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Identifying causes and consequences of allergic disease, asthma, and lung function

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Identifying causes and consequences of allergic disease, asthma, and lung function

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Ulla Wewer, Head of Faculty

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Preface

The work underlying this thesis was carried out from 2014–2017 at the Research Centre for Prevention and Health (RCPH). The Mendelian Randomization studies were performed in collaboration with the Novo Nordisk Foundation Center for Basic Metabolic Research, Section on Metabolic Genetics, University of Copenhagen, Denmark, and the Medical Research Council Integrative Epidemiology Unit, University of Bristol, United Kingdom.

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List of papers

I. Skaaby T, Taylor AE, Jacobsen RK, Paternoster L, Thuesen BH, Ahluwalia TS, Larsen SC, Zhou A, Wong A, Gabrielsen ME, Bjørngaard JH, Flexeder C, Männistö S, Hardy R, Kuh D, Barry SJ, Tang Møllehave L, Cerqueira C, Friedrich N, Bonten TN, Noordam R, Mook-Kanamori DO, Taube C, Jessen LE, McConnachie A, Sattar N, Upton MN, McSharry C, Bønnelykke K, Bisgaard H, Schulz H, Strauch K, Meitinger T, Peters A, Grallert H, Nohr EA, Kivimaki M, Kumari M, Völker U, Nauck M, Völzke H, Power C, Hyppönen E, Hansen T, Jørgensen T, Pedersen O, Salomaa V, Grarup N, Langhammer A, Romundstad PR, Skorpen F, Kaprio J, R Munafò M, Linneberg A. Investigating the causal effect of smoking on hay fever and asthma: a Mendelian randomization meta-analysis in the CARTA consortium. Sci Rep. 2017 May 22;7(1):2224.

II. Skaaby T, Taylor AE, Jacobsen RK, Tang L, Friedrich N, Thuesen BH, Shabanzadeh DM, Paternoster L, Völker U, Nauck M, Völzke H, Munafò M, Hansen T, Pedersen O, Jørgensen T, Grarup N, Linneberg A. Associations of genetic determinants of vitamin B12 and folate status with hay fever and asthma: a Mendelian randomization meta-analysis. European Journal of Clinical Nutrition [Accepted 16 October 2017].

III. Skaaby T, Taylor AE, Thuesen BH, Jacobsen RK, Friedrich N, Møllehave LT, Hansen S, Larsen SC, Völker U, Nauck M, Völzke H, Hansen T, Pedersen O, Jørgensen T, Paternoster L, Munafò M, Grarup N, Linneberg A. Estimating the causal effect of body mass index on hay fever, asthma and lung function using Mendelian randomization. Allergy. 2017 Jul 4. [Epub ahead of print]

IV. Skaaby T, Husemoen LL, Thuesen BH, Fenger RV, Linneberg A. IgE sensitization to inhalant allergens and the risk of airway infection and disease: A population-based study. PLoS One. 2017 Feb 9;12(2):e0171525.

V. Skaaby T, Husemoen LL, Thuesen BH, Jørgensen T, Linneberg A. Lifestyle-Related Factors and Atopy in Seven Danish Population-Based Studies from Different Time Periods. PLoS One. 2015 Sep 15;10(9):e0137406.

VI. Skaaby T, Husemoen LL, Thuesen BH, Fenger RV, Linneberg A. Specific IgE positivity against inhalant allergens and development of autoimmune disease. Autoimmunity. 2015;48(5):282-8.

VII. Skaaby T, Nystrup Husemoen LL, Roswall N, Thuesen BH, Linneberg A. Atopy and development of cancer: a population-based prospective study. J Allergy Clin Immunol Pract. 2014 Nov-Dec;2(6):779-85.

VIII. Skaaby T, Husemoen LL, Thuesen BH, Hammer-Helmich L, Linneberg A. Atopy and cause-specific mortality. Clin Exp Allergy. 2014 Nov;44(11):1361-70.

IX. Skaaby T, Husemoen LL, Thuesen BH, Jeppesen J, Linneberg A. The association of atopy with incidence of ischemic heart disease, stroke, and diabetes. Endocrine. 2015 Mar;48(2):541-50.

Introduction

The prevalence of allergic diseases and asthma has increased for more than 50 years in the industrialized world (1). Asthma is a chronic inflammatory disease of the airways prevalent in all age groups but often starts in childhood. It is characterized by recurrent attacks of wheezing and breathlessness which varying frequency and severity (2). During an attack, the lining of the bronchia swells, and the air flow into and out of the lungs is reduced (3). Allergic hay fever is characterized by an IgE-mediated inflammation of the nasal mucosa (4). Allergic sensitization refers to the production of allergen-specific immunoglobulin E (IgE). Individuals may produce IgE to allergens without developing allergic symptoms if exposed to the allergen.

The number of patients with asthma is currently 300 million, and asthma causes 250,000 deaths each year according to the World Health Organization (WHO) (5). With the increasing trend, the number of patients with asthma is expected to increase to 400 million by 2025 (5). Allergic hay fever affects 10–30% worldwide (1), whereas up to 40% of the populations are allergically sensitized to proteins in the environment (1). In Denmark, the prevalence of asthma has risen from 385,650 cases in 2011 to 427,050 cases in 2016: http://esundhed.dk/sundhedsregistre/uks/Sider/uks.aspx. Allergic hay fever is the most common chronic disease in young to middle aged adults in Denmark (6).

The financial costs associated with allergic disease and asthma are substantial and include both direct medical costs and indirect costs associated with work absence and premature deaths (4). Worldwide, the financial costs of asthma exceed the combined costs of tuberculosis and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (2). The costs of asthma range from $300–$1300 per patient per year in developed countries (5). Costs associated with asthma in the United States (US) increased 6% from $53 billion in 2002 to $56 billion in 2007 (7). In the US, the costs for treating hay fever have doubled to $11 billion in five years.

Patients suffering from allergic diseases and asthma have a reduced quality of life (5). The diseases may cause a significant burden to the patient and the family, and it may restricts the patient's activities for a lifetime (3). Asthma is a chronic condition, and asthmatics usually need continuous medical care (2). Recurrent asthma attacks often cause sleeplessness, fatigue, reduced activity and absence from school and work.

Strategies to tackle these issues have been ineffective so far (4). Since the reasons for the higher sensitization rates and progression of sensitization into allergic diseases are not clear, prevention is challenging (4). The rapid increase in allergic disease and asthma is likely to be explained by changes in environmental factors rather than genetic factors (8). Many lifestyle-related factors, and micronutrients have been proposed to contribute to the rise in allergic diseases and asthma, e.g., smoking (9), obesity (10), and low vitamin B12 and folate status (11). However, the epidemiological evidence to support or refute these hypotheses has been inconclusive. The major reasons for this are confounding and bias (e.g., reverse causation) inherent in observational epidemiological studies. Reverse causation is the case where an association is not explained by the risk factor causing the disease, but by the disease causing the risk factor, e.g., because the disease makes the person change the behavior. The application of the principle of Mendelian Randomization (MR) offers a potential solution to these issues (see next section).

It is well-known that allergic sensitization is associated with higher risk of allergic asthma, allergic rhinitis, atopic dermatitis, and food allergies, but allergic sensitization may have a broader role in various biological processes. The key mediator cells in allergic diseases, the mast cells, have a variety of immunologic functions. These range from the protective immunity against infection with bacteria, viruses, and parasites, and regulation of innate and adaptive immune response, to protection from or promotion of cardiovascular disease, obesity, diabetes, and cancer (12). Mast cells are present in increased numbers in atheromatous plaques, where they may

contribute to coronary artery spasm and plaque eruption (13;14), and many mast cell mediators are associated with diabetes mellitus (15;16). It is not clear whether allergic sensitization is associated with higher risk of these other diseases. In light of the high and increasing prevalence of sensitization, this is important to uncover.

Mendelian randomization

The Mendelian Randomization approach is a novel way of assessing and quantifying *causal effects* in observational studies. Mendelian randomization (MR) refers to the random allocation of alleles during meiosis (17). The MR-method relies on Mendel's Law of Segregation, i.e. allele-pairs separate randomly, and it involves the use of genetic variants that are associated with a specific exposure and are used as proxies or *instrumental variables* (IV) for the exposure. The allocation is assumed to be independent of lifestyle and environmental factors. Unlike a traditional observational study, this allows un-confounded risk estimations that are not explained by reverse causation (17). MR-studies take advantage of genetic differences in exposure to potential risk factors to determine unbiased estimates of their causal effects on disease. E.g., in a Mendelian Randomization study by Holmes et al, the previously misconceived association between alcohol intake and cardiovascular disease was investigated (18). The study found that persons with a genetic variant associated with lower alcohol intake had lower risk of coronary heart disease than persons without the genetic variant. This suggests in contrary to findings from traditional observational studies that *lower* alcohol intake is beneficial for cardiovascular health. This approach has been used for various exposures and diseases, but has barely been applied to allergic diseases and asthma.

The three main assumptions when using an IV are that it is associated with the exposure (the 'relevance' assumption), it is independent of unmeasured confounders (the 'exclusion' assumption), and it is independent of the outcome given the exposure and the

unmeasured confounders (the 'independence' assumption) (see figure 2) (19). This is illustrated by the directed acyclic graph (DAG) in Figure 2.

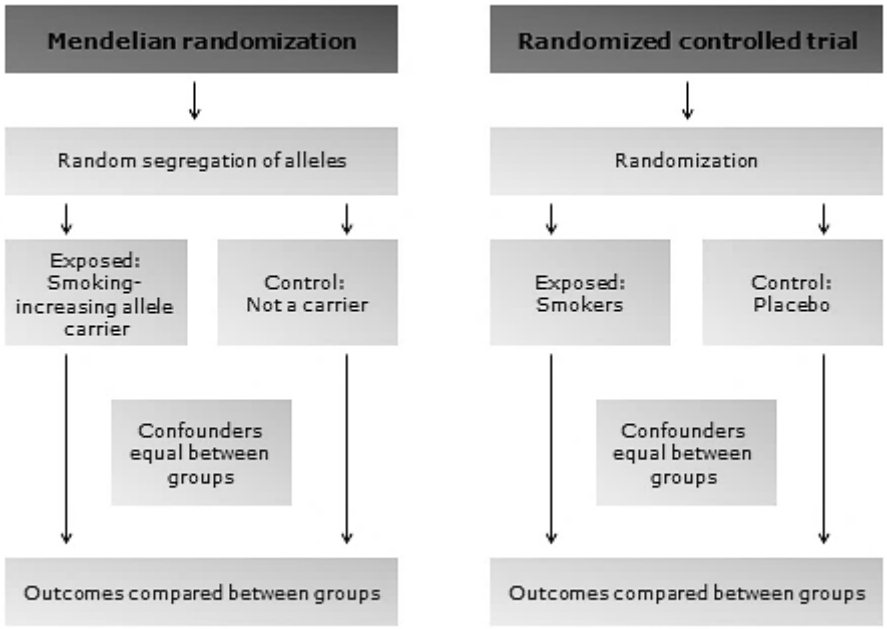


Figure 1. Comparison of a Mendelian Randomization study and a randomized controlled trial of smoking.

In lack of evidence from randomized controlled trials (RCTs), a Mendelian Randomization study can be used to strengthen causal inference. MR-studies are said to mimic randomized controlled trials (See Figure 1). The genetic variants associated with the exposure in question will not be affected by the onset of disease, so the estimates are not biased by reverse causation as mentioned above. A genetic variant is, as opposed to many phenotypes/exposure, often a measure of long-term exposure (20). The single-nucleotide polymorphisms (SNPs) are the most commonly used genetic markers. SNPs are DNA sequence variations in a single nucleotide in the genome.

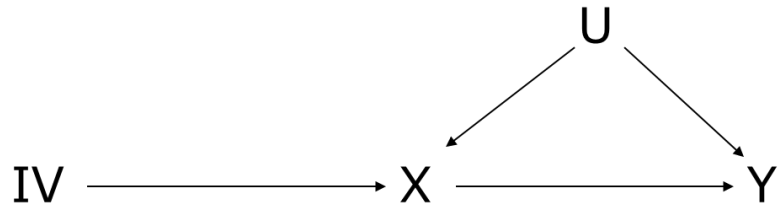


Figure 2. Directed acyclic graph of an instrumental variable (IV) from (21). Abbreviations: IV, instrumental variable; U, unmeasured confounders; X, exposure; Y, outcome.

Mendelian Randomization studies typically require a large number of participants since the genetic biomarkers in general only explain little of the variation of the exposures. There are different types of Mendelian randomization studies (22). Some provide evidence on whether or not a causal association exists, while others allow for the magnitude of the causal effect to be estimated. In the standard one-sample MR analysis, the SNP-exposure and SNP-outcome associations are measured in the same sample. In the two-sample MR approach, the SNP-exposure and SNP-outcome associations are estimated in non-overlapping samples (22).

Objectives

To examine lifestyle-related causes and consequences of allergic disease and asthma. The aims were:

1. To identify causes of allergic disease and asthma by performing a series of predominantly large-scale Mendelian Randomization studies
    1.1. Lifestyle-related factors and allergic sensitization
    1.2. Smoking and allergic disease and asthma
    1.3. Body mass index and allergic disease and asthma
    1.4. Levels of folate and vitamin B12 and allergic disease and asthma
2. To examine the health-related consequences of allergic sensitization
    2.1. Cardiovascular disease and diabetes
    2.2. Autoimmune disease
    2.3. Cancer
    2.4. Cause-specific mortality
    2.5. Airway infection and disease

Material and methods

Study populations

Study populations

Data from the general population studies examined at Research Centre for Prevention and Health (RCPH) (23), other Danish studies, and international studies were included. An overview of collaborating studies is shown in Table 1. I harmonized the eight studies originating from the RCPH and the study from the UK Biobank. The remaining studies were harmonized locally. All the studies included questionnaires, blood sampling, and physical examinations. Relevant variables are described below. The participants gave informed consent, and the studies were approved by the relevant Ethics Committees. The studies were performed according to the Declaration of Helsinki.

The UK Biobank Study is by far the largest included with its more than 500000 participants from across the United Kingdom and aged 40–69 years at recruitment in 2006–2010 (24). The study has both data from questionnaires, physical measures, sample assays, genome-wide genotyping (UK Biobank Axiom array (Affymetrix) or UK BiLEVE array) and longitudinal follow-up for a large number of health-related outcomes. More information can be found on the website: http://www.ukbiobank.ac.uk/.

In the studies of consequences of allergic sensitization (paper IV, VI, VII, VIII, IX), 14849 participants from five population-based studies performed at RCPH (Monica1, Inter99, Health2006, the 1936-cohort and Allergy98) were included. The study of lifestyle factors and allergic sensitization (paper V), in addition, used Health2008 and Health2010, also conducted at the RCPH, which meant a total of 20048 participants. The participants were recruited from the Danish Central Personal Register as random population samples. Descriptions of the studies in this area have previously been published (23;25;26).

I performed paper I in the consortium for Causal Analysis Research in Tobacco and Alcohol (CARTA) that was established to examine the causal effects of tobacco, alcohol and other

lifestyle-related factors on health and sociodemographic outcomes by using Mendelian Randomisation. The consortium is described at the website: http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta/. The analyses were conducted according to my pre-specified analysis protocol that is found here: http://www.bris.ac.uk/expsych/research/brain/targ/research/collaborations/carta/Analysisprotocols.html. I combined data on 231020 participants from 22 studies: the British 1958 Birth Cohort (1958BC), the Study of Health in Pomerania (SHIP) and SHIP-TREND, the Avon Longitudinal Study of Parents and Children (ALSPAC) Mothers, ALSPAC Children, COPSAC2000, the Dan-Monica10, the English Longitudinal Study of Ageing (ELSA), the National FINRISK Study (FINRISK), GOYA Males, Genomics of Overweight in Young Adults (GOYA) Females, Health2006, Health2008, the second wave of the Nord-Trøndelag health study (HUNT2), Inter99, the Cooperative Health Research in the Region of Augsburg (KORA) study, the Middle-aged Span-of-Life (MIDSPAN) Family Study, the MRC National Survey of Health and Development (NSHD), the 1936 Cohort, the UK Biobank, the Netherlands Epidemiology of Obesity (NEO) study, and the Whitehall II (See Table 1).

In paper II, I combined data on 162736 participants from the following nine population-based studies: the Allergy98 Cohort (V), the Monica10 study (27), Health2006 (IX), Health2008 (V), Inter99 (28), the 1936 Cohort (23), the Study of Health in Pomerania (SHIP) (29), SHIP-TREND (30), and the UK Biobank (24) (see Table 1). In paper **Fejl! Henvisningskilde ikke fundet.**, I combined data on 490497 participants from the following seven population-based studies: the Monica10 study (27), Inter99 (28), Health2006 (IX), Health2008 (V), SHIP (29), SHIP-TREND (30), and the UK Biobank (See Table 1) (24).

Table 1. Overview of collaborating studies and available data

Study name	Year	N	Age, years	% Males	Place
1936-cohort (23)	1976–77	~557	40	47	RCPH, Denmark
Monica1 (23)	1982–84	~3,785	Range: 30–60	51	RCPH, Denmark
Monica10 (23)	1993–94	~2,054	Range 41–71	48	RCPH, Denmark
Allergy98 (31)	1997–98	~1,216	Range: 15–77	46	RCPH, Denmark
Inter99 (32)	1999–01	~4,991	Range: 30–60	49	RCPH, Denmark
Health2006 (33)	2006–08	~3,143	Range: 18–69	45	RCPH, Denmark
Health2008 (25)	2008–09	~795	Range: 30–60	44	RCPH, Denmark
Health2010 (26)	2010–12	~1,522	Range: 18–69	44	RCPH, Denmark
COPSAC2000 (34)	1998–01	~543	IQR: 29–37	46	Greater Copenhagen, Denmark
GOYA Females (35;36)	1996–02	~2,016	IQR: 27–33	0	Denmark
GOYA Males (35)	1992–94	~789	IQR: 41–53	100	Copenhagen area, Denmark
Alspac Children (37)	2009–10	~1,549	18	38	Avon, United Kingdom
Alspac Mothers (37)	1999–00	~4,834	IQR: 34–40	0	Avon, United Kingdom
1958 BC (38)	2000	~4,882	42 (45)	50	England, Scotland and Wales
ELSA (39)	1998–01	~5,263	IQR: 55–70	46	England
FINRISK (40)	1992–07	~25,363	IQR: 38–60	47	Finland
HUNT2 (41)	1995–97	~43,211	>20	46	Nord Trøndelag County, Norway
KORA (42)	1994–95	~837	Range: 24–74	48	Augsburg, Germany
MIDSPAN (43)	1996	~2,120	IQR: 41–49	45	Scotland
NEO (44)	2008–12	~5,501	Range: 45–65	48	Netherland
NSHD (45)	1999	~2,484	53	49	England, Scotland and Wales
UK Biobank (24)	2006–10	~500,000	Range: 40–69	49	United Kingdom
Whitehall II (46)	1985–88	~2,304	IQR: 39–49	74	London, United Kingdom
SHIP (47)	1997–01	~4,027	IQR: 36–63	49	West Pomerania, Germany
SHIP TREND (30)	2008–12	~984	IQR: 40–61	44	West Pomerania, Germany

Abbreviations: Allergy98, Copenhagen Allergy study; 1958 BC, British 1958 Birth Cohort; ALSPAC, Avon Longitudinal Survey of Parents and Children; COPSAC2000, Copenhagen Prospective study on Asthma in Childhood; ELSA, English Longitudinal Study of Ageing; FINRISK, Finland Cardiovascular Risk Study; GOYA, Genomics of extremely Overweight Young Adults; HUNT, Nord-Trøndelag Health Study; Inter99, Intervention 1999; IQR, interquartile range; KORA, Cooperative Health Research in the Region of Augsburg; MIDSPAN, the Middle-aged Span-of-Life; Monica, Monitoring of trends and determinants in Cardiovascular Diseases; NEO, Netherlands Epidemiology of Obesity; NSHD, National Survey of Health and Development; RCPH, Research Centre for Prevention and Health; SHIP, Study of Health in Pomerania.

Biomarkers and genetic variants

Genetic biomarkers of possible risk factors as instrumental variables of smoking quantity, serum levels of vitamin B12 and folate, and BMI were used. Table 2 shows an overview of the selected exposures and examples of their genetic biomarkers. SNPs can be used and combined in a number of different ways to resemble instrumental variables. A SNP-score is a single variable that summarizes multiple SNPs in a univariate score by either a simple SNP-score that counts the number of risk alleles, or in a weighted SNP-score where each SNP is weighted by its individual effect on the risk. For smoking, the single-SNP was used additively according to the number of smoking increasing alleles and run as a continuous covariate in the regression analyses (paper I). In paper III, I used a simple genetic risk score by adding the number of BMI-increasing alleles across SNPs (48). Secondarily, I used a weighted genetic risk score (with weights derived from studies different from our own to avoid inflation of the estimates) (49-61). In paper II, I used a multiple SNP approach where the single-SNP estimates were combined in meta-analyses across study populations and across SNPs. In secondary analyses, I used a SNP-score.

Each SNP used as single SNP or in a SNP-score has to be valid. I evaluated the Hardy-Weinberg equilibrium for each SNP and compared minor allele frequencies (MAF) with previously published data. Likewise, the 'F-value' is indicator of statistical power in the MR analysis (62). It is an analogue of the F-statistic for the joint significance of the SNP or SNP-score in the first stage regression. It has been suggested that the F-value should be >10 for the instrument to be good enough. I evaluated the 'F-value' of the instruments.

Table 2. Genetic markers of possible risk factors for allergy and asthma

Exposures	SNPs
B12/folate (63)	rs3742801, rs602662, rs2336573, rs1131603, rs1801222, rs34324219, rs34528912, rs41281112, rs2270655, rs4267943, rs1141321, rs1801133, rs652197, rs778805, rs1047891
Obesity (49-61)	rs10838738, rs10938397, rs10968576, rs11847697, rs12444979, rs13107325, rs1424233, rs1514175, rs1555543, rs17782313, rs1805081, rs206936, rs2112347, rs2241423, rs2287019, rs2568958, rs29941, rs3810291, rs4929949, rs543874, rs713586, rs7647305, rs9939609, rs10146997, rs1121980, rs7138803
Smoking (64-66)	rs1051730

Registry-based diagnoses

A number of unique and comprehensive nationwide registries is available in Denmark. I cross-linked data from the population studies at the RCPH with data from the Danish National Patient Register, the Danish Civil Registration System, the Danish Cancer Register, the Danish National Diabetes Register, and the Danish Registry of Causes of Death. The Danish Civil Registration System contains data on mortality and emigration status etc. (67). The Danish National Patient Register has data on admissions to Danish hospitals since 1977 (68). The admissions are registered by a primary diagnosis and, if applicable, one or more secondary diagnoses coded according to the International Classification of Diseases (ICD). The Danish Registry of Causes of Death has information on the date of death and at least one diagnosis suspected to be the cause of death (69). The Danish Cancer Register contributed data on cancer diagnoses (70;71). It is and has been mandatory to report cases of cancer to the Cancer Register since 1987. The Register was classified in accordance with the modified ICD-7 from 1943-1978 and the ICD-10 from 1978 and onwards (70). The Danish National Diabetes Register enabled identification of incident cases of diabetes (72). The register was started in 1990 and operated until 2012. A person is classified as a diabetic if one of the following criteria is met: hospitalization with a diagnosis of diabetes; measurements of blood glucose five times or more within 1 year, or twice or more per year in a 5-year period; registered chiropody for diabetes; or at least two prescriptions of oral anti-diabetics or

insulin. The participants were followed until 2010 or 2011, depending on which registries were used. A history of the disease in question was defined as a registry-based or self-reported diagnosis prior to examination (baseline), whereas incident disease was defined as a first time (registry-based) diagnosis of the disease during follow-up. An overview of the included registry-based outcomes is shown in Table 3. The corresponding ICD-7, ICD-8, and ICD-10 codes are reported in the papers.

Table 3. Registry-based outcomes

Paper	Outcomes
Autoimmune disease (VI)	Any autoimmune disease, thyrotoxicosis, type 1 diabetes, multiple sclerosis, iridocyclitis, Crohn's disease, ulcerative colitis, psoriasis vulgaris, seropositive, rheumatoid arthritis, and polymyalgia rheumatic
Airway infections and disease (IV)	Pneumonia, other acute airway infection, infection, asthma, other chronic lower airway disease
Cause-specific mortality (VIII)	Neoplasms; endocrine, nutritional and metabolic diseases; mental and behavioral disorders; diseases of the nervous system; diseases of the circulatory system; diseases of the respiratory system; diseases of the digestive system; and infections
IHD, stroke, and diabetes (IX)*	Stroke, IHD, and diabetes
Cancer (VII)	All cancers; all cancers excl. non-melanoma skin cancer, NMSC; cancer of the bronchus and lung; head and neck cancer; colorectal cancer; breast cancer; prostate cancer; cancer of the uterus; cancer of the urinary organs; and malignant melanoma

* A history of these diagnoses also included self-reported ischemic heart disease or myocardial infarction as assessed by the question: "Has a doctor ever/within the last 12 months told you that you had a heart attack?"; ischemic or hemorrhagic stroke as assessed by the question: "Has a doctor ever/within the last 12 months told you that you had a stroke?" or "Has a doctor ever/within the last 12 months told you that you had a brain hemorrhage?"; and diabetes as assesses by the question: "Have you been diagnosed with diabetes?" or "Has a doctor ever/within the last 12 months told you that you have diabetes?". Abbreviations: IHD, ischemic heart disease.

Allergy-related phenotypes

An overview of the main allergy-related outcomes is shown in Table 4. The objective allergy markers include serum total IgE and IgE sensitization as assessed by measurements of serum specific IgE positivity to inhalant allergens. IgE sensitization was dichotomized and defined as a positive test for serum specific IgE (>=0.35 kU) or skin prick test (SPT) (>= 3 mm) to at least one

inhalant allergen. Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were measured by spirometry. Hay fever and asthma were assessed by self-report (see Table 4).

Table 4. Selected allergy-related outcomes

Outcome	Type of data	Example
Hay fever	Self-reported	E.g., a positive answer to the question: "Has a doctor ever told you that you have hay fever?"
Asthma	Self-reported or registry-based	E.g., a positive answer to the question: "Has a doctor ever told you that you have asthma?" or according to the International Classification of Disease
Allergic sensitization	Measured	Measurement of serum specific IgE and/or skin prick test positivity to inhalant allergens
Total IgE	Measured	Measurement of serum total IgE
FEV1	Measured	Measured with spirometry
FVC	Measured	Measured with spirometry

Abbreviations: FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IgE, immunoglobulin E.

Statistical methods

I conducted the statistical analyses using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA), Stata 12.1 or 13 (StataCorp LP, College Station, TX, USA), and R. I performed power calculations in Quanto 1.2.4 (Table 5). If appropriate, I transformed continuous outcomes by the natural logarithm to obtain normal distributions. I used linear and logistic regression for continuous and dichotomous outcomes, respectively. I used multivariate Cox regression analysis to examine the associations between allergic sensitization and mortality or development of disease. Age was used as underlying time axis, and there was delayed entry. The few participants lost to follow-up contributed risk time until the date of their last registered activity.

Table 5. Selected power calculations

Exposure	Genetic variants	Power calculations *
Tobacco	rs1051730 (73)	Requires 3000 smokers with hay fever and 20000 smokers without hay fever to detect an odds ratio of 0.92 for having hay fever per smoking increasing allele.
Alcohol	rs1229984 (74)	Requires 6000 and 18000 ever-drinkers with and without hay fever, respectively, to detect an odds ratio of 0.85 and 14000 and 42000 ever-drinkers with and without hay fever, respectively, to detect an odds ratio of 0.90 among carriers of the protective allele (drinking less) compared to non-carriers. Requires 40000 ever-drinkers to detect an R^2=0.0002/ß=-0.10 for carriers vs. non-carriers of the protective allele (drinking less) for the natural logarithm of serum total IgE (mean=3.4 and standard deviation=1.5) as outcome.
Folate	MTHFR-SNP (63)	Requires 9000 persons with hay fever and 27000 without hay fever to detect an odds ratio of 1.05 for having hay fever per MTHFR effect-allele.
Obesity	FTO-SNP (75;76)	Requires 8000 cases and 24000 controls with and without hay fever, respectively, to detect an odds ratio of 1.05 for carriers vs non-carriers of the FTO-SNP. Requires 52000 cases and 156000 controls with and without hay fever, respectively, to detect an odds ratio of 1.02 for carriers vs non-carriers of the FTO-SNP.

*Power is set to 0.80 and significance level to 0.05. Abbreviations: FTO, fat mass and obesity-associated protein; IgE, immunoglobulin E; MTHFR, methylene tetrahydrofolate reductase.

Pooling of data

The different studies were harmonized, and data were pooled before analyses or were analyzed and then meta-analyzed, or both. I combined the estimates with the inverse variance method in fixed and random effect models. I used the I^2-test to examine the heterogeneity between studies (77;78). The random effects model was based on the method by DerSimonian & Laird and the estimate of heterogeneity from the Mantel-Haenszel model (78).

Mendelian Randomization analysis

The MR-analyses were adjusted for age and gender and stratified if appropriate. I calculated the required number of cases and controls for single-SNP effects and for a range of effect sizes (see Table 5). I performed the multicenter studies (paper I, II and III) according to pre-specified analysis protocols to ensure the uniform study descriptions, definition of variables, and equality of analyses. I wrote the protocols that consist of a number of distinct steps to make the results as rigorous as

possible. Each of the studies contributed a dataset prepared according to the protocol, and this was analyzed by Stata-scripts that I wrote.

The inverse-variance weighted (ivw) estimator was used for the IV-analyses. A one-sample IV-analysis is an IV-analysis where the available studies are included in both the first and second stage analysis, and I used this as primary analysis. In a two-sample IV-analysis, the first and second stage samples have no overlap. I used this design as additional analysis.

I performed a number of additional analyses, e.g., associations of the B12- and folate-associated SNPs excluding SNPs with low first stage F-value (indicator of power) (62), assessed unweighted, weighted, and standardized SNP-scores (Table 6). To assess pleiotropy, i.e. the phenomenon that a gene acts on multiple pathways and therefore may be associated with multiple exposures, I performed MR Egger regression and median regression analyses (22). To substantiate the assumption that the instrument is not associated with unmeasured confounders, I assessed the associations between each single-SNP and a number of possible confounders (paper II) (Table 6).

Table 6. Examples of additional analyses

ANALYSES	SAMPLE	POINT OF ASSESSESMENT	MAIN WEAKNESS
Excluding low-F SNPs	SNPs with F≥10	Relevance assumption Strength of IV	Selection due to internal F-values can induce bias
Adjusting for confounders	E.g., UK Biobank	Exclusion assumption	Can only adjust for measured confounders
MR Egger test and regression	All	Assess bias due to pleiotropy	Wide confidence intervals
Median regression	All	Assess bias due to pleiotropy	Wide confidence intervals
Two-sample IV	All studies divided into two non-overlapping samples	Any weak instrument bias towards zero	Loss of power
Studies with data on all SNPs	Studies with data on all SNPs	Reduce confounding by IV differences across studies	Loss of power
Simple SNP-score	All	Gain power	All SNPs given the same weight Each SNP has to be a valid IV
Weighted SNP-score	All	Gain power Account for SNP effect size	Each SNP has to be a valid IV
Standardized SNP-score	All	Gain power	Each SNP has to be a valid IV
Subgroups in UK Biobank	UK Biobank	Selection bias	Subsample
Self-reported vs. genetically determined Caucasians	UK Biobank	Population stratification	Subsample
SNP-specific estimates for each study	All studies separately	Check for consistency and SNP-outliers	Many subgroup-analyses
Study-specific estimates for each SNP	All SNPs separately	Check for consistency and study-outliers	Many subgroup-analyses

Abbreviations: IV, instrumental variable

Results and comparison with other studies

Causes of allergic disease and asthma

Smoking habits

Smoking has been suggested to increase the risk of allergic symptoms by increased inflammation in the airways but is in some studies associated with a lower prevalence of allergic sensitization (79). Studies have found a number of effects of tobacco smoke on the airways in both humans and animals, e.g., increased permeability, inflammation, and changed gene expression (80;81). Smoking has also been suggested to affect the risk of allergic respiratory disease and asthma (79;82;83), but the evidence is inconclusive (84-89). A systematic review and meta-analysis from 2014 of 34 observational studies (concerning active smoking and hay fever) found no association between smoking and hay fever (79). Our study was the first to assess causality between smoking and allergic disease and asthma using Mendelian Randomization and the largest observational meta-analysis on the subject.

In paper I, both self-reported and genetically determined heavier smoking was associated with lower relative risk of hay fever (Figure 3). In contrast, a meta-analysis of traditional observational studies found no association between smoking and allergic rhinitis (79). The observed inverse association may, however, reflect an immunosuppressive effect of smoking (90;91). It is also possible that allergic persons are more likely not to smoke or to quit smoking. This would imply that the observed association of smoking and hay fever is not causal.

Self-reported and genetically determined heavier smoking was associated with higher relative risk of asthma (Paper I) (Figure 4). Although some studies have found a lack of or an inverse association between smoking and asthma (87-89), the observed positive association in the Mendelian randomization analysis is in line with a number of traditional observational studies (84-

86), e.g., a study that found that smoking increased the incidence of adult-onset asthma (86). This may be due to a smoking-induced airway hyper-reactivity and increased Th2 response (92).

Self-reported and genetically determined heavier smoking was not significantly associated with the risk of allergic sensitization (Figure 5), but former and current smokers had lower odds ratio of allergic sensitization compared to never smokers (Paper I). The observed results are somewhat in contrast to previous studies showing lower risk of allergic sensitization associated with smoking (90;93;94). This may be due to residual confounding in previous studies or to a lack of power to show an effect in the current study.

Figure 3. Observational and Mendelian Randomization analyses of the association of selected exposures and hay fever (I,II,III). For smoking only in current smokers. Abbreviations: CI, confidence interval.

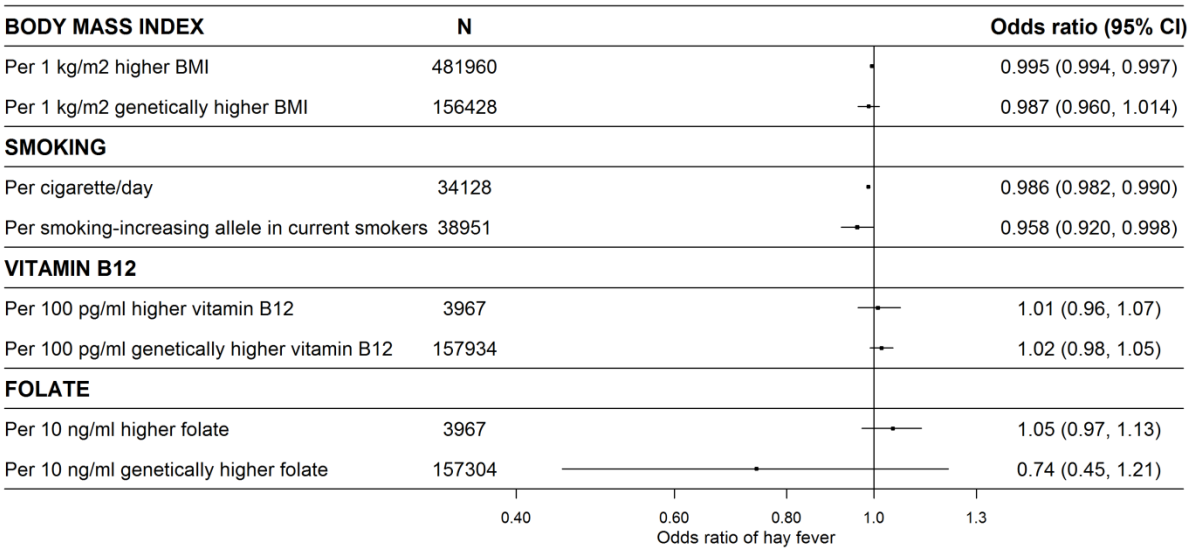


Figure 4. Observational and Mendelian Randomization analyses of the association of selected exposures and asthma (I,II,III). Abbreviations: CI, confidence interval.

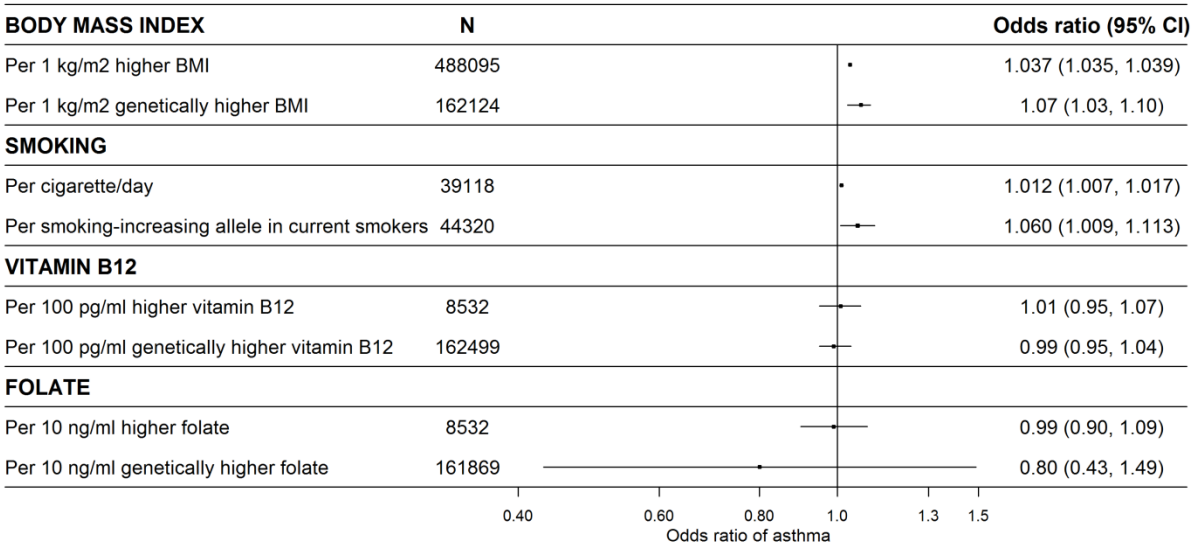
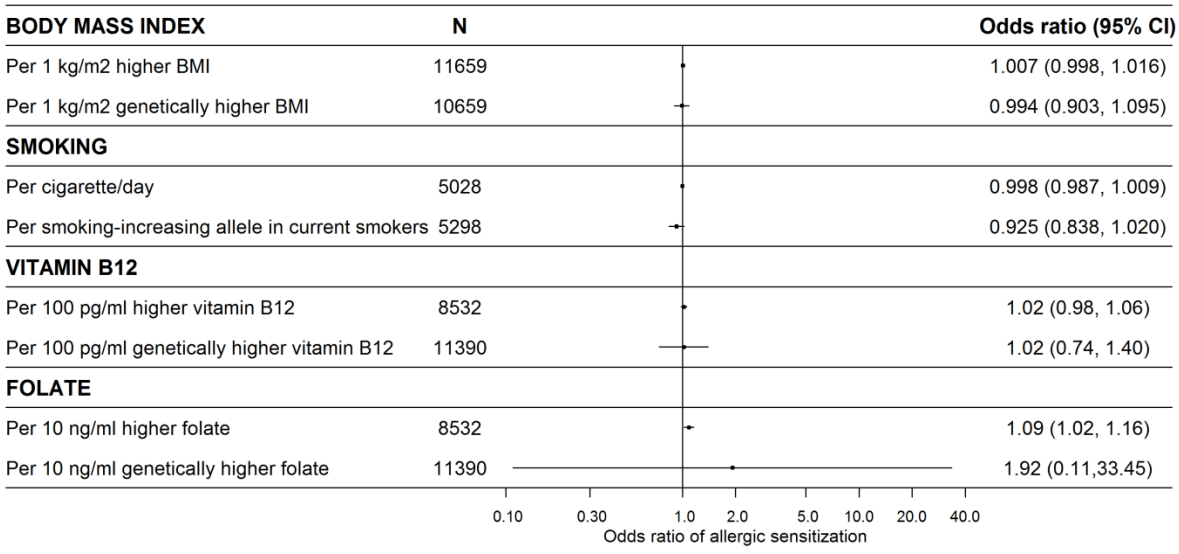


Figure 5. Observational and Mendelian Randomization analyses of the association of selected exposures and allergic sensitization (I,II,III). Abbreviations: CI, confidence interval.



Vitamin B12 and folate

Changes in intake of vitamin B12 and folate have been suggested to play a part in allergic respiratory diseases (82;83;95;96). Low folate intake and serum levels are frequent in countries without fortification with folic acid (97). Folate deficiency changes the cell-mediated immune

response (98), increases the susceptibility to infections (99), and may inhibit the re-methylation cycle (96).

The evidence from adult studies of a possible association between B12 and folate with allergic disease and asthma is scarce. Previously, studies have focused on investigating the effect of folate levels and folate supplementation in pregnancy on risk of allergy and asthma in offspring (100-107). A systematic review of prospective cohort studies concluded that the studies showed conflicting results (108).

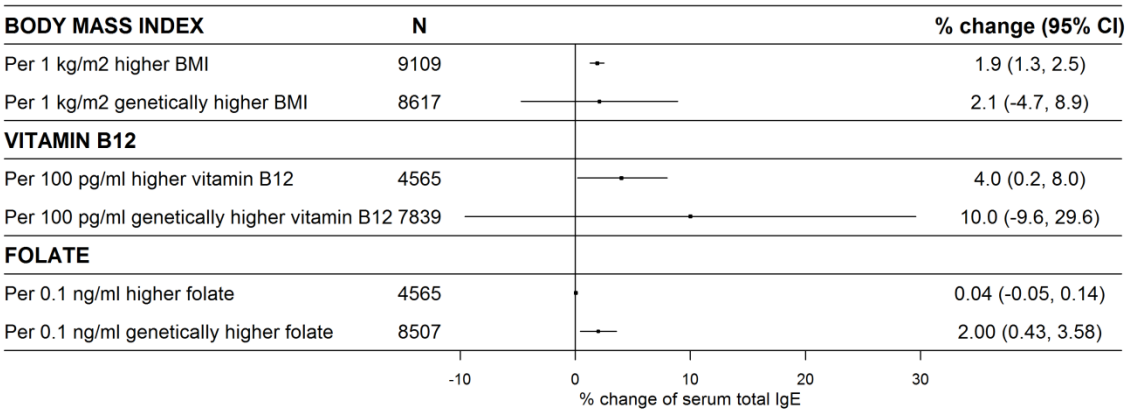
In paper II, instrumental variable analyses showed little evidence for associations between levels of vitamin B12 and hay fever, asthma, allergic sensitization, and log-transformed serum IgE (Figure 3–6). Likewise, there was little evidence for associations between levels of folate and hay fever, asthma, and allergic sensitization, but there was a statistically significant positive association with serum total IgE (Figure 3–6). Our study is the first to assess causality between B12 and folate and allergic disease and asthma by Mendelian Randomization. It is also the largest observational study on the subject.

In line with the observed lack of association in the current study, a study of adults found that folate deficiency was not associated with allergic sensitization (11). In contrast, one study found that folate deficiency was associated with higher risk of allergic sensitization (109), and another found that folate levels were inversely associated with allergic sensitization and high total IgE levels, both in children (110). In contrast, higher serum folate in early childhood was positively associated with allergen sensitization in a third study (111). A study found no effect of folic acid supplementation in pregnancy on offspring risk of specific or total IgE (106).

In line with the observed lack of association with asthma, a meta-analysis found no association between maternal folic acid supplementation in pregnancy and risk of asthma in the offspring (104), and a birth study found that folate and vitamin B12 levels did not affect asthma -

associated outcomes (105). However, an adult study found that folate deficiency was associated with self-reported asthma (11), and a study in children found that folate deficiency was associated with higher risk of severe asthma exacerbations (109).

Figure 6. Observational and Mendelian Randomization analyses of the association of selected exposures and serum total IgE (II,III). Abbreviations: CI, confidence interval; IgE, immunoglobulin E.



Body mass index

Traditional observational studies have shown that body mass index (BMI) is positively associated with asthma (112-117), and to some extent, with decreased lung function (118). The association with hay fever and allergic sensitization is less clear (10;119;120).

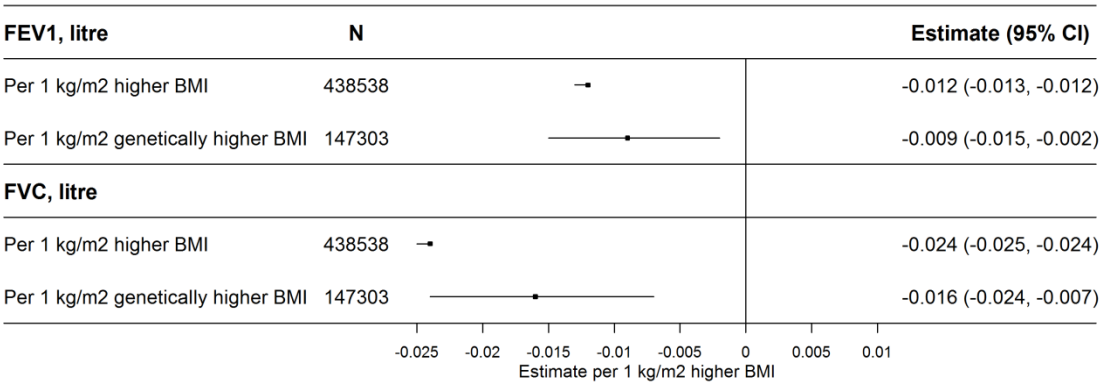
In paper III, a genetically determined higher body mass index was significantly associated with higher relative risk of asthma, but not with hay fever, allergic sensitization or serum total IgE (Figure 3–6). A genetically determined higher body mass index was significantly associated with decreases in FEV1 and FVC (Figure 7). A number of traditional observational studies has examined the association between overweight and asthma. The observed positive association between BMI and asthma is in line with most of the previous studies (113-116;121-126), although not all (127-129). A Mendelian randomization study of 5000 children showed that a higher BMI increased the risk of asthma in childhood (130) (REF). Our study extends these results

into adulthood and by also including lung function measurements. The observed negative association between BMI and lung function is also in line with previous observational studies although they are few (118;131). The observed associations may be explained by 1) increasing BMI leading to lower static lung volumes (132), 2) breathing with smaller tidal volumes leaving some of the myosin-actin in the airways unbroken, further narrowing the airways (133;134), and 3) the obesity-related low-grade inflammation affecting lung function and the risk of asthma.

Evidence from previous studies on BMI and hay fever are inconsistent and rely on traditional observational data (122;124;125;127;128;131;135-138). Paper III extends these results by assessing causality using Mendelian Randomization and by being the largest meta-analysis on the subject. Besides supporting the results of two research studies (129;139), the observed lack of association in the current study is in line with a review of studies including a total of 50,086 individuals (140).

Previous studies have shown conflicting results regarding the association between obesity and allergic sensitization (116). Paper III extends previous results by assessing causality using Mendelian Randomization. The observed lack of association in the MR-study is in line with two studies (124;126). In contrast, two studies found BMI to be positively associated with allergic sensitization (120;121;123;141-144).

Figure 7. Observational and Mendelian Randomization analyses of the association of selected exposures and lung function (III). Abbreviations: FEV1, forced expiratory volume in one second; FVC, forced vital capacity.



Other characteristics

Allergic sensitization was significantly associated with male sex, younger age, and higher education (Paper V). The observed associations are in line with previous studies (93;94;145;146). A possible mechanism underlying the association with age could be that the immune response often decreases with age and may explain the association with age since older people may be less susceptible to allergic reactions and sensitisation.

Allergic sensitization was significantly associated with heavy drinking (Paper V). This is in line with several other studies (94;147;148). However, other studies have found that alcohol consumption was not associated with IgE sensitisation (149) and with prevalent but not incident allergic sensitisation (146). Similarly, a study found no association between alcohol consumption and skin prick test positivity (150). Alcohol may have a direct effect on the B-cells or increase permeability in the gut (151). High alcohol consumption may also increase IgE sensitization to cross-reactive carbohydrate determinants which could interfere with allergy testing (146;152).

Consequences of allergic sensitization

Pneumonia, other infections and respiratory diseases

Studies have suggested that persons with allergic sensitization and allergic disease may be at higher risk of infection (153-156). However, only one previous rather small study investigated the longitudinal association of an objective marker of allergic sensitization and risk of respiratory infection among adults. In paper IV, there was a higher relative risk of pneumonia, asthma, and other chronic lower airway disease for persons with allergic sensitization vs. non-sensitized (Figure 8). There was no association between allergic sensitization and other acute airway infection or infection (Figure 8).

The observed positive association between allergic sensitization and pneumonia is somewhat supported by a number of studies that found association between increasing levels of specific IgE antibodies to common allergens, atopic disease other than asthma, allergic rhinitis, and atopy in adults, and a positive skin prick test in children, to be associated with number and severity of infection (153-156). The observed positive association between allergic sensitization and pneumonia may not be causal. A study found respiratory infections to be strong determinants for onset of asthma in adults (157), and other studies have found more frequent and more severe infections, e.g., pneumonia, in children and adults with allergic sensitization and asthma (158-161). This could suggest that persons more prone to infection develop atopic disease more frequently while keeping their propensity to infections. However, a possible causal association between allergic sensitization and pneumonia may involve 1) a lower protection against infections due to reduced T helper 1 (Th1) cell response among sensitized, and 2) decreased defence against infection and mucosal inflammation in allergic diseases in general, e.g., as seen in patients with atopic dermatitis (153).

Allergic sensitization and autoimmune disease

T helper cells play a key role in the immune system. They release cytokines to regulate immune responses. Proliferating T helper cells can differentiate into Th1 and Th2 cells, respectively. Th1-type cytokines produce a predominantly pro-inflammatory response killing intracellular parasites and perpetuating autoimmune reactions whereas the Th2-type cytokines are associated with promotion of eosinophilic and IgE responses in allergic sensitization and anti-inflammatory response (162). Since uncontrolled pro-inflammatory responses may lead to tissue damage, a mechanism to counteract the responses is necessary. Thus unlike allergic sensitization, autoimmune disease is generally considered to be characterized by a T helper cell 1 (Th1) deviation of the

immune response (163). Since the Th1- and Th2-activation may counteract each other, the two conditions may be inversely associated (164). However, the role of allergic sensitization in the development of autoimmune disease has not been clear (165-168).

In paper VI, the relative risks for atopics vs. non-atopics were not significantly different for autoimmune diseases in general, thyrotoxicosis, type 1 diabetes, multiple sclerosis, iridocyclitis, Crohn's disease, ulcerative colitis, psoriasis vulgaris, seropositive rheumatoid arthritis, or polymyalgia rheumatic (Figure 8).

The observed lack of association between allergic sensitization and autoimmune disease is in line with a cross-sectional study that found no statistically significant association between self-reported atopy and a history of autoimmune disease. However, they also found that having a history of physician-diagnosed hay fever or asthma was associated with a higher risk of having a physician-diagnosed autoimmune disease (166). Our study extended these results by using an objective marker of allergic sensitization; a longitudinal design; standardized registry-based diagnoses; and several additional autoimmune diseases.

Cancer

Allergic sensitization may both prevent and induce cancer (169): on one hand, it may increase the immune system's ability to recognize and destroy cancer cells. On the other, the cycle of inflammation, damage, and repair may increase the risk of cancer. Although inconsistent, asthma, hay fever, and atopic dermatitis have been found to be associated with a number of specific cancer types, e.g., lymphoma, pancreatic cancer, brain tumors, and leukemia (170-173). Our study extends previous studies by using an objective marker of atopy in a large general population sample.

In paper VII there were no statistically significant associations between allergic sensitization and risk of cancer in general, any cancer excluding non-melanoma skin cancer, head

and neck cancer, colorectal cancer, cancer of the bronchus and lung, breast cancer, cancer of the uterus, prostate cancer, urinary cancer, malignant melanoma, and non-melanoma skin cancer (Figure 8).

The observed lack of association between allergic sensitization and incident breast cancer but inverse association with breast cancer mortality in our studies is somewhat inconsistent. So is the existing evidence, although most studies report a lack of association. A meta-analysis of 16 observational studies found no association between allergic disease and breast cancer (169) . However, most of the studies used self-reported atopic disease as exposure rather than allergic sensitization. The observed lack of association in our study is in line with the results of a large case-control study reporting no statistically significant association between breast cancer and specific IgE positivity (170); found no association (174); and a small case-control study between a specific IgE score based on specific IgE to 12 allergens and breast cancer (171). Our results are inconsistent with a study reporting a positive association between a positive skin prick test and incident breast cancer (172). IgE may have a role in recognizing tumor antigens making allergically sensitized better at identifying cancer cells (175). However, lacking supporting evidence, the observed lower breast cancer mortality may be a chance finding.

The observed lack of association between allergic sensitization and prostate cancer is inconsistent with a study of 1005 persons that found specific IgE positivity and a positive skin prick test to inhalant allergens to increase the risk of prostate cancer (173). A meta-analysis reported a positive association between allergic sensitization (but not a history of allergic disease) and prostate cancer (169). The observed lack of association with and colorectal cancer was also in line with previous studies (170;172;174) and a meta-analysis of self-reported atopic disease and colorectal cancer (169). The observed lack of association with lung cancer was in line with some previous

studies (170;172;174) but inconsistent with a meta-analysis that suggested atopic disease to be associated with a higher risk of lung cancer (170).

Allergic sensitization, cardiovascular disease and diabetes

Ischemic heart disease, stroke, and diabetes are some of the most important causes of morbidity and mortality worldwide. Inflammation is known to play a significant role in the pathogenesis, and allergic disease can cause a systemic inflammation that potentially promotes atherosclerosis (176). Of note, allergic sensitization may also protect against cardiovascular disease, since it is associated with a mild hemostatic imbalance (177). In general, results from studies of allergic sensitization as determinant for cardiovascular disease (CVD) and diabetes are inconclusive (178-183). Our study extended previous studies by being a large prospective study, including diabetes.

Analyses showed no statistically significant associations between allergic sensitization and relatively risk of ischemic heart disease, stroke, or diabetes (Figure 8) (Paper IX). Also, allergic sensitization was not associated with blood pressure or serum total cholesterol in (Paper V). The results from previous studies on allergic sensitization and CVD and diabetes are few and the evidence inconsistent. The observed lack of associations between allergic sensitization and IHD and stroke in the current study is somewhat in contrast with previous studies that found inconsistent associations between allergic sensitization and cardiovascular mortality (182), that IgE predicted myocardial infarction in men but not women (180), and that allergic sensitization was inversely related to past myocardial infarction (181).

Mortality

The associations of allergic sensitization with all-cause and specific types of death are unresolved. Allergic sensitization may have an impact on mortality due to a potential role in the pathogenesis

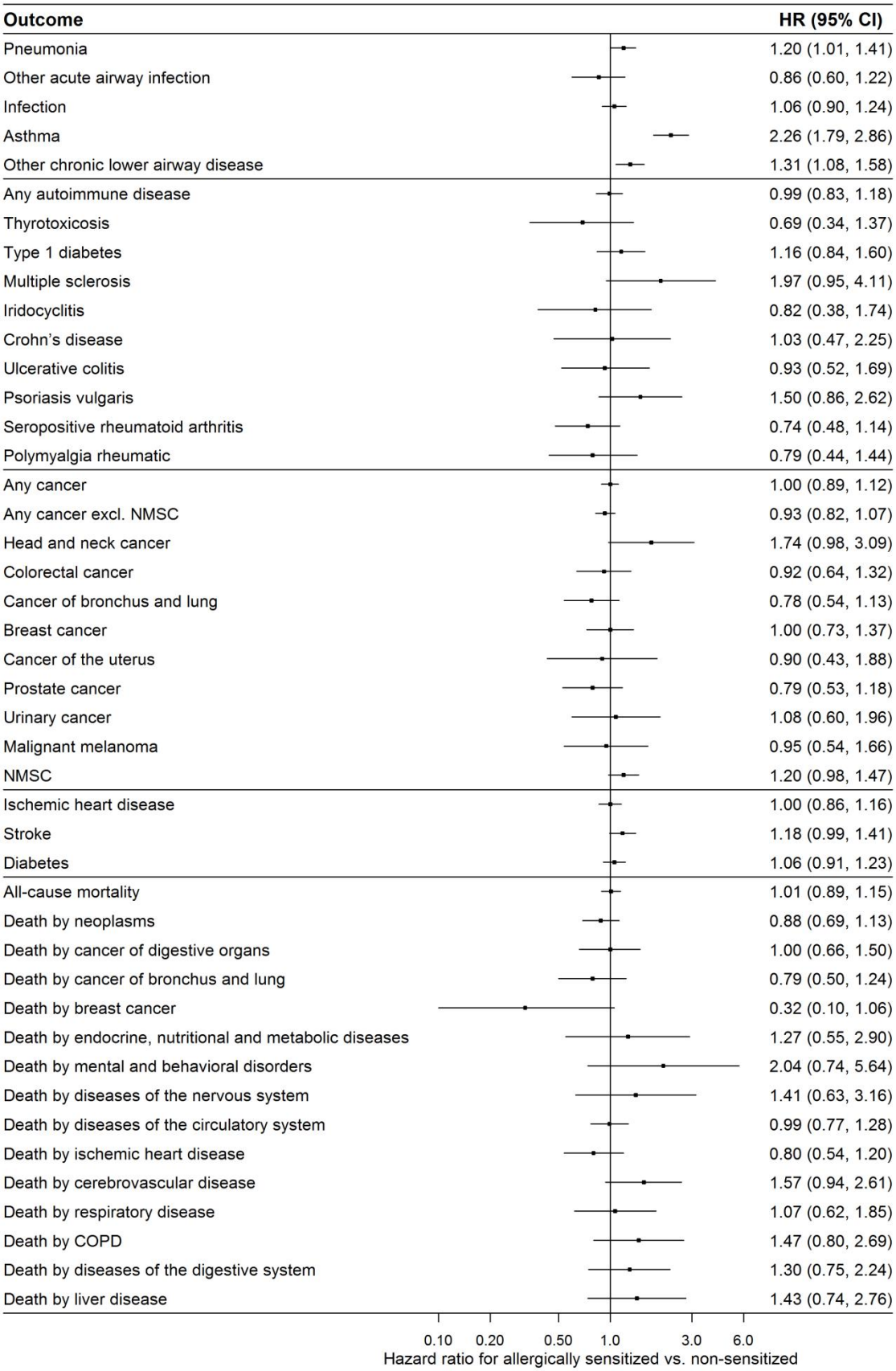
and severity of a number of diseases, e.g., infections (153-156), eosinophilic gastroenteritis (184), coeliac disease (165), chronic obstructive pulmonary disease (COPD) (185), cancer (169;186), and mental disorders such as dementia (187) and depression (188). Our study was the first to examine the association of allergic sensitization with all-cause mortality and specific causes of death.

In paper VIII, there was little evidence for associations between allergic sensitization and mortality in general, death caused by neoplasms or diseases of the circulatory system (Figure 8). The lack of association between allergic sensitization and mortality is in line with a study that found no association between allergic sensitization and cardiovascular, cancer and all-cause mortality (189). There was also little evidence for associations between allergic sensitization and death caused by endocrine, nutritional and metabolic disorders, diseases of the respiratory system, and diseases of the nervous system (Figure 8).

Allergic sensitization has been linked with higher risk of several gastrointestinal diseases but allergic sensitization in relation to gastrointestinal mortality is poorly investigated (165;184;190). In paper VIII, there was a significantly higher risk of dying from diseases of the digestive system (Figure 8). The observed higher risk of dying from liver disease may be explained by the fact that alcohol tends to increase serum total IgE and may increase the risk of being classified as being allergically sensitized (149;191).

In paper VIII, there was a statistically significant higher risk of dying from mental and behavioral disorders (Figure 8). The observed positive association between allergic sensitization and risk of dying from mental and behavioral disorders is somewhat supported by two studies that found persons with asthma, hay fever or eczema to be at higher risk of depression and Alzheimer's disease and dementia, respectively (187;188). However, mental disease comprises a number of unique disease entities which the low number of cases did not allow us to examine in detail.

Figure 8. The associations of allergic sensitization and selected registry-based outcomes.
Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; HR, hazard ratio; NMSC; non-melanoma skin cancer.



Discussion

Main findings

In a cross-sectional study, allergic sensitization was significantly associated with younger age, male sex, heavy drinking, not smoking, and education, but it was not associated with BMI, blood pressure, serum cholesterol, or physical activity. In a Mendelian randomization analysis, a genetically determined heavier smoking was associated with lower risk of hay fever and higher risk of asthma in current smokers. Genetically determined higher BMI was associated with a significantly higher prevalence of asthma and lower lung function (FEV1 and FVC), but not with hay fever or allergy biomarkers. Genetically determined higher levels of vitamin B12 and folate were not associated with hay fever, asthma, or allergic sensitization, but a genetically determined higher level of folate was positively associated with total IgE.

In prospective, registry-based studies, there was little evidence of association between allergic sensitization and incident ischemic heart disease, stroke and diabetes. There was little evidence of an association between allergic sensitization and development of cancer in general, but there were weak signs of a positive association with head and neck cancer. There was also little evidence of association between allergic sensitization and autoimmune diseases.

There was evidence of positive associations between allergic sensitization and asthma, other chronic lower airway diseases, and pneumonia. The positive association with asthma is well-known, while the observed positive association with pneumonia is not generally recognized as a co-morbidity of allergic sensitization. It is, however, possible that undiagnosed asthma underlies the association with pneumonia. There was little evidence of association between allergic sensitization and infections in general.

There was little evidence of an association between allergic sensitization and all-cause mortality. Accordingly, allergic sensitization was not associated with some of the largest contributors to mortality, cardiovascular disease mortality and death caused by neoplasms in general, although there was some evidence of an inverse association with breast cancer mortality. There was, however, some evidence of a positive association between allergic sensitization and death caused by mental and behavioral disorders and gastrointestinal diseases, particularly liver diseases.

Strengths and limitations

With an emphasis on Mendelian Randomization, a selection of the major strengths and limitations are summarized below. For a more detailed discussion, please see the relevant paper.

Mendelian Randomization

Mendelian randomization is a powerful method to assess causality using observational data. Our studies were the first to examine causality using Mendelian Randomization for almost all of the examined associations. Genetic markers of exposure are less likely to be associated with the common confounding factors, they tend to capture long-term levels of exposure, and they are unaffected by the onset of disease and are therefore protected from reverse causation. However, causal inference may be hampered if the Mendelian randomization principles are violated. The validity of the IV is dependent on the three assumptions referred to as ‘relevance’, ‘independence’, and ‘exclusion’ as previously mentioned. The first can be proven, the latter two only falsified (22). When using multiple SNPs, each SNP has to fulfil the requirements of an IV to be a valid instrument, at least in principle (192). The assumptions underlying the estimation of magnitude of

the causal effect are even more stringent as opposed to methods to investigate whether or not a causal association exists (22).

The relevance assumption says that the IV must be associated with the exposure. The risk of violating this assumption may be reduced when choosing biologically plausible SNPs (63). In general, IVs were constructed by SNPs with previously validated and published association with the exposure of interest. E.g., the smoking-associated rs16969968/rs1051730 genotype is known to be strongly and consistently associated with smoking heaviness in smokers, is a solid instrument for smoking, and has shown expected causal associations with increased all-cause mortality, decreased lung function, and BMI (193-198). The strengths of the instruments in our studies were at least acceptable and often very good as assessed by the F-value of the gene-exposure association or by the explained variance in the exposure. As a rule of thumb, the F-value should be above 10. Except for two, all SNPs in paper III had F-values above 10 in the UK Biobank data, and the F-value for the SNP-score was as high as 2000. Exclusion of the few SNPs with less favourable F-values led to similar results (paper II and III).

The independence assumption says that the IV must be independent of the outcome given the exposure and the unmeasured confounders. In general, the independence assumption may be more plausible and intuitive for proteins or serum markers compared to a complex trait like BMI. This assumption can be tried by screening each SNP for associations with common diseases and risk factors and by testing that the SNPs are not in linkage disequilibrium (63). Pleiotropy refers to the situation where a single gene has multiple biological functions (199). It can be formally tested by the MR Egger and median regression. The weighted median regression provides an estimate of the causal effect if at least half of the weights are from valid IVs. In MR Egger, the assumption that the effect of the genetic variants on the outcome is only through the exposure is relaxed by not constraining the intercept to zero. A non-zero intercept is evidence for bias due to pleiotropy. MR-

Egger works only if the sizes of the gene-exposure associations across all genetic variants are independent of their pleiotropic effects. However, MR Egger and median regressions showed little sign of pleiotropy, e.g., for the BMI-associated SNPs and asthma. Regarding the exclusion assumption, the minimal impact of adjusting for potential confounders supported that the assumption was not seriously violated.

Weak instrument bias is a concern in small studies when the genetic variants explain only little variation in the risk factor (200). Weak instrument bias can be introduced in one-sample MR studies since the associations of the genetic variants with the risk factor and outcome are correlated. Therefore, the causal estimate is biased in the direction of the observational estimate. However, in a two-sample Mendelian Randomization analysis any weak instrument bias will be in the direction of the null (22). Results from two-sample Mendelian Randomization analyses of, e.g., hay fever, asthma, FEV1, and FVC were almost identical to the one-sample analyses which means that weak instrument bias did not substantially bias our results. The drawback of two-sample Mendelian Randomization analyses is the loss of power when cutting sample sizes.

The prevalence of outcomes differed between populations, particularly in the studies uses Mendelian randomization which could be due to differences in age, socioeconomic position, and examination period. This may have induced heterogeneity, which was also observed in some of the observational analyses. Other general limitations include the shortage of power well illustrated by wide confidence interval that is a limitation in some of the studies. This is a particular concern in the MR analyses of allergic sensitization and serum total IgE that include substantially fewer people that those of asthma, hay fever, and lung function. Since most genetic markers have small individual effects, genetic risk-scores were made where the multidimensional genetic data were collapsed into a single variable. Risk-scores -as opposed to the individual genetic variants- may be stronger instruments (201). To check for consistency, a number of supplementary analyses were

performed in each study, e.g., in different subsamples, with other adjustments for other possible confounders, using scores and weighting etc. These manipulations most often left the results nearly unchanged.

Registry-based studies

Registry-based data from the Danish National Patient Register, the Danish Civil Registration System, the Danish Cancer Register, the Danish National Diabetes Register, and the Danish Registry of Causes of Death have a very high degree of completeness and gave us almost no loss to follow-up. Classification of causes of death is done according to the rules of the WHO and ICD-10 codes since 1994 (202). Changes in coding practice may, however, affect the comparability and validity of specific causes of death. The Danish National Patient Register is a sound source of data, but over time the content and definitions have changed (203). The payment rates for specific diagnoses changes over time which may cause a drift in the (coding of) diagnoses (203). However, the combination of the national registries has previously been found to be a valid tool for monitoring, e.g., the incidence of myocardial infarction in the population (204).

Conclusion and perspectives

The human and economic costs of allergic disease and asthma are indisputable. Recognizing asthma as a disease of major public health importance, WHO has a key role in coordinating the international collaboration against the disease (2). This international Mendelian Randomization project on causes of allergic disease and asthma is ongoing. It is planned to include further outcomes, e.g., atopic dermatitis, and examining lifestyle related factors such as alcohol and coffee intake, nutritional factors such as intake of milk, and constitutional and hormonal factors such as testosterone levels in males and adult height as exposures. In addition, the UK Biobank data includes registry-based data which enables identification of possible comorbidities of allergic phenotypes, e.g., atopic dermatitis, with great certainty due to the large sample size. The Mendelian Randomization methods are still under rapid development. Statistical and epidemiological groups at the Cambridge and Bristol Universities are leading the development. The methods include how to handle pleiotropy; Egger regression (205), weighted median estimator (206), and random effects IVW meta-analysis (207), and also includes factorial Mendelian Randomization (208).

With the introduction of the UK Biobank data that can be applied for by all researchers, hopefully a trend toward more sharing of data has been initiated. There are several other large biobanks. In the China Kadoorie Biobank, 512891 adults aged 30–79 years from 10 regions were recruited from 2004–2008. Questionnaire data, physical measurements and blood samples were collected and participants are being followed in hospital registries etc. genome-wide association study (GWAS) genotyping on ~100000 participants with ~800,000 SNPs is ongoing (209). The Kaiser Permanente Genetic Epidemiology Research in Adult Health and Aging (GERA) Cohort has undertaken genome-wide genotyping of 100000 participants (210). The Million Veterans cohort began recruiting in 2011, and 397104 veterans have been enrolled in 50 centers

nationwide (211). Data are collected using questionnaires, electronic health records, and blood samples for, e.g., genomic testing.

Our results showed that a number of lifestyle related factors are associated with hay fever and allergic respiratory disease. Taken together with existing evidence, there is evidence that smoking can be causally related to higher risk of asthma and a lower risk of hay fever. While asthma maybe could be added to the list of smoking-induced diseases, the adverse events associated with smoking limit the clinical significance of the observed lower risk of hay fever. There is also evidence that higher BMI is causally related to higher prevalence of asthma and lower lung function, but not with hay fever or biomarkers of allergy. Except for a possible causal role of folate levels on serum total IgE, the results do not support the conclusion that vitamin B12 and folate levels are causally related to the examined allergy and asthma phenotypes. The results show that lifestyle factors may contribute to the rise in allergies and strengthen advice against smoking for reducing chronic diseases. On the other hand, except for a higher risk of pneumonia, asthma and other chronic lower airway disease, allergic sensitization alone is not associated with higher risk of cancer, autoimmune diseases, cardiovascular disease, diabetes, or mortality. Further largescale genetic studies may aid in the development of optimal strategies for the prevention of allergic disease and asthma and its consequences (2;3).

Summary

Introduction

Allergy and asthma have increased. Many lifestyle-related risk factors have been proposed to contribute to the rise in allergic diseases and asthma. Allergic sensitization confers an increased risk of allergic rhinitis, allergic asthma, atopic dermatitis, and food allergies, but it is not clear whether allergic sensitization is associated with other diseases. The aims were to identify causes of allergic disease and asthma by performing a series of Mendelian randomization studies; and to examine health-related consequences of allergic sensitization through registry-based follow-up.

Methods

Data from 25 European and American population-based studies were included in this thesis. The papers included data on 14,849–490,497 participants. The studies had data on allergic respiratory disease, asthma, allergic sensitization, lung function, and lifestyle factors from questionnaires, physical examinations, sample assays, and for the most part also genome-wide genotyping and longitudinal follow-up in disease registries. The data were analyzed in multivariable or instrumental variable regression analyses and combined in fixed and random effects meta-analysis.

Results

Allergic sensitization was significantly associated with male sex, younger age, heavy drinking, never smoking, and higher education, but not with blood pressure, serum total cholesterol, physical activity or body mass index. Mendelian randomization analyses in current smokers showed a lower relative risk of hay fever, higher higher risk of asthma, but no statistically significant difference in allergic sensitization for genetically determined heavier smoking. There was no statistically significant association between genetically determined levels of vitamin B12 and hay fever, asthma,

allergic sensitization, or serum total IgE. Likewise, there was little evidence for associations between genetically determined levels of folate and hay fever, asthma, and allergic sensitization, but a statistically significant positive association with serum total IgE. A genetically determined higher body mass index was significantly associated with higher relative risk of asthma, but not with hay fever or allergic sensitization. Genetically determined higher body mass index was significantly associated with a decrease in forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC).

There were no significantly higher relative risks for allergically sensitized for autoimmune diseases in general, thyrotoxicosis, type 1 diabetes, multiple sclerosis, iridocyclitis, Crohn's disease, ulcerative colitis, psoriasis vulgaris, seropositive rheumatoid arthritis, or polymyalgia rheumatic. No statistically significant associations between allergic sensitization and risk of cancer in general, cancer in general excluding non-melanoma skin cancer, head and neck cancer, colorectal cancer, cancer of the bronchus and lung, breast cancer, cancer of the uterus, prostate cancer, urinary cancer, malignant melanoma, and non-melanoma skin cancer were found. Except for statistically significant higher relative risk of dying from mental and behavioral disorders and diseases of the digestive system among allergically sensitized, there was little evidence for associations with all-cause mortality, death caused by neoplasms, endocrine, nutritional and metabolic disorders, diseases of the respiratory system, diseases of the nervous system, and diseases of the circulatory system. The results showed no higher relative risk of ischemic heart disease, stroke, or diabetes for allergically sensitized vs non-sensitized. There was a higher relative risk of pneumonia, asthma, and other chronic lower airway disease for persons with allergic sensitization vs. non-sensitized, but no association between allergic sensitization and other acute airway infection or infection.

Conclusion

A number of lifestyle related factors are associated with hay fever and allergic respiratory disease. There is evidence that smoking can be causally related to higher risk of asthma and a lower risk of hay fever. There is also evidence that higher body mass index is causally related to higher prevalence of asthma and lower lung function, but not with hay fever or biomarkers of allergy. Except for a possible causal role of folate levels on serum total IgE, the results do not support the conclusion that vitamin B12 and folate levels are causally related to the examined allergy and asthma phenotypes. Allergic sensitization is associated with a higher relative risk of pneumonia, asthma and other chronic lower airway disease. However, allergic sensitization alone is not associated with higher risk of cancer, autoimmune diseases, cardiovascular disease, diabetes, or mortality, and these results are reassuring in a time of increasing incidence of allergic disease.

Dansk resumé

Introduktion

Prævalensen af allergi og astma er steget, og livsstilsrelaterede risikofaktorer kan have bidraget til denne stigning. Allergisk sensibilisering giver øget risiko for allergisk høfeber, allergisk astma, atopisk eksem og fødevareallergi, men det er uklart, om allergisk sensibilisering er forbundet med andre sygdomme. Formålet var at identificere årsagerne til allergisk sygdom og astma ved at udføre en række Mendelsk randomiserings-studier og at undersøge sundhedsrelaterede konsekvenser af allergisk sensibilisering gennem registerbaseret opfølgning.

Metoder

Data fra 25 europæiske og amerikanske befolkningsbaserede undersøgelser blev inkluderet. Undersøgelserne havde information om allergisk respiratorisk sygdom, astma, allergisk sensibilisering, lungefunktion og livsstilsfaktorer fra spørgeskemaer, fysiske undersøgelser, blodprøver og for det meste også genetiske undersøgelser og fremadrettet opfølgning i sygdomsregistre. Data blev analyseret i multivariable eller instrumental variable regressionsanalyser og kombineret i meta-analyse med fixed og random effektmodeller.

Resultater

Allergisk sensibilisering var signifikant forbundet med mandligt køn, yngre alder, stort forbrug af alkohol, aldrig-rygning og højere uddannelse, men ikke med blodtryk, serum total kolesterol, fysisk aktivitet eller body mass index. I analyser med Mendelsk randomisering var der en lavere relativ risiko for høfeber og højere risiko for astma blandt rygere, men ingen statistisk signifikant forskel i allergisk sensibilisering for genetisk bestemt at ryge mere. Der var ingen statistisk signifikant sammenhæng mellem genetisk bestemte niveauer af vitamin B12 og høfeber, astma, allergisk

sensibilisering eller serum total IgE. På samme måde var der ingen sammenhæng mellem genetisk bestemte niveauer af folat og høfeber, astma eller allergisk sensibilisering, men en statistisk signifikant positiv sammenhæng med serum total IgE. Et genetisk bestemt højere body mass index var signifikant forbundet med højere relativ risiko for astma, men ikke med høfeber eller allergisk sensibilisering. Genetisk bestemt højere BMI var signifikant forbundet med et fald i forceret ekspiratorisk volumen i 1 sekund (FEV1) og forceret vital kapacitet (FVC).

Personer med allergisk sensibilisering havde ikke højere relativ risiko for autoimmun sygdom generelt, thyrotoxikose, type 1 diabetes, multipel sklerose, iridocyclitis, Crohns sygdom, colitis ulcerosa, psoriasis vulgaris, seropositiv reumatoid arthritis og polymyalgia rheumatica. Der var ingen statistisk signifikante associationer mellem allergisk sensibilisering og risiko for kræft generelt, kræft generelt ekskl. ikke-melanom hudkræft, hoved- og halskræft, mavetarmkræft, kræft i bronkier og lunge, brystkræft, livmoderkræft, prostatakræft, kræft i urinvejene, malignt melanom og ikke-melanom hudkræft. Bortset fra statistisk signifikant højere risiko for at dø af psykiske og adfærdsmæssige lidelser og sygdomme i fordøjelsessystemet blandt allergisk sensibiliserede, var der ingen sammenhæng med død af alle årsager, dør af neoplasmer, endokrine, ernæringsbetingede og metabolske sygdomme, sygdomme i luftvejene, sygdomme i nervesystemet og kredsløbssygdomme. Resultaterne viste ikke sammenhæng mellem allergisk sensibilisering og iskæmisk hjertesygdom, slagtilfælde eller diabetes. Der var en højere relativ risiko for lungebetændelse, astma og anden kronisk nedre luftvejssygdom for personer med allergisk sensibilisering vs. ikke-sensibiliserede. Der var ingen sammenhæng mellem allergisk sensibilisering og anden akut luftvejsinfektion eller infektion.

Konklusion

Flere forskellige livsstilsrelaterede faktorer ser ud til at være forbundet med høfeber og allergisk sygdom. Vores resultater peger på, at rygning kan medføre en højere relativ risiko for astma og en lavere risiko for høfeber. Højere body mass index er forbundet med højere forekomst af astma og lavere lungefunktion, men ikke med høfeber eller biomarkører for allergi. Der er en mulig årsagssammenhæng mellem folat i blodet og total IgE, men vores resultater viser, at B12-vitamin og folat ikke betyder noget for de undersøgte allergi- og astmavariable i øvrigt. Allergisk sensibilisering er forbundet med en højere risiko for lungebetændelse, astma og anden kronisk nedre luftvejssygdom. Imidlertid er allergisk sensibilisering i sig selv ikke forbundet med højere risiko for kræft, autoimmune sygdomme, hjertekarsygdom, diabetes eller dødelighed. Disse resultater er beroligende i en tid med stigende forekomst af allergisk sygdom.

Abbreviations

AIDS	acquired immunodeficiency syndrome
Allergy98	Copenhagen Allergy study
ALSPAC	Avon Longitudinal Survey of Parents and Children
1958 BC	British 1958 Birth Cohort
BMI	body mass index
CARTA	Causal Analysis Research in Tobacco and Alcohol
CCHS	Copenhagen City Heart Study
CI	confidence interval
COPD	chronic obstructive pulmonary disease
COPSAC	Copenhagen Prospective study on Asthma in Childhood
CVD	cardiovascular disease
DAG	directed acyclic graph
ELSA	English Longitudinal Study of Ageing
FINRISK	Finland Cardiovascular Risk Study
GOYA	Genomics of extremely Overweight Young Adults
GWAS	Genome-wide association study
HIV	human immunodeficiency virus
HR	hazard ratio
HUNT	Nord-Trøndelag Health Study
ICD	International Classification of Disease
IHD	ischemic heart disease
Inter99	Intervention 1999
IQR	interquartile range

IV	instrumental variable
KORA	Cooperative Health Research in the Region of Augsburg
MAF	Minor allele frequency
MIDSPAN	Middle-aged Span-of-Life
Monica	Monitoring of trends and determinants in Cardiovascular Diseases
NA	not applicable/available
NEO	Netherlands Epidemiology of Obesity
NSHD	National Survey of Health and Development
OR	odds ratio
PROSPER	PROspective Study of Pravastatin in the Elderly at Risk
RCT	randomized controlled trial
SHIP	Study of Health in Pomerania
SNP	single nucleotide polymorphism
STROBE	the strengthening the reporting of observational studies in epidemiology
Th cell	T helper cell
US	The United States

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