclusion and exclusion criteria to answer the research questions. The strength of a meta-analysis, when it is well conducted, lies in its ability to combine the results from different small studies that might have been limited by low power to detect a statistically significant effect in association(s) between exposure(s) and outcome(s). The results of a meta-analysis can be used to base medical recommendations on or to provide guidance in the design of future clinical trials.

In this thesis, we used meta-analyses to find the answers to two research questions. The first question was regarding early life exposure to antibiotics and risk of allergies later in life (***Chapter 3.3***) and the second one considered the association between childhood

obesity and asthma severity (***Chapter 3.5***). For both questions the results from earlier studies were conflicting.

However, there are certain aspects in the design of meta-analyses that should be taken into account[45] including the quality assessment of studies included. A quality score for each study included in a meta-analysis is a qualitative measurement to evaluate the biases, strengths, and weaknesses of primary studies. A low quality score should lead to exclusion of a study. Including low-quality studies in a meta-analysis leads to entering fundamental biases from the primary studies into the meta-analysis, after which it is difficult to detect which and the magnitude of the errors entered. In our two meta-analyses, we used the Newcastle-Ottawa Scale (NOS) which is one of the more comprehensive instruments for assessing the quality of observational studies in meta-analyses[46]. Using the NOS checklist, we assessed the quality of studies included in our two meta-analyses. Each study was evaluated independently by three authors on eight items categorized into three groups including the selection of the study group, the comparability of the groups and the assessment of either the exposure or outcome of interest for different studies. In cases of disagreement, consensus between the authors was reached after discussion. When a study met ≥5 NOS criteria, the study was considered to be of high quality and included in our meta-analyses. Studies with a NOS score <5 were excluded from the meta-analysis (***Chapter 3.5***) or included in meta-analysis (***Chapter 3.3***) but applying a sensitivity analysis to assess the effect of studies with low quality scores on the pooled results. Another important aspect to take into account is heterogeneity. The term heterogeneity applies for any kind of variability among individual studies in a systematic review including a) clinical heterogeneity (variability in the participants, interventions and outcomes), b) methodological heterogeneity (variability in study design) and c) statistical heterogeneity (variability in the confounding effects being evaluated in the different individual studies). Heterogeneity is one of the most common flaws in meta-analyses that can be avoided by applying a systematic approach to meta-analysis. In our meta-analyses, we used strict inclusion and exclusion criteria and only included those studies with the same definitions of exposures and outcomes. We tested the robustness of our findings using multiple subgroups and/or sensitivity analyses taking into account the study design, study size, and children's characteristics to assess the effect of clinical and methodological heterogeneity on the pooled results. Finally, publication bias and selective reporting of outcomes are other major points of attention in a meta-analysis. Studies with no significant associations are more likely not to be published (publication bias) and studies that were not positive were also often published in a way that conveyed a positive outcome (selective reporting). These biases will lead to an overestimation of the effect. An exemplary example of publication bias is the case of the efficacy of antidepressant agents. Turner et al. showed that according to the published literature 48 out of the 51 studies were reported to have positive results on antidepressant's efficacy. While according to the FDA, 38 out of the 74 registered studies had positive results; there was no overlap between these two sets of confidence intervals[47]. In the meta-analyses in this thesis, we evaluated the magnitude of publication bias by statistical methods. A graphical method (funnel plot) to show the effect

estimates of individual studies ordered by the number of patients in a study (or a variance estimate of the study effects) was used to detect possible publication bias.

CLINICAL IMPLICATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Given the higher risk of CVD risk factors among children with T1DM and that CVD is the major cause of death after the age of 30 years (33.6%) in patients with childhood-onset T1DM[48]; there is a strong need for detecting, screening and preventing diabetes-related CVD risk factors once diabetes is diagnosed. Although, presentation of CVD risk factors might already occur at young age CVD is a long-term complication of T1DM[49].

A high rate of poor glycemic control in pediatric population with T1DM (average rate is 13%) especially in adolescents is a major concern. The increased risk of hypertension has been found to be associated with poor glycemic control during 18 years of follow-up[5]. It is, therefore, important to intensively control glucose levels (HbA1c<58 mmol/mol) to lower the risk of hypertension in children with T1DM[6].

More importantly, undertreatment of CVD risk factors in pediatric T1DM populations is a big concern. Our studies (***Chapter 2.1 & 2.2***) and others showed that there is substantial undertreatment of CVD risk factors in both hypertension and hypercholesterolemia. Current guidelines[6,50] recommend screening, diagnosis and treatment of hypertension and hypercholesterolemia as follows: in children with T1DM, blood pressure should be measured at every routine visit. Pharmacological treatment of hypertension (defined as systolic and/or diastolic blood pressure that is ≥95th percentile for age, gender, and height) in diabetic children should be considered as soon as the diagnosis is confirmed. In children with hypercholesterolemia, after the age of 10 years, addition of a statin is suggested in those who, despite lipid-lowering diet and lifestyle changes, continue to have LDL cholesterol ≥130 mg/dL (3.4 mmol/L) or in children with multiple CVD risk factors.

Although, there is a lack of longitudinal data on the efficacy of CV medications in children with T1DM treatment of both hypertension and hypercholesterolemia needs to be implemented. Implementing educational programs on guidelines-based treatment directed at health care providers and patients with T1DM might improve CVD management of these children and thereby prevent CVD complications later in life.

It is still unclear whether there are causal relations between early life risk factors and the occurrence of asthma/asthma exacerbations and allergy during childhood. Therefore, further prospective research is warranted to clarify the underlying mechanisms and reach novel strategies for the prevention and treatment.

CONCLUSIONS

The studies included in this thesis focused on different aspects related to pediatric chronic disorders including T1DM, asthma and allergy. We have investigated the trends in prevalence and incidence rates of CVD risk factors, asthma and related-medications use in

children with T1DM, evaluated genetic and environmental risk factors associated with the occurrence of childhood asthma/allergy, and finally studied risk factors associated with asthma exacerbations in children.

Our studies in children with T1DM demonstrated that these children have higher prevalence rates of CVD risk factors and CV drugs use than age and gender-matched children without diabetes. However, there was a substantial undertreatment of hypertension and hypercholesterolemia in children with T1DM. When realizing that T1DM patients later in life have a substantial higher risk for CVD than non-diabetics, screening and adequate treatment of CVD risk factors is of utmost importance.

There are strong indications that genetic and environmental factors increase the risk of asthma and allergy in children. The GRS developed in adults with asthma also predicted asthma in children. The role of antibiotics early in life as a risk factor for asthma and allergies (hay fever, eczema and food allergy) needs further elucidation. However, breast-feeding was shown as a protective factor for asthma exacerbations.

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Chapter 5 Appendices

Chapter 5.1 English summary

The worldwide increasing rate in number of children growing up with chronic diseases is a major concern. Chronic diseases are a leading cause of morbidity and mortality worldwide. Although the epidemiology of childhood type 1 diabetes mellitus (T1DM), asthma and allergy is already well documented, very few studies have measured risk factors, complications and treatment thereof in this population.

The aims of the studies in this thesis were to explore:

1. *Comorbidities and co-medications use in childhood T1DM including cardiovascular disease (CVD) risk factors, and asthma*
2. *Risk factors (genetic and environmental) associated with the occurrence of childhood asthma/asthma exacerbations/allergy*

In ***Chapter 1***, ***the general introduction***, an overview was given with respect to the epidemiology and current knowledge on childhood chronic diseases, related risk factors and complications.
T1DM, asthma and allergy are common chronic disorders in children. Susceptibility to these chronic disorders results from interactions between genetic disease susceptibility and environmental factors. T1DM, asthma and allergy are complex diseases, which need continuous medical care as well as multifactorial risk reduction strategies. These children are typically at increased risk of serious health problems and complications that require early treatment.

In ***Chapter 2***, ***trends in comorbidities related to T1DM in children***, we studied whether children with T1DM are at increased risk of CVD risk factors (hypertension and hypercholesterolemia) and CVD events compared with age and gender matched children without diabetes mellitus using two different databases; PHARMO in the Netherlands (***Chapter 2.1)*** and CPRD in the UK (***Chapter 2.2***).

In ***Chapter 2.1***, ***cardiovascular medication use and cardiovascular disease in children and adolescents with type 1 diabetes: a population-based cohort study,*** in a retrospective cohort study between 1999 and 2009 we investigated the 5-years prevalence and incidence rates of CV medication and CVD before and after the onset of diabetes in children aged <19 years with T1DM (defined as children with at least two insulin prescriptions) (n=925). The results were further compared with a 4 times larger reference cohort (n=3,591) of the same age, gender. First insulin dispensing was selected as the index date. Our findings showed a statistically higher prevalence rate of CV medication use in the T1DM cohort before (2.2% vs. 1.0%, p<0.001) and after (9.2% vs. 3.2%, p<0.001) the index date. Angiotensin converting enzyme inhibitors (ACEIs) (2.0%) and statins (1.5%) were shown as the most prevalent CV medications in the T1DM cohort after the index date. The highest incidence rate of cardiovascular medication use was observed in the first year after the index date (28.1 per 1000 person-years). Furthermore, as expected, the number of children hospitalized due to CVD was extremely low in the two cohorts after the index date.

In ***Chapter 2.2***, ***time trends in the epidemiology of CVD risk factors, diseases and medication use in children and adolescents with T1DM: a CPRD study***, using the same study design as the PHARMO study but a different population (CPRD from the UK) and a roughly 5-times larger sample size (n=22,241) we intended to study the long-term trends in CVD risk factors, CVD events and treatment of CVD risk factors in children with T1DM from 2 years before up to 20 years after the onset of diabetes compared with children without diabetes. We also evaluated undertreatment of hypertension and hypercholesterolemia followed by identifying the determinants of undertreatment. Subjects were followed from 01/01/1987 until the end of study period (30/10/2015). The findings in this study showed that the annual prevalence rates of hypertension (0.64% vs. 0.34%, p=0.007 and 35.2% vs. 11.4%, p<0.001), hypercholesterolemia (0.91% vs. 0.05%, p<0.001and 64.8% vs. 5.0%, p<0.001) and cardiovascular (CV) medication use (0.59% vs. 0.27%, p=0.002 and 37.0% vs. 3.6%, p<0.001) were substantially higher in the T1DM cohort compared with the reference cohort one year before and 20 years after the index date, respectively. Moreover, a substantial number of children in the T1DM cohort was not treated with medication for at least a period of one year after the index date even though they were hypertensive (76%) or had diagnosis of hypercholesterolemia (98%). Of the available determinants only age was associated with undertreated hypertension in the T1DM cohort (p=0.04).

In ***Chapter 2.3***, ***asthma related medication use and exacerbations in children and adolescents with T1DM****, b*etween 1999 and 2009, and applying the same study design and database as Chapter 2.1, we investigated asthma medication use and the occurrence of asthma exacerbations up to 5 years before and after the onset of T1DM in children younger than 19 years. Our findings showed that the prevalence and incidence rates of asthma medication use in the T1DM cohort (23.2% and 7.8 per 1000 person-year, respectively) was significantly higher than the reference cohort (18.3% and 6.8 per 1000 person-year, respectively) one year after the onset of diabetes. No statistically significant difference between the two cohorts was observed in the use of specific types of asthma medication e.g. long acting beta agonists and short acting beta agonists except for short acting muscarinic antagonists that were significantly more used in the T1DM cohort (5.5%) compared with the reference cohort (0.62%) after the onset of diabetes. Our study also showed that the incidence rate of asthma medication use declined over time with a peak in the T1DM cohort the first year after the onset of diabetes.

In ***Chapter 3***, ***risk factors associated with asthma/allergy in children***, we explored the role of risk factors including genetic and environmental factors (antibiotics consumption and breast-feeding) on the development of childhood asthma/asthma exacerbations and allergy. Several high-risk alleles that are strongly associated with respiratory disorders have been recently studied in genome-wide association studies (GWASs). Furthermore, there are indications that gut microbiome plays a role in modifying the risk of asthma and allergy. Reduced gut microbial diversity during the first months of life influences immune function leading to an imbalanced ratio of T helper type 1 and T helper type 2 cells, and

therefore, might increase the risk of allergic responses. However, the conclusions from epidemiological studies are inconsistent.

In ***Chapter 3.1***, ***genetic variation and the association with asthma and lung function in children: results of the WHISTLER cohort study,*** we aimed to assess if genetic variation that is associated with adult asthma onset and lung function is also associated with the susceptibilities to these diseases in children. In this study, we used the wheezing illnesses study (WHISTLER) cohort, which is a prospective population-based birth cohort study on determinants (including early life lung function) and prediction of wheezing illnesses. Two "genetic risk scores" (GRSs) that are also called allele scores, gene scores or genotype scores were constructed based on single nucleotide polymorphisms (SNPs) previously found to be associated with adult asthma and lung function separately; such that a higher score was associated with a higher risk of asthma and lung function. Medical record and parental-report of physician-diagnosis asthma in any time during the course of follow-up (2001-2014) was used to define asthma cases in this study. Airway resistance (R_{rs}) and compliance (C_{rs}) of the pulmonary system were measured before the age of 2 months; interrupter resistance (Rint) was assessed at the age of 5 years. It appeared in 1,249 children that that the asthma GRS was strongly associated with the onset of asthma disease before age 8 years. The hazard ratio (HR) was 1.71, 95% confidence interval (CI): 1.16-2.51. Children with GRS≥2 (median GRS is 2) were estimated to have a significant higher probability of asthma compared with those with GRS below 2 (log rank p=0.03). Furthermore, genetic variation associated with adult lung function was not associated with lung function in neonates and young children (n=481). A higher lung function GRS was associated with lower C_{rs} (-6.32 mL · kPa-1/score point, 95% CI: -11.5; -1.10, p = 0.018). However, this association was no longer statistically significant after including age and gender in the model (-4.29 kPa-1/score point, 95%CI: -9.36; 0.78, p = 0.0975). Moreover, the lung function GRS was not associated with Rint at age 5 years.

In ***Chapter 3.2***, ***use of antibiotics in early childhood and the risk of asthma onset/ asthma exacerbations,*** we studied the effect of early life exposure to antibiotics (as a marker for influence on the gut microbiome) on asthma susceptibility and asthma exacerbations. Using four different databases including Generation R (n=7,393), SEATON (n=924), PACMAN (n=674) and BREATHE (n=806), our findings showed that early life (first three years of life) exposure to antibiotics was statistically significantly associated with subsequent asthma in a meta-analysis of the Generation R and SEATON data (OR: 2.18, 95% CI: 1.04-4.60; I^2: 76.3%). However, there was no statistically significant association between antibiotics consumptions and risk of asthma exacerbations later in life in a meta-analysis of the PACMAN and BREATHE data (OR: 0.93, 95% CI: 0.65-1.32; I^2: 0.0%).

In ***Chapter 3.3***, ***early life antibiotics exposure increases the risk of developing allergic symptoms later in life: A meta-analysis,*** the role of early life antibiotic exposures on risk of allergy including hay fever, eczema, food allergy or atopy including positive skin

prick testing (SPT) or elevated allergen-specific serum/plasma IgE levels later in life was studied. Epidemiologic studies evaluating the association between early life antibiotics use and the risk of allergy/atopy later in life are conflicting. For this, PubMed and Web of Science databases were searched for observational studies published from January 1966 through November 11, 2015 to perform a meta-analysis. Overall pooled estimates of the ORs were obtained using fixed or random-effects models. Our findings in this study revealed that early life exposure to antibiotics is statistically significantly associated with increased risk of hay fever (OR: 1.23, 95% CI: 1.13-1.34; I^2: 77.0%) in 21 studies, eczema (OR: 1.26, 95% CI: 1.15-1.37; I^2: 74.2%) in 22 studies and food allergy (OR: 1.42, 95% CI: 1.08-1.87; I^2: 80.8%) in 3 studies. However, no association was found for antibiotics exposure during early in life and objective atopy measurements including positive SPT or elevated allergen-specific serum/plasma IgE levels.

In ***Chapter 3.4, breast-feeding is associated with a decreased risk of asthma exacerbations later in life,*** the effect of breast-feeding on asthma severity including poor asthma control (ACQ≥0.75) and asthma exacerbations (measured by oral corticosteroids use and/or emergency department visits due to asthma) was studied. Epidemiological studies reported inconsistent results for the relation between breast-feeding and risk of asthma/allergies. Moreover, the association between breast-feeding and asthma severity was not already studied. Using the PACMAN database a large cohort of pediatric asthma medication users (n=960), we found that breast-feeding was statistically significantly associated with a decreased risk of asthma exacerbations later in childhood (OR: 0.59, 95% CI: 0.38-0.93). After stratification for duration of breast-feeding the ORs were 0.53, 95% CI: 0.31-0.91 for <6 months and 0.68, 95% CI: 0.41-1.12 for ≥6 months.

In ***Chapter 3.5, childhood obesity in relation to poor asthma control and exacerbations- A meta-analysis,*** was studied. Studies that reported about this association were conflicting and therefore, in a meta-analysis including 14 studies we assessed whether there is association between obesity and poor asthma control or risk of exacerbations in asthmatic children, and also whether gender modified these associations. Our findings in this study revealed that obesity was associated with an increased risk of asthma exacerbations in children and young adults with asthma (n=46,070); OR: 1.17, 95% CI: 1.03-1.34; I^2:54.7%. Obese children appeared not to have poorer asthma control compared to non-obese peers (n=4,973); OR: 1.23, 95% CI: 0.99-1.53; I^2: 0.0%. Gender did not modify this effect; after stratification by gender the effect estimates for girls and boys were similar.

In ***Chapter 4***, ***the general discussion***, we discussed our key findings in comparison with literature in a broader context, the strengths and limitations of the studies described in this thesis and finally implications for future research and for clinical practice.

Chapter 5.2 Samenvatting

Wereldwijd neemt het aantal kinderen dat opgroeit met een chronische ziekte toe. Chronische ziekten zijn een belangrijke oorzaak van morbiditeit en mortaliteit. Epidemiologische gegevens van type1 diabetes mellitus (T1DM), astma en allergie op kinderleeftijd zijn goed gedocumenteerd, echter er zijn relatief weinig studies verricht naar de risicofactoren, complicaties en behandeling van deze ziekten.

De doelstellingen van de studies in dit proefschrift zijn als volgt:
1) Evaluatie van co-morbiditeit en co-medicatie bij kinderen met T1DM waaronder met name risicofactoren van hart- en vaatziekten (HVZ) en astma
2) Risicofactoren (genetische- en omgevingsfactoren) van astma , astma-exacerbaties en allergie bij kinderen

In ***hoofdstuk 1***, ***de algemene inleiding***, is een overzicht gegeven van de epidemiologie, risicofactoren en complicaties van een aantal chronische ziekten (T1DM, asthma en allergie) bij kinderen.
Bij het ontstaan van deze ziekten spelen genetische factoren in interactie met omgevingsfactoren een rol. T1DM, astma en allergie zijn complexe ziekten waarvoor continu medische zorg nodig is. Om complicaties op latere leeftijd zoveel mogelijk te vermijden zijn vroege herkenning en adequate behandeling belangrijk.

In ***hoofdstuk 2***, ***trends in co-morbiditeit gerelateerd aan T1DM bij kinderen***, is onderzocht in welke mate kinderen met T1DM een hoger risico hebben op risicofactoren van HVZ, namelijk hypertensie (hoge bloeddruk) en hypercholesterolemia (verhoogd cholesterol), en HVZ (zoals hartinfarct) in vergelijking met kinderen van dezelfde leeftijd en geslacht maar zonder T1DM. Deze studies werden uitgevoerd in twee verschillende databases; PHARMO in Nederland (***hoofdstuk 2.1***) en CPRD in het Verenigd Koninkrijk (***hoofdstuk 2.2***).

In ***hoofdstuk 2.1***, ***HVZ-medicatie en HVZ bij kinderen en adolescenten met T1DM*** is een cohortonderzoek beschreven. In een retrospectief cohortonderzoek tussen 1999 en 2009 werden de 5-jaars prevalentie en incidentie van HVZ medicatie en HVZ vóór en na de diagnose diabetes bij kinderen <19 jaar met T1DM (gedefinieerd als kinderen met ten minste twee insuline voorschriften) (n=925) onderzocht. De resultaten van deze kinderen werden vergeleken met een vier keer groter controle cohort (n=3,591) van dezelfde leeftijd en geslacht. Per kind met T1DM en de gematchte controles werd de datum van de eerste insuline afgifte geselecteerd als de index datum. Onze resultaten toonden een statistisch significante hogere prevalentie van HVZ medicatie in de kinderen met T1DM ten opzichte van de controle groep zowel voor (2,2% versus 1,0%, p <0,001) als na (9,2% versus. 3,2%, p <0,001) de index datum. Na de indexdatum bleken ACE-remmers (2,0%) en statines (1,5%) de meest gebruikte HVZ medicatie bij de kinderen met T1DM. De hoogste incidentie van HVZ medicatie werd waargenomen in het eerste jaar na de index datum (28,1 per 1000 persoonsjaren). Zoals verwacht bleek in beide groepen dat het aantal kinderen dat in een ziekenhuis opgenomen was voor HVZ zeer laag.

In ***hoofdstuk 2.2***, ***tijdtrends in de epidemiologie van HVZ risicofactoren, ziekten en medicatie gebruik bij kinderen en adolescenten met T1DM: een CPRD studie***, wordt met behulp van dezelfde studie opzet als de PHARMO-studie, maar in een andere populatie (CPRD) in een ongeveer 5-maal grotere steekproef (n=22,241) een vergelijkbare studie beschreven. De lange termijn trends in HVZ risicofactoren, HVZ en de medicamenteuze behandeling van HVZ werden vanaf 2 jaar voorafgaand aan de diagnose T1DM tot 20 jaar na deze diagnose vergeleken met kinderen zonder diabetes. Tevens werden onderbehandeling van hypertensie en hypercholesterolemia en determinanten van deze onderbehandeling bestudeerd. De deelnemers werden gevolgd vanaf 1 januari 1987 tot en met het einde van de studie periode (30 oktober 2015). De bevindingen van deze studie toonden aan dat bij T1DM kinderen versus de controle kinderen de jaarlijkse prevalenties van hypertensie (0,64% versus 0,34%, p=0,007 en 35,2% versus 11,4%, p <0,001), hypercholesterolemie (0,91% versus 0,05%, p <0,001 en 64,8% versus 5,0%, p <0,001) en HVZ medicijngebruik (0,59% versus 0,27%, p=0,002 en 37,0% versus 3,6%, p <0,001), respectievelijk 1 jaar voorafgaand aan de diagnose T1DM en 20 jaar na de diagnose aanzienlijk hoger waren. Tevens bleek dat een aanzienlijk aantal kinderen met T1DM gedurende ten minste een periode van een jaar medicamenteus onbehandeld te zijn voor hypertensie (76%) en hypercholesterolemie (98%). Van de determinanten die in de database beschikbaar waren om het verband met onderbehandeling te bestuderen, bleek alleen leeftijd geassocieerd met onderbehandelde hypertensie (p=0,04).

In ***hoofdstuk 2.3***, ***astma gerelateerde medicatie gebruik en astma exacerbaties bij kinderen en adolescenten met T1DM***, is een studie beschreven waarin het gebruik van astma medicatie en het optreden van astma exacerbaties de 5 jaar voorafgaand aan de diagnose T1DM bij kinderen <19 jaar tot aan 5 jaar na deze diagnose is vergeleken met een controle groep kinderen zonder diabetes gedurende de kalender periode 1999-2009. Het bleek dat de prevalentie en incidentie van astma medicatie gebruik gedurende het eerste jaar na de diabetesdiagnose bij de T1DM kinderen (23,2% en 7,8 per 1000 persoonsjaren, respectievelijk) statistisch significant hoger waren dan in de controlegroep (18,3% en 6,8 per 1000 persoonsjaren, respectievelijk). Geen statistisch significante verschillen werden tussen de twee groepen kinderen waargenomen voor wat betreft het gebruik van specifieke astmamedicatie bijvoorbeeld de langwerkende beta-agonisten of kortwerkende beta-agonisten behalve de kortwerkende muscarine antagonisten die aanzienlijk meer gebruikt werden bij de kinderen met T1DM (5,5% versus 0,62%). De studie toonde ook aan dat de incidentie van astma medicatie bij de T1DM kinderen na een piek in het eerste jaar na de diagnose daalde.

In ***hoofdstuk 3***, ***risicofactoren van astma en allergie bij kinderen***, staat een studie beschreven waarin risicofactoren, waaronder genetische en omgevingsfactoren (antibiotica consumptie en borstvoeding) van astma, astma-exacerbaties en allergie werden bestudeerd. Recent is uit genoombrede associatiestudies (GWAS) gebleken dat verschillende hoog-risico-allelen sterk geassocieerd zijn met aandoeningen van de luchtwegen. Tevens zijn er aanwijzingen dat de dikkedarm flora (de bacterieën die in de dikkedarm

groeien) een rol spelen bij het ontwikkelen van astma en allergie. Een afgenomen diversiteit van de bacterieën in de dikke darm gedurende de eerste levensmaanden leidt tot veranderingen in het immuunsysteem, met name een ongunstige verhouding van de T-helper type 1 en T-helper type 2 cellen (deze cellen spelen een rol in het afweersysteem), en kan hierdoor bijdragen aan een verhoogd risico op allergische reacties. Echter, de resultaten van gepubliceerde epidemiologische studies zijn tegenstrijdig.

In ***hoofdstuk 3.1***, ***genetische variatie en de associatie met astma en longfunctie bij kinderen: resultaten van de Whistler cohort studie***, staat een studie beschreven waarin werd bestudeerd of genetische variatie die bij volwassen samenhangt met het ontwikkelen van astma of longfunctie ook een rol spelen bij kinderen voor wat betreft hun gevoeldigheid voor astmaontwikkeling en longfunctie. In deze studie werd gebruik gemaakt van het WHISTLER cohort, waarin een groep kinderen vanaf jonge leeftijd wordt gevolgd ter bestudering van risicofactoren voor de ontwikkeling van zieken met een piepende ademhaling. Twee genetische risico scores (GRSs) werden gebouwd op basis van mutaties in het DNA die eerder afzonderlijk in verband zijn gebracht met astma en longfunctie bij volwassenen. De scores werden zo gebouwd dat een hogere score samenhangt met een hoger risico op astma of een afgenomen longfunctie. In deze studie werd aangenomen dat een kind astma heeft indien deze diagnose gedurende de observatietijd (2001-2014) in het medisch dossier werd teruggevonden en/of door de ouders in een enquete werd genoemd. Luchtwegweerstand uitgedrukt in R_{rs} en de mate van rekbaarheid (compliantie) van de longen uitgedrukt in C_{rs} werden gemeten vóór de leeftijd van 2 maanden; een andere luchtwegweerstandstest uitgedrukt in Rint werd op 5-jarige leeftijd gemeten. Het bleek op basis van 1249 kinderen dat de astma-GRS sterk samenhangt met de ontwikkeling van astma voor de leeftijd van 8 jaar. Het relatieve risico, uitgedrukt in de hazard ratio (HR), was 1,71, 95% betrouwbaarheidsinterval (BI): 1,16-2,51 hetgeen betekent dat als de score met een punt stijgt het risico op astma met 71% toeneemt. Ook bleek indien de GRS in twee groepen werd verdeeld dat kinderen met een mediane GRS van ≥2 een significant hogere kans op astma hadden in vergelijking met kinderen met een GRS <2 (log rank p=0,03). Verder bleek bij 481 jonge kinderen dat de genetische variatie die bij volwassenen samenhangt met longfunctie bij kinderen hier niet mee samenhangt. Een hogere longfunctie-GRS die zonder correctie van verstorende variabelen statistisch significant samenhing met een lagere C_{rs} (-6,32 ml · kPa-1/score punt, 95% BI: -11,5;-1,10; p=0,018) bleek niet meer significant samen te hangen na correctie voor leeftijd- en geslachtsverschillen (-4,29 kPa-1/score punt, 95% BI: -9,36; 0,78; p=0,0975). Bovendien bleek de longfunctie-GRS niet samen te hangen met Rint op 5-jarige leeftijd.

In ***hoofdstuk 3.2***, ***gebruik van antibiotica op jonge leeftijd en het risico van astma en astma exacerbaties***, staat de studie beschreven waarin het effect van gebruik van antibiotica op jonge leeftijd (als marker voor een verandering van de bacteriële flora in de dikke darm) op het ontstaan van astma en astma exacerbaties werd bestudeerd. Voor het onderzoek werd gebruik gemaakt van vier verschillende databases, namelijk Generation R (n=7,393), SEATON (n=924), PACMAN (n=674) en BREATHE (n=806). In

een meta-analyse op basis van de Generation R en SEATON gegevens bleek dat antibiotica gebruik gedurende de eerste drie levensjaren statistisch significant samenhangt met de ontwikkeling van astma op latere leeftijd (odds ratio (OR): 2,18, 95% BI: 1,04-4,60; I^2:76,3%). Op basis van de PACMAN en BREATHE gegevens bleek er echter geen statistisch significant verband tussen antibioticagebruik op jonge leeftijd en het optreden van astma exacerbaties bij kinderen met astma (OR: 0,93; 95% BI: 0,65-1,32; I^2: 0,0%).

In ***hoofdstuk 3.3***, ***blootstelling aan antibiotica op jonge leeftijd verhoogt het risico op het ontwikkelen van allergische symptomen op latere leeftijd: een meta-analyse,*** staat een studie beschreven waarin de samenhang van antibiotica op jonge leeftijd met verschillende uitingen van allergieën zoals hooikoorts, eczeem, voedselallergie of atopie met inbegrip van positieve huidpriktest (SPT) of verhoogde allergeen-specifieke serum/plasma IgE spiegels op latere leeftijd werden bestudeerd. Eerder zijn tegenstrijdige resultaten gepubliceerd over de relatie tussen antibioticagebruik op jonge leeftijd en de latere ontwikkeling van allergie en/of atopie. Alle relevante publicaties gepubliceerd vanaf januari 1966 tot 11 november 2015 werden achterhaald en een meta-analyse werd uitgevoerd. Het bleek na statistische samenvatting van de studies dat blootstelling aan antibiotica op jonge leeftijd statistisch significant samenhangt met hooikoorts (OR: 1,23; 95% BI: 1,13-1,34; I^2: 77,0% op basis van 21 studies), eczeem (OR: 1,26, 95% BI: 1,15-1,37; I^2: 74,2%, in 22 studies) en voedselallergie (OR: 1,42, 95% BI: 1,08-1,87; I^2: 80,8%, in drie studies). Er werd echter geen verband gevonden tussen het gebruik van antibiotica en objectief gemeten biomarkers van atopie namelijk een positieve SPT of verhoogde allergeen-specifieke serum/plasma IgE spiegels.

In ***Hoofdstuk 3.4***, ***borstvoeding verlaagt het risico op astma-exacerbaties op latere leeftijd***, staat een studie beschreven waarin het effect van borstvoeding op de ernst van astma vastgesteld met een gevalideerd meetinstrument (matige astmacontrole indien de ACQ≥0.75 is) en astma exacerbaties (gemeten door middel van het gebruik van orale corticosteroïden en/of bezoek van een eerstehulp afdeling in een ziekenhuis vanwege astma) werden bestudeerd. Gepubliceerde epidemiologische studies laten wisselende resultaten zien voor wat betreft de relatie tussen borstvoeding en het risico op astma/allergieën. Verder was het verband tussen borstvoeding en de ernst van astma nog niet eerder onderzocht. Met behulp van de PACMAN databank bleek binnen een grote groep van kinderen die allemaal gebruik maakten van astma-medicatie (n=960), dat borstvoeding statistisch significant samenhangt met een lager risico op astma exacerbaties op latere kinderleeftijd (OR: 0,59; 95% BI: 0,38-0,93). Na opsplitsing voor de duur van de borstvoeding waren de OR: 0,53; 95% BI: 0,31-0,91 voor korter dan 6 maanden en 0,68; 95% BI: 0,41-1,12 voor langer dan 6 maanden.

In ***hoofdstuk 3.5***, ***het verband tussen obesitas bij kinderen en matige astmacontrole en astma exacerbaties - een meta-analyse***, staat een studie beschreven naar het verband tussen obesitas en astma. Eerder waren 14 studies gepubliceerd naar dit verband waarbij tegenstrijdige resultaten werden gerapporteerd. Dit vormde de aanleiding om op

basis van deze studies een meta-analyse te verrichten. Het bleek dat obesitas samenhing met een hoger risico op astma exacerbaties bij kinderen en jong volwassenen met astma (n=46,070); OR: 1,17; 95% BI: 1,03-1,34; I^2: 54,7%. Obese kinderen bleken een matigere astma controle te hebben in vergelijking met niet-obese kinderen (n=4,973); OR: 1,23; 95% BI: 0,99-1,53; I^2: 0,0%. De resultaten waren voor jongens en meisjes vergelijkbaar (geen effectodificatie).

In ***hoofdstuk 4***, ***de algemene discussie***, worden de belangrijkste bevindingen van dit proefschrift gepresenteerd, in de context gebracht met bestaande literatuur, de sterke en zwakke punten van de studies besproken en ten slotte de implicaties hiervan voor toekomstig onderzoek en de klinische praktijk besproken.

In ***Hoofdstuk 3.4***, ***borstvoeding verlaagt het risico op astma-exacerbaties op latere leeftijd***, staat een studie beschreven waarin het effect van borstvoeding op de ernst van astma vastgesteld met een gevalideerd meetinstrument (matige astmacontrole indien de ACQ≥0,75 is) en astma exacerbaties (gemeten door middel van het gebruik van orale corticosteroïden en/of bezoek van een eerstehulp afdeling in een ziekenhuis vanwege astma) werden bestudeerd. Gepubliceerde epidemiologische studies laten wisselende resultaten zien voor wat betreft de relatie tussen borstvoeding en het risico op astma/allergieën. Verder was het verband tussen borstvoeding en de ernst van astma nog niet eerder onderzocht. Met behulp van de PACMAN databank bleek binnen een grote groep van kinderen die allemaal gebruik maakten van astma-medicatie (n=960), dat borstvoeding statistisch significant samenhangt met een lager risico op astma exacerbaties op latere kinderleeftijd (OR: 0,59; 95% BI: 0,38-0,93). Na opsplitsing voor de duur van de borstvoeding waren de OR: 0,53; 95% BI: 0,31-0,91 voor korter dan 6 maanden en 0,68; 95% BI: 0,41-1,12 voor langer dan 6 maanden.

In ***hoofdstuk 3.5***, ***het verband tussen obesitas bij kinderen en matige astmacontrole en astma exacerbaties - een meta-analyse***, staat een studie beschreven naar het verband tussen obesitas en astma. Eerder waren 14 studies gepubliceerd naar dit verband waarbij tegenstrijdige resultaten werden gerapporteerd. Dit vormde de aanleiding om op basis van deze studies een meta-analyse te verrichten. Het bleek dat obesitas samenhing met een hoger risico op astma exacerbaties bij kinderen en jong volwassenen met astma (n=46,070); OR: 1,17; 95% BI: 1,03-1,34; I^2: 54,7%. Obese kinderen bleken een matigere astma controle te hebben in vergelijking met niet-obese kinderen (n=4,973); OR: 1,23, 95% BI: 0,99-1,53; I^2: 0,0%. De resultaten waren voor jongens en meisjes vergelijkbaar (geen effectodificatie).

In ***hoofdstuk 4***, ***de algemene discussie***, worden de belangrijkste bevindingen van dit proefschrift gepresenteerd, in de context gebracht met bestaande literatuur, de sterke en zwakke punten van de studies besproken en ten slotte de implicaties hiervan voor toekomstig onderzoek en de klinische praktijk besproken.

Chapter 5.3 Acknowledgments

Looking back to the year of 2012, I see myself lost in academia. After many years working as a pharmacist and expertise in the Ministry Of Health in Iran, I decided to start a new journey in a new country, far away from home, and in a new field of science; doing research.
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Utrecht University, 24th of October 2016
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Chapter 5.5 List of publications

LIST OF PUBLICATIONS RELATED TO THIS THESIS:

Fariba Ahmadizar, Soulmaz Fazeli Farsani, Patrick C. Souverein, Marja M. J. van der Vorst, Anthonius de Boer, Anke H. Maitland-van der Zee. *Cardiovascular medication use and cardiovascular disease in children and adolescents with type 1 diabetes: a population-based cohort stud.* Pediatr diabetes, 2015 Aug; 17(6): 433-40. DOI: 10.1111/pedi.12302.

Fariba Ahmadizar, Patrick C. Souverein, Hubertus G. M. Arets, Anthonius de Boer, Anke H. Maitland-van der Zee. *Asthma related medication use and exacerbations in children and adolescents with type 1 diabetes*. Pediatr pulmonol, 2016 May; 1-9. DOI: 10.1002/ppul.23428.

Fariba Ahmadizar, Susanne J. H. Vijverberg, Hubertus G. M. Arets, Anthonius de Boer, Jason E. Lang, Meyer Kattan, Colin N.A. Palmer, Somnath Mukhopadhyay, Steve Turner, Anke H. Maitland-van der Zee. *Childhood obesity in relation to poor asthma control and exacerbations- A meta-analysis*. Eur Respir J*,* 2016, Sep; 1-11. DOI: 10.1183/13993003.00766-2016.

PAPERS CURRENTLY UNDER REVIEW:

Fariba Ahmadizar, Patrick Souverein**,** Anthonius de Boer**,** Anke H. Maitland-van der Zee. *Time trends in epidemiology of cardiovascular risk factors, diseases and medication use in children and adolescents with type 1 diabetes: a CPRD study.*

Fariba Ahmadizar, M. Leusink, Ali Arabkhazaeli, Susanne J. H. Vijverberg, Geertje W. Dalmeijer, Anthonius de Boer, C.S. Uiterwaal, C.K. van der Ent, N.C. Onland-Moret, A.H. Maitland-van der Zee. *Genetic variation and the association with asthma and lung function in children: results of the WHISTLER cohort study.*

Fariba Ahmadizar, Susanne J. H. Vijverberg, Hubertus G. M. Arets, Anthonius de Boer, Steve Turner, Graham Devereux, Ali Arabkhazaeli, Patricia Soares, Somnath Mukhopadhyay, Johan Garssen, Colin N.A. Palmer, Liesbeth Duijts, Evelien R. van Meel, Aletta D. Kraneveld, Anke H. Maitland-van der Zee. *Use of antibiotics in early childhood and the risk of asthma onset/asthma exacerbations.*

Fariba Ahmadizar, Susanne J. H. Vijverberg, Hubertus G. M. Arets, Anthonius de Boer, Jason E. Lang, Johan Garssen, Aletta Kraneveld, Anke H. Maitland-van der Zee. *Early life antibiotics exposure increases the risk of developing allergic symptoms later in life: A meta-analysis.*

Fariba Ahmadizar, Susanne J. H. Vijverberg, Hubertus G. M. Arets, Anthonius de Boer, Johan Garssen, Aletta D. Kraneveld, Anke H. Maitland-van der Zee. *Breast-feeding is associated with a decreased risk of asthma exacerbations later in life.*

LIST OF PUBLICATIONS NOT-RELATED TO THIS THESIS:

Nooshin Mohammad Hosseini, Fatemeh Soleymani, **Fariba Ahmadizar.** *The amazing world of bacteria*. 2008, Andishe mandegar, Book in Persian.

Fatemeh Soleymani, **Fariba Ahmadizar**, Fanak Fahimi. *Drug and Therapeutic Committee.* 2009, Andishe mandegar, Book in Persian.

Fariba Ahmadizar, Nasrin Khoshnevis, Naser Hadavand. *Practical Information in Pharmacy for pharmacists*. 2010, Hayyan, Tehran, Book in Persian.

Fariba Ahmadizar, Fatemeh Soleymani, Mohammad Abdollahi. *Study of Drug-Drug Interactions in Prescriptions of General Practitioners and Specialists in Iran 2007-2009*. Iran J Pharm Res. 2011;10 (4):921-31.

Nasrin Khoshnevis **Fariba Ahmadizar**, Mahtab Alizadeh, Mohammad Esmail Akbari. *Nutritional Assessment of Cancer Patients in Tehran, Iran.* Asian Pac J Cancer Prev. 2012: 13(4):1621-6.

Fatemeh Soleymani, **Fariba Ahmadizar**, Alipasha Meysamie, Mohammad Abdollahi. *A survey on the factors influencing the pattern of medicine's use: Concerns on irrational use of drugs*. J Res Pharm Pract. 2013; 2 (2):59-63.

Fariba Ahmadizar, N.Charlotte Onland-Moret, Anthonius de Boer, Geoffrey Liu, Anke H. Maitland-van der Zee. *Efficacy and safety assessment of the addition of bevacizumab to adjuvant therapy agents in cancer patients: A Systematic review and Meta-analysis of Randomized Controlled Trials***.** PLoS One. 2015 Sep; 2;10 (9). DOI: 10.1371.

Chapter 5.6 About the author

Originally from Iran, Fariba received the doctorate of pharmacy degree in 1999 from Shahid Beheshti University of Medical Sciences and Health Services (SBUM) in Tehran. She joined the Food and Drug Organization (FDO) immediately after graduation where she has worked in the Rational Use of Drug (RUD) committee. She worked about 3 years in community and hospital pharmacies in addition to her own pharmacy. She also worked as a lecturer on pharmacology in the Kurdistan University of Medical Sciences and in the RUD for almost 4 years.

Fariba has awarded a scholarship from the exceptional talents program from the Iranian Ministry Of Health; an exclusive honor to study PhD abroad. She started her PhD program at the division of Pharmacoepidemiology and Clinical Pharmacology under the supervision of Prof. dr. Anthonius de Boer and Prof. dr. Anke-Hilse Maitland-van der Zee in 2012. In 2015, she also started the Master of Epidemiology at the University Medical Center Utrecht; part of the program was a research project related to the risk of asthma in children with type 1 diabetes.

During her PhD training she has been working on several projects related to effectiveness and safety of drugs in adult patients with cancer as well as risk factors and treatment of pediatric chronic disorders including type 1 diabetes, asthma and allergy. Furthermore, she has presented her studies at international congresses (ICPE, EAACI and ERS). As a PhD student she also supervised a master student with a project entitled “Pharmacogenetic associations of IL1RL1 variants with asthma severity in Dutch children on ICS treatment.

Throughout the course of her PhD studies, Fariba obtained increasing research independence and is now ready to pursue her own research project on difficult to treat childhood type 1 and type 2 diabetes.