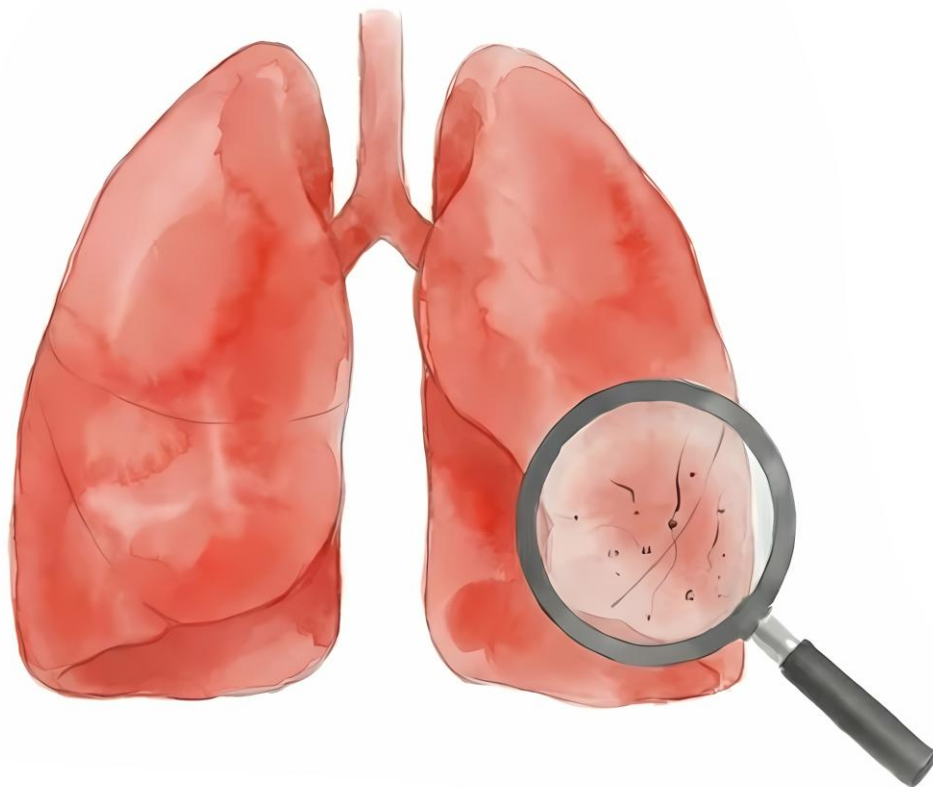




Risk-behaviours, non-atopic comorbidities, and inflammatory biomarkers of adolescents with asthma



PhD Thesis

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Title of thesis: Risk-behaviours, non-atopic comorbidities, and inflammatory biomarkers of adolescents with asthma

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Trine Mølbæk-Engbjerg

Copenhagen, January 2026

ABBREVIATIONS

ADHD = Attention Deficit Hyperactivity Disorder

AHR = Airway Hyperresponsiveness

AIC = Akaike Information Criterion

ASRS = The Adult ADHD Self-Report Scale

BIA = Bioelectrical Impedance Analysis

BIC = Bayesian Information Criterion

BMI = Body Mass Index

COPSAC₂₀₀₀₀ = Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀

FeNO = Fractional exhaled Nitric Oxide

FEV₁ = Forced Expiratory Volume at 1 second.

FVC = Forced Vital Capacity

GA = Gestational Age

IgA = Immunoglobulin A

Ig = Immunoglobulin E

IgM = Immunoglobulin M

IgG = Immunoglobulin G

IQR = Interquartile Range

LCT = Latent Class Trajectories

LMM = Linear Mixed Models

NLR = Neutrophils – Lymphocytes Ratio

PD20 = Provoking dose, 20%

SD = Standard deviation

SOP = Standard Operating procedures

sRaw = Specific airway resistance

Th2 = T helper 2 cells

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PUBLICATIONS INCLUDED IN THE THESIS

PAPER I **Mølbæk-Engbjerg T**, Vahman N, Mikkelsen M, Fink NR, Christensen ED, Brustad N, Sass L, Løvenhøj H, Strandberg-Larsen K, Groot J, Andersen AMN, Vinding R, Schoos AMM, Stockholm J, Bønnelykke K, Chawes B

Design of the 18-year follow-up of the Danish COPSAC2000 birth cohort¹

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PAPER II **Mølbæk-Engbjerg T**, Vahman N, Ali M, Skov F, Vinding R, Horner D, Brustad N, Thorsen J, Schoos AMM, Stockholm J, Bønnelykke K, Chawes B

Risk behaviours and non-atopic comorbidities of adolescents with asthma²

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Longitudinal trajectories of inflammatory biomarkers and their association with asthma

Manuscript prepared for submission

ENGLISH SUMMARY

Background

Asthma is an inflammatory disease in the airways that often debuts in childhood or adolescence. The disease is the most common chronic disease in childhood and represents a large burden to the affected families and society. Living with asthmatic disease may also increase the risk of other health conditions. Several studies found that adolescents with chronic diseases have an increased risk of developing depression and anxiety, and a connection between asthma and neurodevelopmental disorders has been identified. Finally, other studies have observed an association between dyslipidaemia, obesity, and asthma. It is not clear why these relations are observed, but one hypothesis is that asthma and other non-communicable diseases share genetic and environmental risk factors, leading to low-grade systemic inflammation that drives these diseases.

In this PhD thesis, we explore the following:

1. We outline the design of the 18-year follow-up visit from the COPSAC₂₀₀₀ cohort
2. We investigate associations between asthma, risk behaviours, and non-atopic comorbidity.
3. We explore associations between systemic inflammatory biomarkers collected through childhood and adolescence and asthma status at 18 years.

Methods and results

This PhD thesis is based on data from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₀₀), a high-risk cohort comprising 411 children born to mothers with asthma. The children were followed closely from one month of age to 18 years with extensive examinations and data collection.

Paper I. Design of the 18-year follow-up of the Danish COPSAC2000 birth cohort, BMJ Paediatrics Open, published

In **Paper I**, we outline the design of the 18-year follow-up visit for the COPSAC₂₀₀₀ cohort, in which risk factors for atopic diseases, obesity, and neuropsychiatric disorders are identified through extensive environmental characterisation, deep clinical phenotyping, and

longitudinal biosampling. This paper outlines the data that serve as the basis for the analyses conducted in **Papers II** and **Paper III**.

Paper II. Risk behaviours and non-atopic comorbidities of adolescents with asthma, World Allergy Organization Journal, published

In **Paper II**, we investigate the association between asthma status at the 18-year follow-up visit of the COPSAC₂₀₀₀ cohort and risk behaviours and non-atopic comorbidities. We include obesity and neuropsychiatric diseases captured from medical records and electronic questionnaires on behavioural traits and psychopathology. The study shows that neuropsychiatric comorbidity and self-destructive behaviour are associated with asthma at 18 years of age.

Paper III. Longitudinal trajectories of inflammatory biomarkers and their association with asthma, manuscript prepared for submission

In **Paper III**, we longitudinally examine inflammatory biomarkers across childhood and adolescence in COPSAC₂₀₀₀ to identify associations between trajectories of these biomarkers and asthma, and asthma-related outcomes at 18 years. We found an association among platelet trajectories, airway obstruction (FEV1), and airway hyperresponsiveness (PD20), suggesting that platelet levels over time are involved in the pathophysiology of asthma.

Conclusion

To conclude, **Paper I** provides knowledge of the cohort used in the thesis. **Paper II** identifies risk behaviours and non-atopic comorbidities associated with asthma and contributes to knowledge of the general health of adolescents living with asthma. **Paper III** explores biomarkers of systemic inflammation across childhood and adolescence and their association with asthma at 18 years, advancing our understanding of asthma pathogenesis and the role of platelets.

DANISH SUMMARY

Baggrund

Asthma er en inflammatorisk sygdom i luftvejene som typisk debuterer i barndommen. Sygdommen er den hyppigst forekommende, kroniske sygdom i barndommen og udgør en stor belastning for de berørte familier og for samfundet, men at leve med astma kan også øge risikoen for andre sygdomme som påvirker ens helbred. Flere studier har påvist, at unge, som lever med en kronisk sygdom, er i øget risiko for at udvikle depression og angst, og der er ligeledes påvist en sammenhæng imellem astma og neuropsykiatriske lidelser. Endelig har andre studier påvist en sammenhæng imellem dyslipidemi, fedme og astma. Det er fortsat uklart, hvad der ligger til grund for disse observationer, men en hypotese er, at astma og andre ikke-smitsomme sygdomme deler fælles genetiske- og miljømæssige risikofaktorer, som medfører en kronisk, stille inflammation som er den underliggende årsag til sygdommene.

I denne PhD afhandling undersøger vi følgende:

1. Vi beskriver designet af 18 års opfølgningsbesøget for COPSAC₂₀₀₀ kohorten.
2. Vi undersøger sammenhænge imellem asthma, risikoadfærd og ikke allergisk komorbiditet.
3. Vi undersøger sammenhænge imellem inflammatoriske biomarkører, opsamlet igennem barndommen og ungdommen, og astma ved 18 år.

Metoder og resultater

Afhandling er baseret på data fra fødselskohorten, Copenhagen Prospective study on Asthma in Childhood (COPSAC₂₀₀₀), som er en højrisiko kohorte der består af 411 børn, født af mødre med astma. Børnene blev fulgt regelmæssigt fra de var en måned gammel og indtil de var 18 år med klinisk kontroller og omfattende dataindsamling.

Paper I. Design of the 18-year follow-up of the Danish COPSAC2000 birth cohort, *BMJ Paediatrics Open*, published

I **Paper I**, beskriver vi designet af 18 års opfølgningsbesøget for COPSAC₂₀₀₀ kohorten, hvor vi identificerer risikofaktorer for allergisk sygdom, fedme, og neuropsykiatrisk sygdom via

en omfattende og dybdegående dataindsamling som bl.a. indeholder milføfaktorer og biologisk materiale.

Paper II. Risk behaviours and non-atopic comorbidities of adolescents with asthma, World Allergy Organization Journal, published

I **Paper II** undersøger vi sammenhængen imellem astma ved 18 år, risikoadfærd og ikke allergisk-komorbiditet. Vi inkluderede fedme, og neuropsykiatrisk sygdom indsamlet fra patientjournaler, og elektroniske spørgeskemaer omhandlende selvskadende adfærd, og mental sundhed. Studiet viser en sammenhæng imellem neuropsykiatrisk komorbiditet, selvskadende adfærd og astma ved 18 år.

Paper III. Longitudinal trajectories of inflammatory biomarkers and their association with asthma, manuskript klargjort til submission

In **Paper III**, undersøger vi longitudinalt, inflammatoriske biomarkører igennem barndommen og ungdommen i COPSAC₂₀₀₀ kohorten for at påvise sammenhænge imellem specifikke mønstre for biomarkørerne og astma, samt astma-relaterede outcomes ved 18 år. Vi fandt en sammenhæng imellem specifikke mønstre for trombocytter igennem barndommen og ungdommen, og luftvejsobstruktion (FEV1) og luftvejshyperreaktivitet (PD20) ved 18 år. Disse fund indikerer at udviklingen i trombocytter over tid kan være involveret i patofysiologien ved astma.

Konklusion

I **Paper I** bidrager vi med viden om kohorten som ligger til grund for studierne inkluderet i afhandlingen. I **Paper II** identificerer vi risikoadfærd og ikke allergisk-komorbiditet som er associeret til astma og som bidrager med viden om den generelle sundhed hos unge med astma. I **Paper III** undersøger vi associationen imellem inflammatoriske biomarkører igennem barndommen og ungdommen og astma ved 18 år, og fremmer derved vores forståelse for patofysiologien ved astma og trombocytternes rolle.

INTRODUCTION

RESEARCH ENVIRONMENT

I joined the Copenhagen Prospective Studies on Asthma in Childhood in February 2021 and worked in the COPSAC clinic throughout my time as a PhD student. Besides joining the clinic in Gentofte, I was also part of a team responsible for the ABC clinic in Næstved, including planning and running visits, handling acute cases, and applying for funding for the clinic. The ABC clinic is a satellite clinic of COPSAC, following approximately 200 out of the 700 children from the COPSAC₂₀₁₀ cohort. During my time as a PhD student, I have spent half of my time collecting data and running clinical visits.

The data used in the thesis are pre-collected data from the 18-year follow-up visit in the COPSAC₂₀₀₀ cohort. During my time in COPSAC, I have collected identical data. This data will not be used in the thesis, but will be passed on for future research, a key point in the COPSAC spirit.

CLINICAL WORK AND DATA COLLECTION

During my time as a PhD student, I carried out clinical visits at COPSAC in Gentofte and at the ABC-clinic in Næstved. The visits were carried out in collaboration with the rest of the clinical team, including physicians and nurses. The visits included scheduled follow-up visits and acute visits concerning asthma, allergy, and eczema. During the visits, I was responsible for patient examination, diagnostics, and appropriate treatment. Furthermore, I participated in data collection using a wide array of assessments and questionnaires. During the visits, I was in close contact with the children and their families from both COPSAC₂₀₀₀ and COPSAC₂₀₁₀, including planning scheduled visits, conducting diagnostics, treating acute symptoms, and gathering data on atopic diseases. Alongside the work with the two exciting cohorts, I assisted with the inclusion of children in the COPSAC_{ACUTE} study, which was conducted in pediatric departments at the hospitals. After one year, I assisted with the clinical follow-up visit in the COPSAC clinic.

Besides participating in the work described above, a main assignment during my PhD has involved the COPSAC_{SEVERE}. Together with my colleagues in the COPSAC_{SEVERE} team, I have been responsible for implementing and running the study. The COPSAC_{SEVERE} is a national,

observational study of children and adolescents with severe asthma, recruited from pediatric departments across Denmark. The children in the cohort were followed for one year. Alongside study management, I participated in patient recruitment and inclusion, clinical baseline visits, acute visits, and the final one-year follow-up visit.

When I joined COPSAC, the 10-year follow-up of the COPSAC₂₀₁₀ cohort was ongoing, and COPSAC_{SEVERE} and COPSAC_{ACUTE} had just begun. Later, we conducted the 13-year visit, also from the COPSAC₂₀₁₀ cohort, which was a scheduled six-hour visit that included a broad range of clinical examinations, assessments, and questionnaires. I participated in these visits until they were completed at the end of my time.

RESEARCH EXCHANGE

From November to December 2024, I joined Professor Stuart Turvey's research group in the Division of Allergy and Immunology at the Department of Paediatrics, British Columbia Children's Hospital, Vancouver, Canada. The group is responsible for the Canadian Health Infant Longitudinal Development (CHILD) study in the Vancouver region. The study is a multicentre, longitudinal, and population-based birth cohort study of approximately 3,500 children enrolled prenatally and followed through childhood and adolescence. The study aims to investigate how environmental factors interact with genetic predispositions to influence children's health and development, such as the COPSAC cohorts.

During my stay, I gained valuable insight into how another research institution is run and how data is collected and handled, and I received input for our research projects.

BACKGROUND

CHILDHOOD ASTHMA

Asthma is the most common chronic, non-communicable disease in childhood ^{3,4}. In Western countries, one in five preschool children is diagnosed with asthma, and up to one-third suffer from frequent asthma-like symptoms ^{5,6}. Among adolescents, one in ten is diagnosed with asthma ⁵. This causes an economic burden to the patients, their families, the health care system, and, even more importantly, a significant social burden and impact on the quality of life ⁵.

The disease is characterized by chronic inflammation in the airways and reversible episodes of bronchial hyperresponsiveness, causing symptoms such as coughing, shortness of breath, and chest tightness ^{3,4,7,8}. Initially, the symptoms can be few, which can make the diagnosis difficult ^{9,10}. Traditionally, asthma was perceived as a homogeneous disease ¹¹. Today, we understand asthma as a much more diverse disease concerning clinical expression, and a disease influenced by the age of the child, gender, genetics, and environmental exposures ^{3,8,10,12}. The underlying mechanism of the disease is heterogeneous and is the consequence of an intricate interplay between environmental and lifestyle-induced factors and genetics ^{4,9,13}. It is suggested that proneness to asthma may be established in early childhood or even before birth, and implies a disrupted immune system development, leading to low-grade systemic inflammation, including airway inflammation ¹¹

Though asthma can resolve in some patients in childhood with mild disease, for most, the disease will persist in the long-term and become irreversible ¹⁴. It is not possible to cure asthma; it is only possible to treat and relieve the intensity and frequency of symptoms with medication ^{14,15}.

RISK BEHAVIOURS, COMORBIDITIES AND ASTHMA

Among adolescents, risk behaviours are a common part of teen life and are defined as voluntary actions that increase the risk of negative health consequences, although they also help establish autonomy and stimulate independence ¹⁶. Living with a chronic disease can influence the individuals' attitude towards risk behaviours. Research shows that adolescents and young adults living with a chronic disease are more likely to favor risk behaviours, e.g.,

alcohol consumption or cigarette smoking, compared to adolescents not live with a chronic disease ^{17,18}. Among American adolescents, the group living with asthma was more prone to report cigarette smoking, drug use, and binge drinking compared to the adolescents without asthma ^{2,17}.

Also, it has been discussed whether living with asthma also increases the risk of comorbidities, as other non-communicable, lifestyle-related diseases ^{2,19,20}. An overlap between asthma, depression, and anxiety is observed ¹⁹ and asthma, is related to neurodevelopmental disorders ²⁰ and obesity ²¹. A meta-analysis found a significant overlap between asthma and attention deficit hyperactivity disorder (ADHD) ²⁰ and further, asthma is associated with dyslipidaemia ²² and obesity ²¹. These observations propose a shared genetic, environmental, or lifestyle-induced mechanism in early life as a commonality between asthma and non-atopic comorbidities ^{1,2,19,20,22-24,10,13,25-28}.

INFLAMMATORY MARKERS AND ASTHMA THROUGH CHILDHOOD AND ADOLESCENTS

Asthma is an inflammatory disease of the airways ^{3,29,30}. In the past, the disease was perceived as one driven by T-helper 2 cells (Th2), causing Type 2 airway inflammation involving the release of cytokines and the production of Immunoglobulin E (IgE) with upregulation of eosinophils^{31,32}. Today, we know that this understanding of asthma is simplified ^{29,30}. In general, asthma is considered heterogeneous concerning the clinical presentation, the phenotypes, and the cellular responses; the endotypes ^{10,11,31}. The best described endotype is Th2 high, characterized by a high level of eosinophils ³³, whereas Th2 low inflammation is less studied ³¹, however, it is hypothesized that other inflammatory biomarkers are involved, such as neutrophils, lymphocytes, and platelets ³⁴⁻³⁶.

The role of neutrophilic inflammation in asthmatic patients has been studied in several studies, which find that neutrophilic inflammation dominates in certain asthma endotypes, differentiating them from endotypes dominated by eosinophils ^{35,37-39}. Furthermore, an association is found between elevated blood Neutrophil-Lymphocyte Ratio (NLR) and asthma⁴⁰, as well as between increased systemic inflammation and severe asthma ³⁶. In addition, platelets are involved in the inflammatory response by releasing spasmogens and interacting with other inflammatory cell types ⁴¹. Interestingly, research shows that platelets

express high-affinity receptors for IgE, and platelet upregulation is associated with severe allergic asthma ^{34,42}.

Finally, IgE is known to be central to the inflammatory response, and it is further suggested that other immunoglobulins, such as Immunoglobulin M (IgM), Immunoglobulin A (IgA), and Immunoglobulin G (IgG), are involved in the pathogenesis. One study finds that low levels of Immunoglobulin G (IgG) are associated with severe asthma ⁴³.

AIM AND OBJECTIVES

The overall aim of this PhD thesis was to enhance our understanding of asthma in adolescence and the relation to non-atopic comorbidities and risk behaviours. Furthermore, we aimed to longitudinally examine inflammatory biomarkers to identify systemic inflammation and its association with asthma and asthma-related outcomes.

Our hope for this work is to improve the understanding of the relationship between asthma and other non-communicable diseases and the link to low-grade systemic inflammation

The specific objectives of the three studies are outlined below.

- I. The aim of the first study was to outline the design of the 18-year follow-up visit from the COPSAC₂₀₀₀ cohort, where risk factors of atopic diseases, obesity, and neuropsychiatric disorders were identified through an extensive characterisation of the environment, together with a deep clinical phenotyping and longitudinal biosampling.
- II. The aim of the second study was to investigate the association between asthma status at the 18-year follow-up visit of the COPSAC₂₀₀₀ cohort and risk behaviours and non-atopic comorbidities.
- III. The aim of the third study was to explore inflammatory biomarkers longitudinally through childhood and adolescence in COPSAC₂₀₀₀, aiming to identify associations between trajectories of inflammatory biomarkers and asthma and asthma-related outcomes at 18 years.

Conclusion

To conclude, **Paper I** provides knowledge of the cohort used in the thesis. **Paper II** identifies risk behaviours and non-atopic comorbidities associated with asthma and contributes to knowledge of the general health of adolescents living with asthma. **Paper III** explores biomarkers of systemic inflammation across childhood and adolescence and their association with asthma at 18 years, advancing our understanding of asthma pathogenesis and the role of platelets in its inception.

METHODS

DESIGN, SETTING, AND PARTICIPANTS

Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) is a research institution located at Herlev-Gentofte Hospital focusing on pediatric asthma research. It was founded more than 25 years ago, aiming to develop evidence-based prevention strategies for childhood asthma. The Research institution consists of two mother-child birth cohorts. The COPSAC₂₀₀₀ and COPSAC₂₀₁₀. COPSAC₂₀₁₀ is a population-based longitudinal cohort including 736 pregnant women and their 700 children, and clinical follow-up is still ongoing. This thesis is based on data from the COPSAC₂₀₀₀ cohort, which is described in more detail below.

COPSAC₂₀₀₀

The COPSAC₂₀₀₀ is a single-center, prospective mother-child birth cohort including 411 individuals, all born to mothers with asthma. It is a high-risk cohort, as all mothers have a history of asthma. All mothers were recruited during pregnancy from the Danish National Birth Cohort (DNBC), and they all lived in the Capital region of Copenhagen, Denmark ⁴³. The DNBC is a prospective cohort including more than 100,000 women enrolled from all regions of Denmark ⁴⁴. The main aim of the DNBC is to investigate the causal link between early life exposures and disease manifestation later in life ^{1,2}.

Children were enrolled at one month of age if they fulfilled the inclusion criteria. These were: 1. A gestational age > 36 weeks, 2. A history without any mechanical ventilation or any respiratory tract infections, and 3. Children without any severe congenital abnormalities ⁴³. After inclusion, the children visited the COPSAC research unit for scheduled clinical visits every six months until the age of 7, and again at ages 12 and 18. Furthermore, the children visited the COPSAC research unit if any acute airway symptoms or skin symptoms appeared ^{1,2}.

Figure 1.1 Flowchart COPSAC₂₀₀₀¹

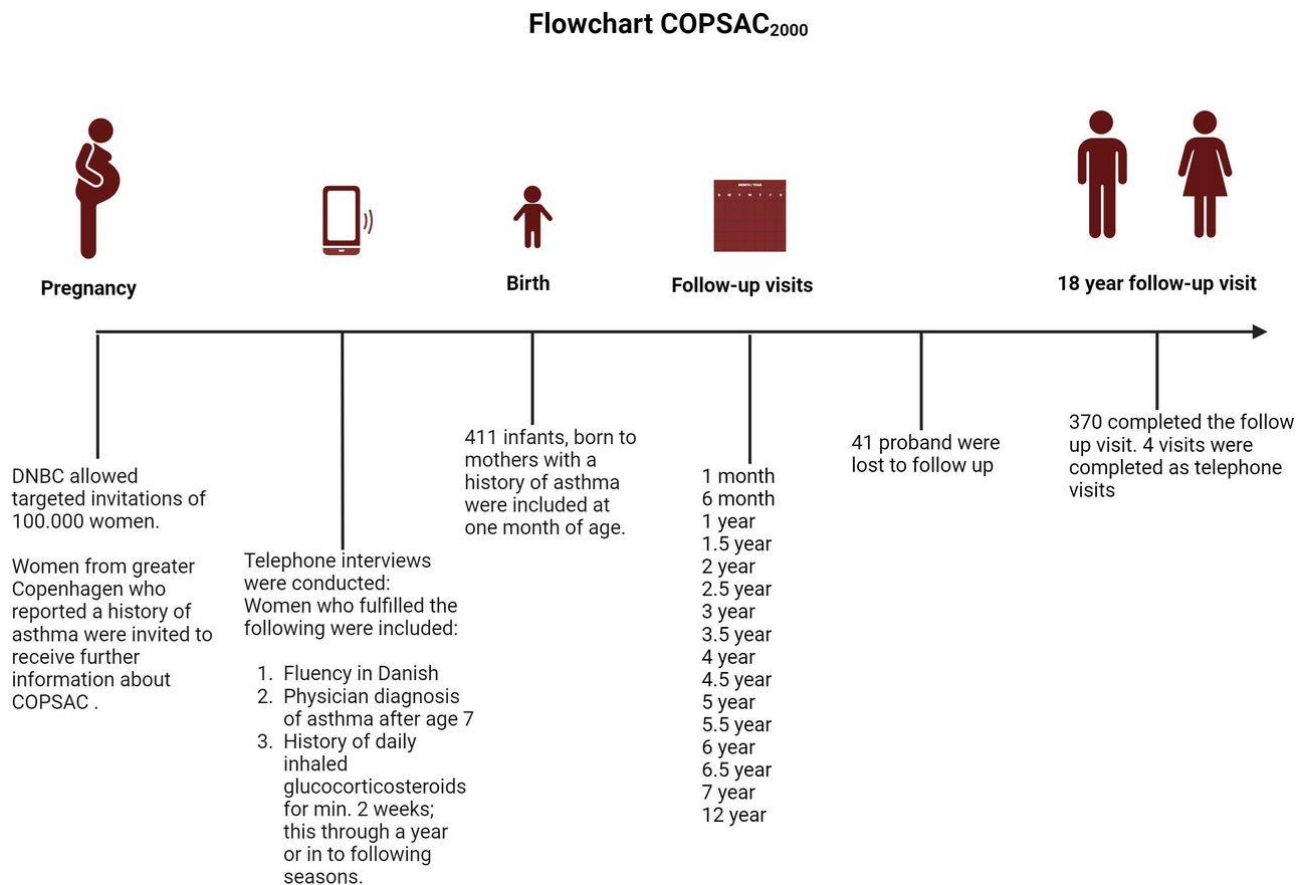


Figure 1.1. Flowchart COPSAC₂₀₀₀. Copyright by T. Mølbaek-Engbjerg, BMJ Paediatrics Open¹

The 18-year follow-up visit was extensive, providing a thorough characterization of the environment, exposures, and risk behaviours ¹. This, along with a wide collection of biomaterials such as hair, blood, urine, feces, throat and skin swaps, nasal lining fluid and scraping, and a deep metabolic characterization and multi-organ investigations ¹. The metabolic status was characterized by a meal challenge after fasting, together with continuous flash glucose monitoring and a food photo diary in the following two weeks ¹. Also, physical activity was measured using an omnidirectional accelerometer that continuously assessed activity, and a gastrointestinal questionnaire regarding symptoms and the Bristol Stool Chart was completed ^{1,45}. Bones and muscles were characterized by

measuring Bioelectrical Impedance Analysis (BIA), handgrip strength, and bone density ^{1,46}. The vibration perception threshold was measured using a biothesiometer and cardiorespiratory fitness was estimated using the Danish Step test ^{1,47}. Finally, multiple ophthalmological assessments were collected. We measured the axial length of the eyes, uncorrected visual acuity, refractive error, and best-corrected visual acuity. Also, we obtained a three-dimensional image of the macula and the optic disk and a color fundus photography ^{1,48}.

At the visit, we conducted a physical examination, primarily focusing on skin details, diagnosing eczema in subjects with skin issues following Hanifin & Rajka criteria ^{1,49}. We diagnosed allergic rhinitis based on allergic sensitization and a corresponding history of sneezing, blocked, runny, or itching nose upon relevant exposure in the past 12 months, in periods without a concurrent cold ^{1,50}.

Figure 1.2 18-year follow-up visit¹

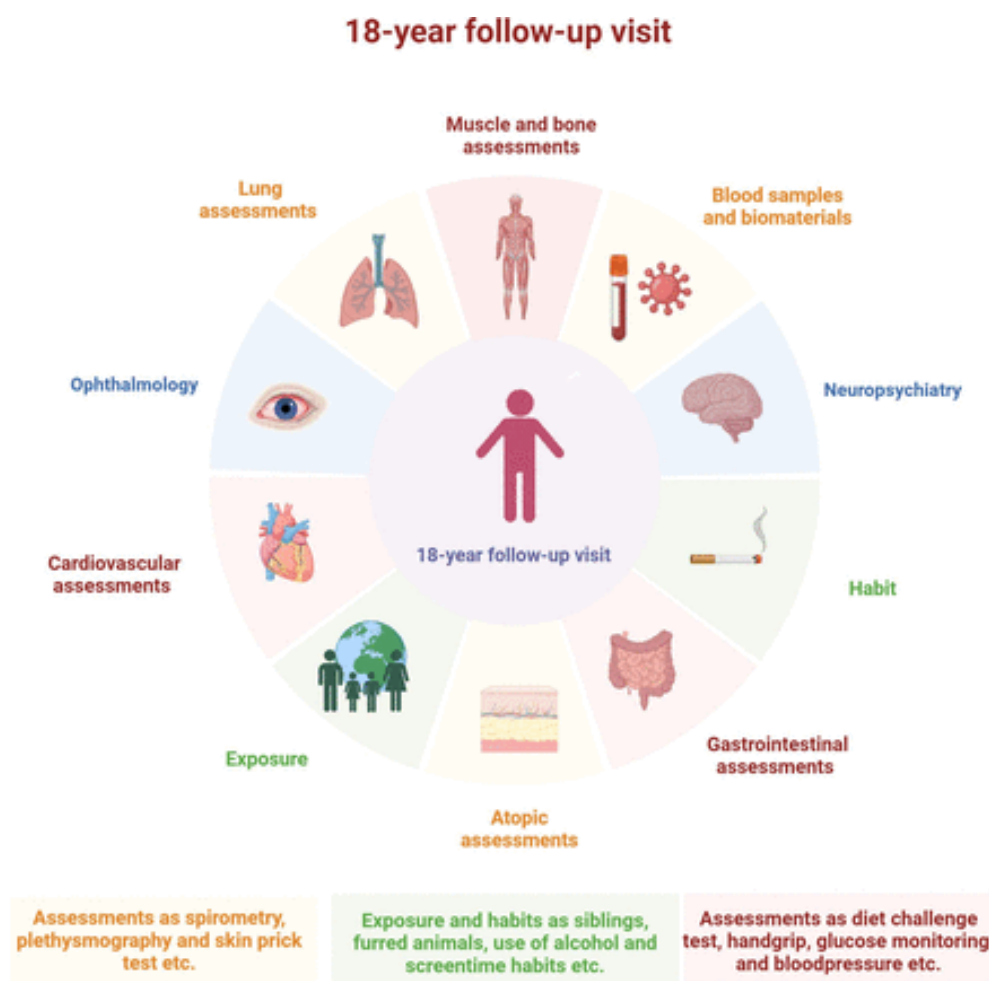


Figure 1.2. 18-year follow-up visit. Copyright by T. Mølbaek-Engbjerg, BMJ Paediatrics Open¹

ENVIRONMENTAL EXPOSURES, HABITS AND RISK BEHAVIOURS

In **Paper I** and **Paper II**, we included environmental information such as educational level, other children living at home, exposure to pets, passive smoking, and fireplaces ^{1,2}.

In **Paper I** and **Paper II**, we included information on habits and risk behaviours thoroughly. At the 18-year follow-up visit, we interviewed the participants about their risk behaviours, including alcohol consumption, binge drinking, tobacco smoking, drug use, screen time use, and self-destructive behaviour. The participants were interviewed without their parents being present ^{1,2}.

Alcohol consumption: We asked the participants if they had ever consumed more than one unit of alcohol. Further, they were asked about their alcohol consumption in the past year. This included frequency of drinking (never, max once per month, 2-4 times per month, 2-3 times per week, or $\geq$ four times per week), and numbers of units per event (0 units, 1-2 units, 3-4 units, 5-6 units, 7-9 units, or $\geq$ 10 units) ². At last, they were asked about the number of episodes in which they consumed five or more units of alcohol on a single event, i.e., binge drinking ^{1,2}.

Tobacco smoking: We collected information about tobacco smoking from the participants. They were asked details on their current smoking status, frequency of smoking (daily, weekly, less than weekly, or never), and estimated daily cigarette consumption over the past four weeks. We defined current smoking as smoking within the last week, including daily smoking, and occasional smoking was defined as smoking less than weekly, but within the previous four weeks ^{1,2}.

Drug use: We interviewed the participant about their previous experience with drug use. The information was collected by answering an online questionnaire (part of the Depression, Anxiety, and Stress Scale (DASS21 ⁵¹), and, if relevant, which substances they had tried, including cannabinoids, amphetamine, ecstasy/3,4-methylenedioxy-N-methylamphetamine (MDMA)/fantasy, cocaine, opiates (such as heroin, morphine, or methadone), Lysergic Acid Diethylamide (LSD), sedatives, inhalation of lighter gas, inhalation of nitrous oxide ("laughing gas"), hallucinogenic mushrooms, or others ^{1,2,51}. They were then asked to specify

the frequency of their drug use, which was grouped as never, once, less than once per month, monthly, weekly, almost daily, or daily ^{1,2}.

Self-destructive behaviour: We got details on the frequency of self-destructive behaviours, e.g. cutting, scratching, hitting, and overeating, from a part of the DASS21 online questionnaire ^{1,2,51}.

Screen time: We interviewed participants about their screen time habits over the past year, distinguishing between screen time on a telephone, a tablet, a TV, or a computer. Screen time was grouped based on use during weekdays (Monday to Thursday) and weekends (Friday to Sunday), as the average number of hours spent on screen during leisure time ^{1,2}. The total screen time was taken as a sum of TV screen, telephone, tablet, and computer, and weekday and weekend screen time were combined ². High daily screen time use was defined as exceeding 8 hours per day ².

NON-ATOPIC COMORBIDITY

Neuropsychiatric diagnosis: In **Paper I** and **Paper II**, we included neuropsychiatric diagnosis as a clinical outcome. The diagnoses were obtained from medical records based on ICD-10 criteria, including any neuropsychiatric diagnoses, past and present. The diagnoses were grouped into the following categories: ADHD, autism spectrum disorder, anxiety, depression, eating disorder, mental retardation, personality disorder, schizophrenia, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), and others ^{1,2}.

At the 18-year visit, three electronic questionnaires on behavioural traits and psychopathology were completed: 1. The Strengths and Difficulties Questionnaire (SDQ) ^{53,54}, 2. The Adult ADHD Self-Report Scale (ASRS) ⁵⁵, and 3. The DASS21 ⁵¹ (**Appendix Paper I, Supplementary**) ².

BMI and obesity: Weight was measured under standard conditions using a calibrated digital scale ^{1,2}. Height was measured by a Harpenden stadiometer (Holtain Ltd, Crymych, Dyfed, Wales) as previously described ^{1,2,43}. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and categorised according to the WHO definition as underweight: BMI<18.5; normal weight: 18.5–25; overweight: 25–30, and obesity: >30 ⁵⁶.

BLOOD BIOCHEMISTRY MEASURES

In **Paper III**, we used venous blood samples collected fasting from the participants at the beginning of the visit at ages 4, 5, 6, 7, 12, and 18 years. We employed longitudinal assessments of blood neutrophil, lymphocyte, eosinophil, and platelet counts, NLR, and total IgE, IgA, IgM, and IgG levels. To measure haematological biomarkers, the blood samples were collected in Vacuette tubes containing K₃ EDTA, and cell concentrations were measured as 10⁹ cells/L using the ADVIA 2120i Hematology system from Siemens Healthcare Diagnostics. Immunoglobulins were measured in blood samples collected in Vacuette tubes containing a Lithium-Heparin anticoagulant mixture. The material was mixed with antisera to produce immune complexes, which were available for measurements, and the concentration of immunoglobulins was measured as g/L using Vitros 5.1 F. s from Ortho-Clinical Diagnostics. We included all participants in the analysis who had at least 2 blood biochemistry measurements for any of the collected biomarkers during scheduled visits.

LUNG FUNCTION TESTS

We measured lung function tests by spirometry (MasterScope Pneumoscreen, Erich Jäeger, Würzburg, Germany) ^{1,2,57,58}. Forced Expiratory Volume at 1 second (FEV1) and Forced Vital Capacity (FVC) were recorded at baseline and again after inhalation of a short-acting beta-2 agonist ^{1,2}. Specific airway resistance (sRaw) was assessed by whole-body plethysmography (MasterScope Bodybox, Erich Jäeger, Würzburg, Germany) ^{1,2,59}. All measurements were conducted in triplicate. Airway obstruction was measured with FEV1.

Airway hyperresponsiveness (AHR) was assessed by a methacholine challenge test by inhaling a dose of ≤8 mg/ml methacholine in combination with an exercise test ^{1,2,60}. The provoking dose, 20% (PD20) was recorded. Bronchial reactivity was assessed by methacholine provocation using the APS Pro, CareFusion, 234 GmbH, Germany ⁶⁰.

Inflammation in the airways was measured with Fractional exhaled nitric oxide (FeNO) using the Denox 88 version 88 DX0256, Switzerland ⁶¹.

ASTHMA DIAGNOSIS

In **Paper I**, **Paper II** and **Paper III**, the asthma diagnosis at 18 years is used as a clinical outcome. The asthma diagnosis was measured by the COPSAC physicians following a

predefined SOP ^{1,2,10,43} and the final diagnosis used in the analysis following the European Respiratory Society recommendations ⁶², an asthma diagnosis was confirmed if at least two of the following criteria were fulfilled: 1. Obstructive lung function (FEV₁<80% and/or FEV₁/FVC<80% and/or sRaw>1.6kPa/s); 2. Bronchodilator reversibility (change of FEV₁≥12% and/or sRaw≥30% from baseline after inhalation of short-acting beta-2-agonist); 3. Airway inflammation (FeNO≥25 ppb); and/or 4. Bronchial hyperresponsiveness as measured by methacholine challenge test (drop in FEV₁≥20% from baseline by a dose of methacholine ≤8 mg/ml) and/or exercise test (≥10% decrease in FEV₁ from baseline) ^{1,2}. Remission was defined as no symptoms or asthma treatment for one year ^{1,2}.

STATISTICAL CONSIDERATIONS

Data management and processing were conducted with the statistical software R (R Foundation for Statistical Computing, R version 4.0.3 (2020-10-10), and R version 4.4.0 (2024-04), Vienna, Austria) using a significance cut-off of p-values of 0.05.

Statistical approach of Paper I: We analysed the data descriptively using counts (N%), mean ± standard deviation (SD), and median Interquartile Range (IQR). Missing values were treated as missing observations. A t-test or X² test was run to assess differences between genders. We used the Wilcoxon test to handle non-normal distributions.

Statistical approach of Paper II: We analyzed the associations between asthma and risk behaviours, obesity, and neuropsychiatric disorders by utilizing logistic regression models. The models were run as a univariate and multivariable regression analysis adjusted for gestational age (GA), sex, mother's age, breastfeeding, smoking during pregnancy, mother's education, and household income.

In the multivariable model, we added risk behaviours and non-atopic comorbidities: current and occasional smoking, binge drinking, drug use, high daily screen time use, self-destructive behaviour, obesity, and neuropsychiatric disorder. We wanted to elucidate which variables explained the most and simplify the model. Therefore, a backward selection procedure was used, dropping the parameter with the highest p-value and retaining only the variable with $p < 0.10$ in the final model.

At last, we examined the interaction with sex by adding a post-hoc analysis stratified for sex. Given the study's explorative nature, no adjustment for multiple comparisons was made.

Statistical approach of Paper III: We explored inflammatory blood biochemistry measures longitudinally through childhood and adolescence, focusing on identifying associations between trajectories of inflammatory blood biochemistry and asthma, and asthma-related outcomes at 18 years. To do this, we proceed as follows. First, we examined the cross-sectional association at 18 years between selected blood biochemistry measures, asthma diagnosis, and asthma-related outcomes, such as FEV1, FeNO, sRaw, and PD20 (AHR). To do this, we used linear regression models. Second, we used linear mixed models (LMM) to examine the longitudinal association between inflammatory blood biochemistry measures and asthma and asthma-related outcomes at 18 years. Third, by using latent class trajectory (LCT), we examined the same associations.

In the LMM analyses, blood biochemistry measures were used to produce longitudinal models. We used fixed effects with a variable intercept and random effects with both variable intercepts and variable slopes. We included participants' ID numbers to avoid repeated measurements. To produce the models, we included a baseline level at age 4 years (intercept) and the age-related change from 4 to 18 years (slope). This was applied for each biomarker, allowing each child to have their own rate of change over time. Then, we extracted the subject-specific intercepts and slopes from the model and examined their associations with asthma and asthma-related outcomes using linear regression. Finally, we scaled the estimated intercepts and slopes, and the estimates in the following model were therefore interpreted per 1 SD.

Furthermore, we applied LCT modelling to longitudinal blood biochemistry data to identify clear developmental patterns over time. First, we evaluated the model performance from alternative polynomial age functions (linear, cubic, and quadratic) by comparing them using the Bayesian Information Criterion (BIC). Then, for each selected growth function, we fitted the models with two to four latent classes. At last, we evaluated the model fit utilizing a combination of goodness-of-fit metrics, including BIC, entropy, and class size. We selected

the optimal model for each biomarker based on the lowest BIC value, highest entropy, reasonable class size, and clinical interpretation of the final trajectories ⁶³.

In the cross-sectional analysis, LMM and LCT models were produced unadjusted at first, followed by adjusting for sex, gestational age (GA), mother's age at delivery, mother's highest completed education, and household income at the child's second birthday, smoking and antibiotic use during pregnancy, and passive smoke exposure in childhood.

ETHICAL CONSIDERATIONS

The cohort used for **Paper I**, **Paper II** and, **Paper III** was approved by the local Ethics Committee and the Danish Data Protection Agency. The studies followed the guiding principles of the Declaration of Helsinki. The approvals for COPSAC₂₀₀₀ from the Local Ethics Committee were H-B-2008-093 and H-16039498. The approvals for COPSAC₂₀₀₀ from the Danish Data Protection Agency were 2015-41-3696. The participants' parents provided written informed consent before enrolment. The participant gave written content as the reach the age of 18 years ^{1,2}.

RESULTS

PAPER I. DESIGN OF THE 18-YEAR FOLLOW-UP OF THE DANISH COPSAC₂₀₀₀ BIRTH COHORT

In **Paper I**, we outlined the design of the 18-year follow-up visits from the COPSAC₂₀₀₀ cohort, bringing knowledge of the health and habits of Danish adolescents.

Demographics: Out of the 411 children in the cohort, 370 (90%) completed the 18-year visit. The mean age of the participants was 17.7 years, and 49.4% were males. The cohort consisted of participants from urban (47%) and rural (53%) environments, with an even distribution. Demographics are detailed in **Table 1.1**.

Environmental exposures and habits: Among participants who completed the 18-year follow-up, 61.1% were susceptible to passive smoking, and 29.7% were susceptible for >100 days per year. A total of 31.4% were currently smoking, and of these, 24.1% smoked daily. Of the participants, 68.7% drank > 4 units per event, and 22.2% drank > 10 units per event. Furthermore, 23.8% had experience with drug use. Among the participants, the mean screen time was 6.0 hours per day, including games, phones, and television. The mean screen time was higher among males vs. females: 6.7 vs. 5.9 hours ($p < 0.01$) (**Table 1.1, Figure 1.2**).

Anthropometric assessments: Among participants, males and females had an even distribution of BMI, and we did not observe a significant difference between the genders (p -values > 0.51). At the 18 year-visit, the mean (SD) BMI was 22.8 kg/m² (0.39) for males and 23.3 kg/m² (0.42) for females (**Table 1.1, Figure 1.2**).

Table 1.1 Demographics¹

Cohort	DNBC*****		COPSAC2000		
Demographics	All N (%)	All N (%)	Female N (%)	Male N (%)	p-value****
Gender	44485 (58.6 female)	370 (90)	188 (50.8)	182 (49.2)	0.35
Parents' divorce	13394 (30.1)	142 (38.4)	74 (39.4)	68 (37.4)	0.77
Race (Caucasian)	N/A	355 (96.0)	183 (97.3)	172 (95.0)	0.34
Proband education					
Elementary school, completed	N/A	357 (96.5)	182 (96.8)	175 (96.2)	1.00
College, started	35280 (83.6)	288 (77.8)	155 (82.4)	133 (73.1)	0.07
Tradesman, started	4331 (10.3)	38 (10.3)	9 (4.8)	29 (15.9)	< 0.01
University, started	361 (0.9)	1 (0.3)	-	1 (0.51)	-
Parents education, completed					
Elementary school, mother	N/A	13 (3.51)	8 (4.26)	5 (2.74)	0.41
Elementary school, father	N/A	17 (4.60)	11 (5.85)	6 (3.30)	0.23
High school, mother	N/A	6 (1.62)	6 (3.19)	-	-
High school, father	N/A	7 (1.89)	5 (2.66)	2 (1.10)	0.26
Tradesman, mother	N/A	94 (25.4)	39 (20.7)	55 (30.2)	0.10
Tradesman, father	N/A	102 (27.6)	47 (25.0)	55 (30.2)	0.43
University, medium long, mother	N/A	125 (33.8)	62 (33.0)	63 (34.6)	0.93
University, medium long, father	N/A	70 (18.9)	35 (18.6)	35 (19.2)	1.00
University, long, mother	N/A	69 (18.6)	41 (21.8)	28 (15.4)	0.12

University, long, father	N/A	82 (22.2)	40 (21.3)	42 (23.1)	0.83
Fireplace					
1-100 days	N/A	137 (37)	63 (33.5)	74 (40.7)	0.20
> 100 days	N/A	38 (10.3)	18 (9.6)	20 (11.0)	0.53
Furred animals					
Cats, yes	N/A	100 (27.0)	52 (27.7)	48 (26.4)	0.87
Dogs, yes	N/A	181 (48.9)	96 (51.1)	85 (46.7)	0.46
Other children younger, yes	N/A	218 (58.9)	105 (55.9)	113 (62.1)	0.47
Other children older, yes	N/A	87 (23.5)	46 (24.5)	41 (22.5)	0.89
Current smoking	9639 (21.7)	116 (31.4)	58 (30.9)	58 (31.9)	0.38
Every day*	3335 (34.6)	28 (24.1)	15 (25.9)	13 (22.4)	0.71
Min ones every week*	2193 (22.8)	37 (31.9)	15 (25.9)	22 (37.9)	0.25
Min ones every month*	4111 (42.7)	51 (44.0)	28 (48.2)	23 (39.7)	0.48
Number of cigarettes smoked in the last 4 weeks**					
1-4 per day	2879 (52.1)	82 (80.4)	36 (73.5)	46 (86.8)	0.35
5-9 per day	1326 (24)	11 (10.8)	9 (18.4)	2 (3.8)	0.08
> 9 per day	1189 (21.5)	9 (8.8)	4 (8.1)	5 (9.4)	0.74
Passive smoking					
1-100 days per years	N/A	116 (31.4)	58 (30.9)	58 (31.9)	0.92
> 100 days per year	N/A	110 (29.7)	61 (32.4)	49 (26.9)	0.29
Drug use ever	14673 (33)	87 (23.8)	31 (16.8)	56 (30.8)	0.69
Hash / Pot	13907 (94.8)	60 (70.0)	23 (74.2)	37 (66.1)	0.06

Cocaine	1804 (12.3)	22 (25.3)	8 (25.8))	14 (25.0)	0.26
Laughing gas (NO)	1464 (10)	18 (20.7)	3 (9.7))	15 (26.8)	0.01
Ecstasy / MDA / Fantasy	1317 (9)	12 (13.9)	5 (16.1))	7 (12.5)	0.75
Others***	2979 (20.3)	34 (39.1)	11 (35.5)	23 (41.1)	0.04
Alcohol; drinking frequency					
Never	3273 (7.4)	3 (1.0)	-	3 (1.6)	0.14
Max once per month	10017 (22.5)	89 (24.1)	49 (26.1)	40 (22.0)	0.46
> 1 per month	21195 (70.1)	253 (68.4)	124 (66.0)	129 (70.9)	0.46
Alcohol; units					
1-4	11853 (28.8)	88 (23.8)	51 (27.1)	37 (20.3)	0.26
5-9	21952 (53.3)	172 (46.5)	93 (49.5)	79 (43.4)	0.41
> 10	7407 (18)	82 (22.2)	29 (15.4)	53 (29.1)	0.91
BMI < 18.5 (%) Underweight	4395 (9.9)	32 (8.7)	17 (9.20)	15 (8.2)	0.72
BMI 18.5-24.99(%) Normal weight	32336 (72.7)	249 (67.8)	121 (65.4)	128 (70.3)	0.66
BMI 25-30 (%) Overweight	5770 (13)	57 (15.5)	31 (16.8)	26 (14.3)	0.51
BMI > 30 (%) Obese	1984 (4.5)	29 (7.9)	16 (8.7)	13 (7.1)	0.58
Screen time					
Total screen time hours, median (IQR)	N/A	6.0 (4.5-7.0)	5.90 (4.5-6.5)	6.7 (5.0-7.9)	< 0.01
Asthma, current	N/A	93 (25.1)	46 (24.4)	47 (25.8)	0.85
Self-injury	N/A	73 (21)	56 (31.1)	17 (9.9)	0.92
Self-esteem all (mean/sd)	N/A	3.58 / 0.80			
1		5 (1.35)	3 (3.23))	2 (1.10)	1.00
2		18 (4.86)	12 (12.9)	6 (3.30)	0.26

3		142 (38.4)	81 (87.1)	61 (33.5)	0.08
4		162 (43.8)	77 (82.80)	85 (46.7)	0.29
5		40 (10.8)	14 (15.1)	26 (14.3)	0.05
Neuropsychiatric disorder ever	N/A	62 (16.8)	30 (16.0)	32 (17.6)	0.80

Table 1.1 Demographics

*Only for current smokers.
 ** Only for current smoker. Data available from 102 proband.
 *** Etc. Amphetamine, sedatives, LSD, opiates, euphoric mushrooms, lighter gas.
 **** t-test comparing significance between the gender
 *****Absolute and relative frequencies for a sample of complete response observations from the 18-year follow-up of Danish National Birth Cohort participants.

Blood samples and Immunological outcomes: The results of the blood samples are provided in the supplementary appendix, where blood samples are presented with mean (SD) or median (IQR) and percentage of the participants placed outside the normal reference interval (**Appendix Paper I, Supplementary, Tables S1, S3**).

Figure 1.2 Illustration of the assessments described at the 18-year visit¹

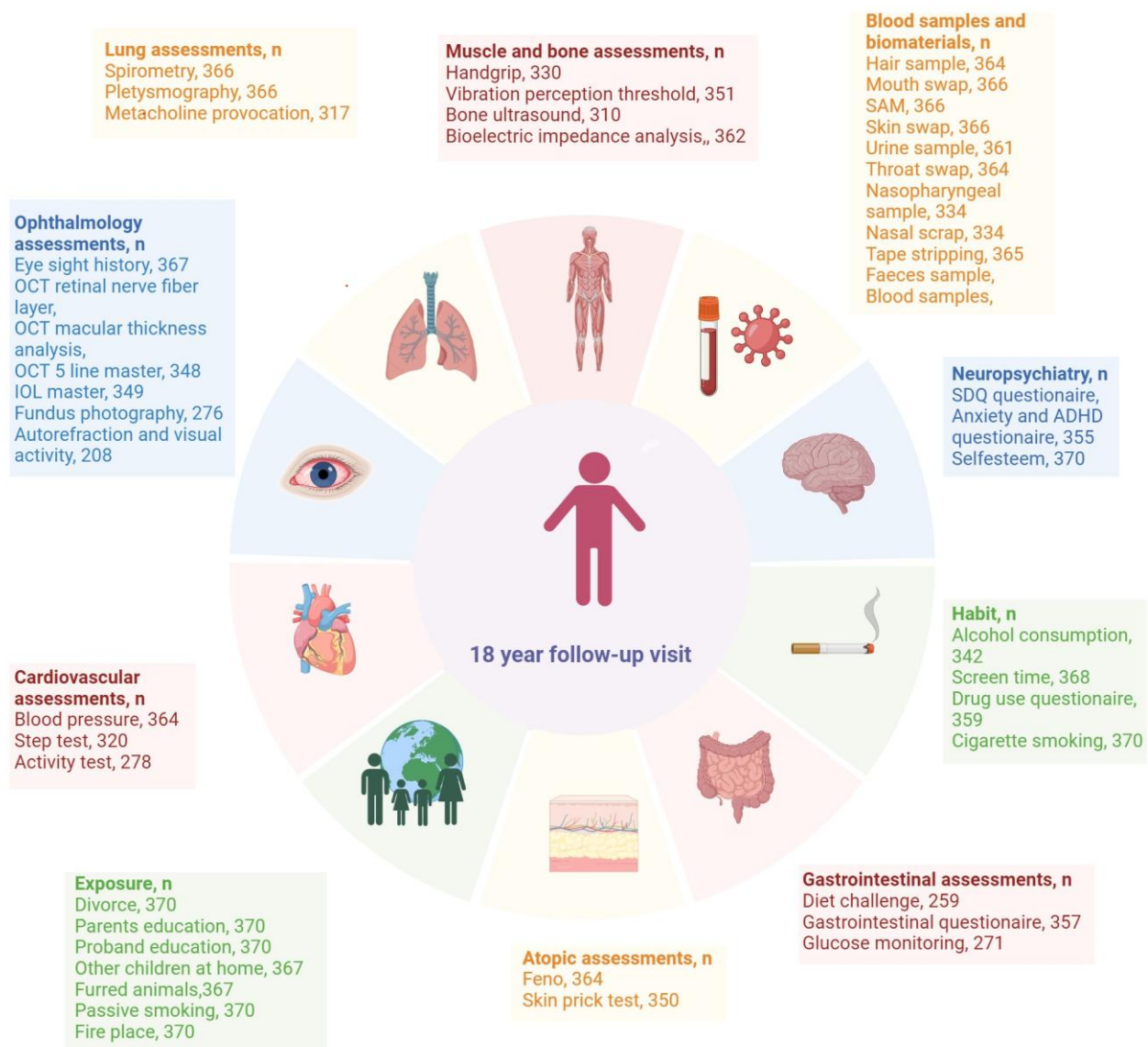


Figure 1.2 Illustration of the assessments described at the 18-year visit

ADHD, attention deficit hyperactivity disorder; SDQ, Strengths and Difficulties Questionnaire; OCT, optical coherence tomography; IOL, Intraocular Lens. Copyright by T. Mølbaek-Engbjerg, BMJ Paediatrics Open¹

Atopic assessment and diagnoses: At age 18, 25.1% of the participants were diagnosed with asthma. Furthermore, 38.6% of participants had allergic rhinitis, and 10.3% had atopic dermatitis (**Table 1.2**). There was no difference between the genders in relation to the presence of asthma at age 18; however, we observed that more females than males had atopic dermatitis ($p=0.05$), and we noticed a trend of more allergic rhinitis among males compared to females ($p=0.06$). Furthermore, we noticed that, broadly, more males than females had unfavourable lung functions and allergy test compared to females.

Results from the spirometry showed that among the participants 46.8% had an FEV1 < 90% and 9.5% of the participants FEV1 < 80%. When examine airway resistance, 7.6% had sRaw > 1.6 kPa/s, and FENO > 25 ppb was observed in 28.1% of the participants. A positive methacholine provocation test, specified as a reduction of 20% in FEV1 after inhalation of a dose <8 µmol/ml methacholine, was seen among 4.6% of the participants. According to the skin prick test of the participants, it was positive for food in 7.6% and aeroallergens in 47.3%. (**Table 1.2**).

Table 1.2 Atopic assessments and diagnoses¹

Atopic assessments and diagnoses	All N (%)	Females N (%)	Males N (%)	p-value****
Spirometry, FEV 1*				
FEV1, L < 80%	35 (9.5)	11 (5.9)	24 (13.2)	0.03
FEV1, L < 90%	173 (46.8)	93 (49.5)	80 (44.0)	0.31
sRaw				
sRaw kPa/s > 1.6 (%)	28 (7.6)	12 (6.4)	16 (8.8)	0.52
Methacholine provocation, median PD20 / µmol **	225.40	186.2	298.06.00	0.01
% < cut off	17 (4.6)	8 (4.3)	9 (5.0)	
Feno > 25 ppb*** (%)	104 (28.1)	40 (21.3)	64 (35.2)	< 0.01
IgE total x 103 IU/L, Median (IQR) ***	80.0 (28.0 -238.1)	69.4 (25.0-216.5)	103.9 (32.4- 262.6)	0.06
Positive specific IgE any kU/L, N (%) ***	192 (51.9)	80 (42.6)	112 (61.5)	< 0.01
Positive specific IgE food kU/L, N (%) ***	72 (19.5)	34 (18.1)	38 (20.9)	0.6
Positive specific IgE inhalation kU/L, N (%) ***	188 (50.8)	78 (41.5)	110 (60.4)	< 0.01
Positive SPT any (%)	176 (47.6)	75 (39.9)	101 (55.5)	< 0.01
Positive SPT food (%)	28 (7.6)	11 (5.9)	17 (9.3)	0.23
Positive SPT inhalation (%)	175 (47.3)	74 (39.4)	101 (55.5)	< 0.01
Asthma, current (%)	93 (25.1)	46 (24.4)	47 (25.8)	0.86
Asthma, ever (%)	164 (44.3)	77 (41.0)	87 (47.8)	0.44
Rhinitis, current (%)	143 (38.6)	62 (33.0)	81 (44.5)	0.06
Atopic dermatitis, current (%)	38 (10.3)	25 (13.3)	13 (7.14)	0.05

Table 1.2. Atopic assessments and diagnoses

*Calibrated for sex, height, and age.

**PD20 is the median dose of methacholine that causes a 20 % reduction in FEV1. The test is positive when a 20 % reduction in FEV1 is caused

by a PD20 < 8 µmol.

***Normal range Feno < 25 ppb

**** Significance between the gender is calculated with t-test. In cases of not normal distributed data Wilcoxon test has been used.

PAPER II. RISK BEHAVIOURS AND NON-ATOPIC COMORBIDITIES OF ADOLESCENTS WITH ASTHMA

In **Paper II**, we examine the association between asthma and risk behaviours, obesity, and neuropsychiatric comorbidities at 18 years of age.

Demographics: A total of 370 (90%) of the 411 participants in the cohort completed the 18-year follow-up visit. Of the participants, 93 (25.1%) had coexisting asthma. Demographics are detailed in **Table 2.1**.

Table 2.1 Demographic information of the probands included in the COPSAC₂₀₀₀ cohort at the 18-year visit²

Demographics, N (%)	All N (%) N=370	Current asthma N=93	Not current asthma N=277	p-value
Allergic rhinitis, current	143 (38.6)	62 (66.7)	81 (29.2)	< 0.001
Atopisk dermatitis, current	38 (10.3)	15 (16.1)	23 (8.3)	0.19
Parents' divorce	142 (38.4)	38 (40.9)	104 (37.5)	0.66
Race (Caucasian)	355 (96.7)	89 (95.7)	266 (96.0)	1.00
Fireplace				0.92
1-100 days	137 (37.0)	36 (38.7)	101 (36.5)	
> 100 days	38 (10.3)	9 (9.7)	29 (10.5)	
Furred animals				
Cats, yes	98 (26.5)	21 (22.6)	77 (27.8)	0.40
Dogs, yes	179 (48.4)	43 (46.2)	136 (49.1)	0.74
Passive smoking				0.03

1-100 days per years	116 (31.4)	36 (38.7)	80 (28.9)	
> 100 days per year	110 (29.7)	18 (19.4)	92 (33.2)	
Other children younger, yes	217 (58.6)	46 (49.5)	171 (61.7)	0.12
Other children older, yes	87 (23.5)	26 (28.0)	61 (22.0)	0.45
Proband education				0.38
Elementary school, completed	357 (96.5)	91 (97.8)	266 (96.0)	
College, started	288 (77.8)	72 (77.4)	216 (78.0)	
Tradesman, started	38 (10.3)	5 (7.94)	33 (12.0)	
University, started	1 (0.3)	1 (1.1)	-	
Parents education, completed				0.33
Elementary school, mother	13 (3.5)	5 (5.4)	6 (2.2)	
Elementary school, father	17 (4.6)	4 (4.3)	13 (4.7)	
High school, mother	6 (1.6)	1 (1.1)	5 (1.8)	
High school, father	7 (1.9)	4 (4.3)	3 (1.1)	
Tradesman, mother	94 (25.4)	33 (35.5)	61 (22.0)	
Tradesman, father	102 (27.6)	23 (24.7)	79 (28.5)	
University, medium long, mother	125 (33.8)	29 (31.2)	96 (34.7)	
University, medium long, father	70 (18.9)	12 (12.9)	58 (20.9)	
University, long, mother	69 (18.6)	16 (17.2)	53 (19.1)	
University, long, father	82 (22.2)	25 (26.9)	57 (20.6)	

Table 2.1 Demographics
The table presents demographic information of the probands included in the COPSAC₂₀₀₀ cohort at the 18-year visit.

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Risk behaviours and the association with asthma

Alcohol consumption: Alcohol consumption among participants with asthma showed that 64.5% reported drinking more than once per month, and at events where they drank, 75.2% drank ≥ 5 units (**Table 2.2**). We did not observe any significant association between asthma status and binge drinking. This result was similar in both the univariate and multivariable models (**Table 2.3**).

Tobacco smoking: In the group of participants with asthma, 26.9% were smokers (daily, weekly, or occasionally) compared to 32.9% of those without asthma (**Table 2.2, Figure 2.1**). We did not observe any significant association between asthma status and tobacco smoking (**Table 2.3**).

Drug use: When exploring experience with any drug use among the participants, we observed a tendency. Drug use seemed less common among participants with asthma compared to participants without asthma in the univariate analysis: 16.1% vs. 26.0%, OR=0.57 (95% CI, 0.30-1.05), $p=0.08$ (**Table 2.3, Figure 2.1**). We performed a backward selection procedure on the multivariable model showing an inverse association; reduced drug use and risk of asthma: adjusted OR=0.59 (0.29-0.96), $p=0.045$ (**Table 2.3, Figure 2.1**).

Screen time: Among all participant the median screen time per day was 6.0 hours. Among participants with asthma, we observed a tendency. Participants with asthma had higher daily screen time use, >8h/day: 25.8% compared to participants without asthma, 16.6%, OR=1.74 (0.98-3.04), $p=0.053$ (**Table 2.2**). Despite this, we did not see a significant association in the multivariable and backward selection models (**Table 2.3**).

Self-destructive behaviour: In the cohort, 26.9% of the participants with asthma described experience with self-destructive behaviour compared with 17.3% in the group of participants without asthma (**Table 2.2**). In the univariate analysis, we observed a significant association between asthma and increased self-destructive behaviour, OR=1.84 (1.03-3.21), $p=0.035$, although this was not observed in the multivariate model or after the backward selection. These models just showed a tendency to increase the risk of self-destructive behaviour, OR=1.76 (0.93-3.29), $p=0.08$ (**Table 2.3**).

A heatmap is included in the supplementary, showing the correlations between risk behaviours included in the study (**Appendix Paper II, Supplementary, Figure S1**).

Table 2.2 Risk Behaviours²

Risk behaviours	All N=370	Current asthma N = 93	Not current asthma N = 277	p-value*
Alcohol; drinking frequency, N=342 (92.4)				0.46
Never	3 (1.0)	-	3 (1.0)	
Max once per month	89 (24.1)	26 (28.0)	61 (22.0)	
> 1 per month	253 (68.4)	60 (64.5)	193 (69.7)	
Alcohol; units				0.28
1-4	88 (23.8)	16 (17.2)	71 (25.6)	
5-9	172 (46.5)	51 (54.8)	120 (43.3)	
> 10	82 (22.2)	19 (20.4)	63 (22.7)	
Smoking, N=189 (51.1)	116 (31.4)	25 (26.9)	91 (32.9)	0.34
Current smoking†	65 (17.6)	16 (17.2)	49 (17.7)	
Occasional smoking†	51 (13.8)	9 (9.7)	42 (15.2)	
Former smoker	73 (19.7)	18 (19.4)	55 (19.9)	
Daily	28 (7.6)	5 (5.4)	23 (8.3)	0.35
1-4 cigarettes per day	11 (39.3)	3 (60.0)	8 (34.7)	
5-9 cigarettes per day	8 (28.6)	2 (40.0)	6 (26.1)	
> 9 cigarettes per day	9 (32.1)	-	9 (39.1)	
Drug use ever	87 (23.8)	15 (16.1)	72 (26.0)	<0.01
Cannabinoids	60 (70.0)	9 (60.0)	51 (70.8)	
Cocaine	22 (25.3)	5 (33.3)	17 (23.6)	
Laughing gas (NO)	18 (20.7)	1 (6.7)	17 (23.6)	
Ecstasy / MDMA / Fantasy	12 (13.9)	4 (26.7)	8 (11.1)	
Others‡	34 (39.1)	7 (46.7)	27 (37.5)	
Screen time, N=367 (99.2)				

Total screen time hours, median (IQR)	6.0 (4.5 - 7.0)	6.0 (4.5-8.0)	6.0 (4.5-7.0)	0.93
High daily screen time use	70 (18.9)	24 (25.8)	46 (16.6)	0.01
Self-destructive behaviour	73 (19.7)	25 (26.9)	48 (17.3)	<0.01

Table 2.2 Risk behaviours

The table presents descriptive information on risk behaviour from probands in the cohort. The information is presented in groups of asthma yes/no.

†Current smoking was defined as daily or weekly smoking, and occasional smoking was defined as smoking less than weekly.

‡Etc. Amphetamine, sedatives, LSD, opiates, euphoric mushrooms, lighter gas.

*Significance between the genders is calculated with a t-test. In cases of non-normally distributed data, the Wilcoxon test has been used.

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Non-atopic comorbidity and the association with asthma

Neuropsychiatric disorders: Examine all participants; 16.8% had been diagnosed with a neuropsychiatric disorder in childhood or adolescence (**Table 2.4, Appendix paper II, supplemental Figure S2**). Among adolescents with asthma, the risk of neuropsychiatric comorbidity was doubled compared with adolescents in the cohort without asthma: 24.7% vs. 14.1%, OR=2.01 (1.11-3.56), p=0.019. This result was consistent across the multivariate analysis OR=2.17 (1.04-4.48), p=0.037, and the backward selection procedure OR=2.04 (1.00-4.12), p=0.049; **Table 2.3**). ADHD was diagnosed in 10.8% of adolescents with asthma vs. 5.8% in adolescents without asthma and was the most common neuropsychiatric diagnosis in the cohort (**Table 2.4**).

Obesity: In the cohort, 15.1% of participants with asthma were overweight, with a BMI of 25.1-30.0 kg/m², and 10.8% as having obesity, with a BMI > 30 kg/m². Among participants without asthma, this was 15.5% and 6.9%, respectively (**Table 2.4**). We did not find a significant association between the BMI categories and asthma at 18 years (**Table 2.3, Figure 2.2**). In the stratified analyses, we observed an association between asthma and obesity in females, OR=3.48 (1.18-10.33), p=0.02. There was no association among males, OR=1.55 (0.66-3.44), p=0.30, indicating a gender difference (p-interaction=0.04) (**Appendix paper II, Supplementary, Table S1**).

Figure 2.1 Distribution of risk behaviour, BMI, and neuropsychiatric disorders in adolescents with vs. without asthma²

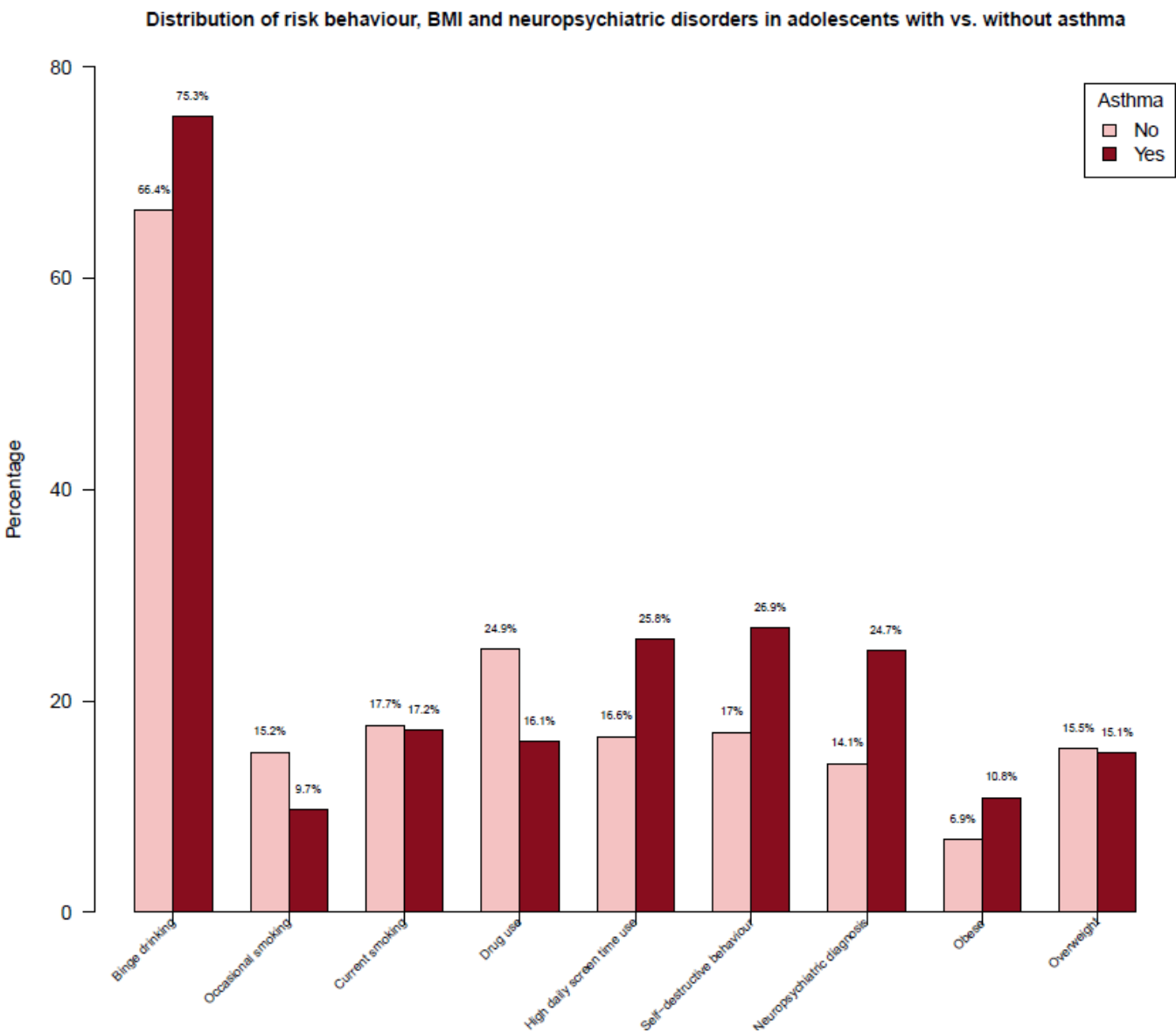


Figure 2.1. Showing distribution of risk behaviours, BMI, and neuropsychiatric disorders in adolescents with vs. without asthma. Copyright by T. Mølbæk-Engbjerg, The World Allergy Organization journal²

Table 2.3 Asthma associations²

Asthma associations	Univariate model			Adjusted model			Backward selection model		
Predictors	OR	CI	P	OR	CI	P	OR	CI	P
Binge drinking†	1.66	(0.93 – 3.08)	0.10	1.70	(0.88 – 3.43)	0.12			
Current smoking‡	0.86	(0.44 – 1.62)	0.64	0.50	(0.17 – 1.29)	0.17	-	-	-
Occasional smoking‡	0.56	(0.24 – 1.19)	0.15	0.55	(0.21 – 1.33)	0.20	-	-	-
Drug use (yes)	0.57	(0.30 – 1.05)	0.081	0.73	(0.32 – 1.62)	0.44	0.59	(0.29-0.96)	0.045
High daily screen time use§	1.74	(0.98 – 3.04)	0.053	1.68	(0.83 – 3.35)	0.15	-	-	-
Self -destructive behaviour (yes)	1.84	(1.03 – 3.21)	0.035	1.65	(0.81 – 3.30)	0.16	1.76	(0.93-3.29)	0.079
Neuropsychiatric disorder¶	2.01	(1.11 – 3.56)	0.019	2.17	(1.04 – 4.48)	0.037	2.04	(1.00 – 4.12)	0.049
Obesity	1.55	(0.66 – 3.44)	0.30	1.67	(0.61 – 4.36)	0.30	-	-	-

Table 2.3: Association between risk behaviours, obesity, and neuropsychiatric disorders.

The adjusted model is adjusted for sex, GA, mother's age, mother's educational level, household income, smoking during pregnancy, and breastfeeding. All risk behaviours are included in the multivariable model: binge drinking, smoking, drug use, high daily screen time use, self-destructive behaviour, neuropsychiatric disorder, and obesity.

†Binge drinking refers to alcohol units ≥ 5 .

‡Current smoking refers to smoking daily or weekly, and occasional smoking refers to smoking less than weekly.

§ High daily screen time use > 8 hours / day.

¶Neuropsychiatric disorder ever through childhood.

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Table 2.4 BMI and neuropsychiatric disorders²

	All	Current asthma	Not current asthma	p-value*
BMI and neuropsychiatric disorders, N (%)	N = 370	N=93	N=277	
BMI, N=367 (99.1)				0.58
BMI < 18.5	32 (8.7)	6 (6.45)	26 (9.39)	
BMI 18.5-24.9	249 (67.8)	64 (68.8)	185 (66.8)	
BMI 25-30	57 (15.5)	14 (15.1)	43 (15.5)	
BMI > 30	29 (7.90)	10 (10.8)	19 (6.9)	
ASRS questionnaire, N=348 (94.0) ‡	4.0 (2.0-7.0)	4.5 (2.0-7.0)	4.0 (2.0-7.0)	0.97
SDQ - Total difficulty score, N=347 (93.7) §	74 (21.3)	22 (25.0)	52 (20.1)	0.59
SDQ - Daily functioning score (Impact score) §	95 (27.4)	23 (26.1)	72 (27.8)	0.94
Neuropsychiatric disorder†	62 (16.8)	23 (24.7)	39 (14.1)	0.02
ADHD	26 (7.0)	10 (10.8)	16 (5.8)	0.16
Depression	8 (2.2)	4 (4.3)	4 (1.4)	0.11

Anxiety	12 (3.2)	2 (2.2)	10 (3.6)	0.74
Autisme	13 (3.1)	7 (7.5)	5 (1.8)	0.01
Eating disorder	11 (3.0)	3 (3.2)	8 (2.9)	1.00
Personality disorder	2 (0.5)	-	2 (0.7)	-
Mental retardation	3 (0.8)	1 (1.1)	2 (0.7)	1.00
PTSD	5 (1.4)	2 (2.2)	3 (1.1)	0.60
Schizophrenia	2 (0.5)	1 (1.1)	1 (0.4)	0.44
OCD	9 (2.4)	3 (3.2)	6 (2.2)	0.70

Table 4: BMI and neuropsychiatric disorders

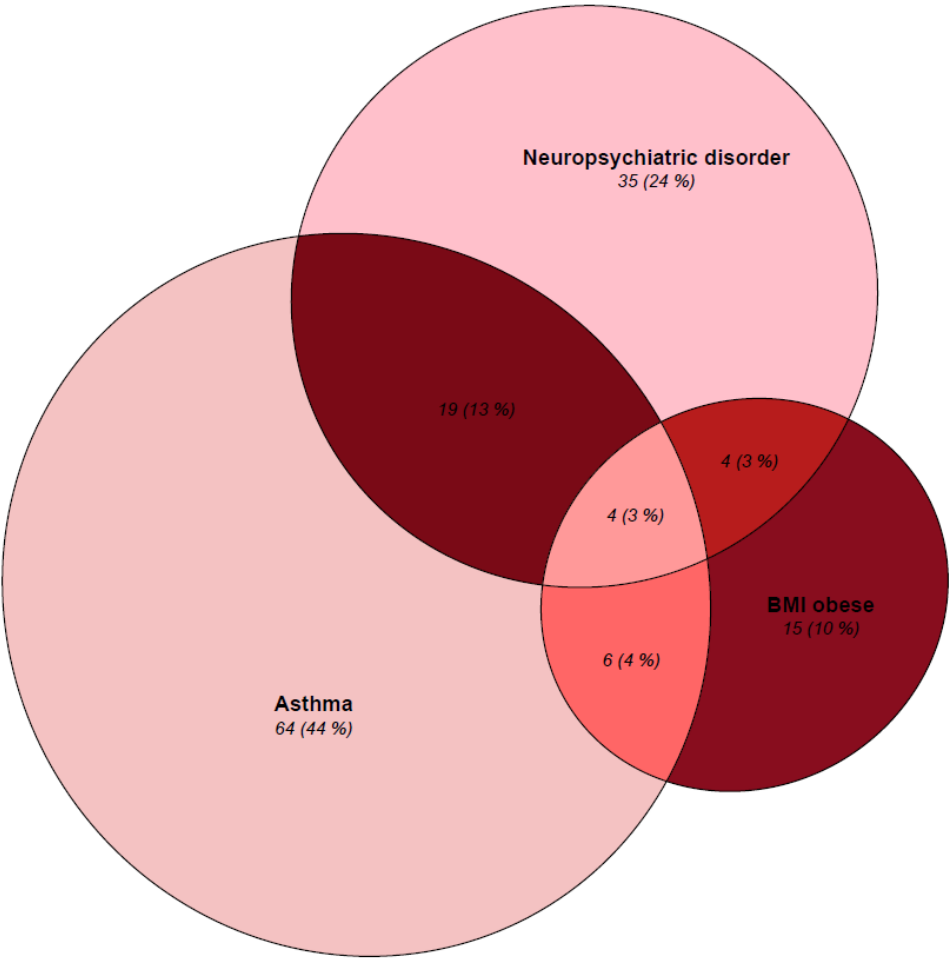
†Neuropsychiatric disorders include a group of other diagnoses. This group includes 7 individuals. Some of the probands have more than one neuropsychiatric diagnosis, which is why the number of specific categories does not sum to 62.

‡ASRS (Adult ADHD self reported scale), data from 348 proband, 88 with asthma and 260 without asthmatic disease.

§SDQ-questionnaire. Data available from 347 probands.

*Fishers test

Figure 2.2²



²**Figure 2.2.** Venn diagram showing the relationship of the 147 participants with asthma and/or neuropsychiatric disorder, and/or obesity (BMI $\geq$ 30), N (%)
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PAPER III. LONGITUDINAL TRAJECTORIES OF INFLAMMATORY BIOMARKERS AND THEIR ASSOCIATION WITH ASTHMA

In **Paper III**, we explored whether trajectories of inflammatory biomarkers through childhood were associated with the development of asthma and asthma-related outcomes at 18 years.

Cohort characteristics: Of the 411 children included in the COPSAC₂₀₀₀ cohort, 370 (90%) completed the 18-year follow-up visit, and all had data on either asthma diagnosis, airway obstruction (FEV1 and sRaw), airway inflammation (FeNO), or AHR (PD20). Baseline characteristics for participants with follow-up vs. without follow-up are presented in **Table S1 (Appendix Paper III, Supplementary Table S1)**, elucidating significant differences such as socioeconomic status, as well as risk factors, including solely breastfeeding at age 3 months and maternal smoking during pregnancy.

Blood samples were available in 385 (93.7%) of the children included in the cohort. Among those participants, 217 (56.4%) had biochemistry at all four timepoints, 85 (22.1%) had from three timepoints, 53 (13.8%) had from two timepoints, and 30 (7.8%) had only biochemistry available from one timepoint at age 4, 5, 6, 7, 12, or 18 years.

Cross-sectional analysis at 18 years: We did not observe any significant association between blood biochemistry measures and asthma in the unadjusted and covariate-adjusted multivariable analyses (**Table 3.1, Appendix Paper III, Supplementary, Table S2**). In the unadjusted analysis, we viewed a significant association between increased platelet count and decreased FEV1 (**Appendix, paper III, Table S2**), although we did not find significant associations between biochemistry measurement and FEV1 or sRaw in the adjusted analyses (**Table 3.1**). In the adjusted analyses, airway inflammation, evaluated by FeNO, was significantly associated with higher eosinophil count (beta-estimate=22 ppb FeNO per 10^9 cells/L (95% CI, 9-36), $p=0.001$) and higher IgE level (beta-estimate=0.01 ppb FeNO per IgE g/L (0.01-0.02), $p<0.001$) (**Table 3.1**). Furthermore, a significant association was found between AHR, a higher lymphocyte count, and a lower PD20 (estimate = -1422 (-2809 to -36), $p=0.044$), i.e., greater AHR (**Table 3.1**).

Table 3.1 Cross-sectional 18 years

Table 3.1															
Blood biochemistry	Asthma			FEV1			FeNO			sRaw			PD20		
	OR	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	0.91	(0.70-1.17)	0.489	0.01	(-0.04-0.05)	0.740	0.09	(-2.25-2.43)	0.938	-0.02	(-0.04-0.01)	0.262	121.41	(-415-658)	0.658
Eosinophils	0.83	(0.15-3.43)	0.816	0.07	(-0.20-0.34)	0.621	22.46	(9.22-35.70)	0.001	-0.04	(-0.21-0.12)	0.622	-1388.57	(-4457-1680)	0.375
Lymphocytes	0.61	(0.30-1.22)	0.176	0.10	(-0.02-0.21)	0.119	-2.13	(-8.23-3.96)	0.493	-0.03	(-0.11-0.04)	0.393	-1422.13	(-2809 to -36)	0.044
Platelets	1.00	(0.99-1.00)	0.159	-0.00	(-0.00-0.00)	0.472	-0.04	(-0.09-0.01)	0.140	0.00	(-0.00-0.00)	0.835	-6.71	(-19-6)	0.281
Ratio Neutrophils / Lymphocytes	1.01	(0.68-1.47)	0.945	-0.01	(-0.08-0.06)	0.755	0.04	(-3.47-3.56)	0.981	-0.01	(-0.06-0.03)	0.553	496.65	(-300-1293)	0.222
IgG	1.03	(0.87-1.23)	0.705	0.00	(-0.03-0.03)	0.887	0.52	(-1.05-2.09)	0.517	0.01	(-0.01-0.02)	0.590	-179.84	(-553-194)	0.345
IgA	0.76	(0.46-1.20)	0.252	0.03	(-0.05-0.12)	0.445	2.03	(-2.14-6.21)	0.340	-0.01	(-0.06-0.04)	0.688	-154.47	(-1140-831)	0.759
IgM	1.82	(0.93-3.55)	0.079	-0.08	(-0.21-0.04)	0.186	-2.65	(-8.94-3.65)	0.410	0.07	(-0.00-0.15)	0.056	-537.68	(-2075-1000)	0.493
Total-IgE	1.00	(1.00-1.00)	0.674	-0.00	(-0.00-0.00)	0.257	0.01	(0.01-0.02)	<0.001	0.00	(-0.00-0.00)	0.920	-0.20	(-1.8-1.4)	0.812

Table 3.1. Cross-sectional 18 years - multivariable. The table shows the cross-sectional analysis between biochemistry measurements and atopic outcomes at 18 years.

Linear mixed model analysis of childhood biochemistry measurements

Asthma: In the covariate-adjusted LMM analyses, we found an association between neutrophil count over time and asthma at 18 years. We observed that lower neutrophil count at age 4 years (baseline), (intercept OR=0.77 per 10^9 cells/L (0.59-0.98), $p=0.036$) and increasing neutrophils through childhood and adolescents, i.e., from age 4 to 18 years (slope, OR=1.30 per 10^9 cells/L (1.01-1.68), $p=0.040$) were associated with an increased risk of asthma (**Table 3.2.1**). Further, NLR was associated with asthma at 18 years. The analysis showed that lower NLR at baseline was associated with increased risk of asthma (intercept OR=0.77 (0.60-0.98), $p=0.038$). Moreover, the analysis showed that a higher eosinophil count at baseline was associated with increased risk of asthma at 18 years (intercept OR=1.51 per 10^9 cells/L (1.17-1.98), $p=0.002$), and moreover, increased levels of total-IgE at baseline and also over time were associated with asthma (intercept OR=1.28 per IgE g/L (1.00-1.63), $p=0.049$, and slope OR=1.37 per IgE g/L (1.07-1.77), $p=0.015$) (**Table 3.2.1**).

Airway obstruction: In the FEV1 analysis, we observed a significant association between baseline platelet count and FEV1 at 18 years. The analysis showed that increased platelet count at baseline was associated with reduced FEV1 (intercept beta-estimate=-0.07 L per 10^9 cells/L (-0.12 to -0.03), $p=0.002$) (**Table 3.2.2**). Furthermore, we observed a trend of association between a higher lymphocyte count at baseline and lower FEV1 (intercept beta-estimate=-0.04 L per 10^9 cells/L (-0.09-0.00), $p=0.077$) and a significant association between an increasing lymphocyte count over time and higher FEV1 at 18 years (slope beta-estimate=0.05 L per 10^9 cells/L (0.00-0.10), $p=0.044$) (**Table 3.2.2**). We did not find any significant association in the adjusted LMM analysis of sRaw (**Appendix, Paper III, Table 2.4**).

Airway inflammation: The analyses of FeNO and blood eosinophil count showed an association between an elevated eosinophil count at baseline and elevated FeNO level at 18 years (intercept beta-estimate=3.84 ppb per 10^9 cells/L (1.53-6.15), $p=0.001$). The development in eosinophils over time, hence, the slope, was not significant (slope beta-estimate=1.75 ppb per 10^9 cells/L (-0.57-4.06), $p=0.139$) (**Table 3.2.3**). In addition, we observed that an elevated level of total-IgE at baseline and increasing total-IgE levels over time were associated with elevated FeNO at 18 years (intercept beta-estimate=3.91 ppb per

g/L (1.64-6.18), $p=0.001$, and slope beta-estimate=4.84 ppb per g/L (2.55-7.14), $p<0.001$) (Table 3.2.3).

Airway hyperresponsiveness: We did not observe any significant associations between the development of the selected blood biochemistry measures and AHR, as indicated by PD20 at 18 years (Appendix, Paper III, Table 2.5).

Results from the univariate unadjusted analyses are presented in the supplementary appendix (Appendix, Paper III, Supplementary, Table S3.1-S3.5).

Table 3.2.1 The longitudinal effect of inflammatory biomarkers in LMM models on atopic outcomes, asthma

Table 3.2.1 The longitudinal effect of inflammatory biomarkers in LMM models on asthma, multivariate			Asthma			
Blood samples	OR	Intercept CI	p-value	OR	Slope CI	p-value
Neutrophils	0.77	(0.59-0.98)	0.036	1.30	(1.01-1.68)	0.040
Eosinophils	1.51	(1.17-1.98)	0.002	0.78	(0.61-1.01)	0.059
Lymphocytes	10.99	(0.78-1.27)	0.961	0.95	0.73-1.21	0.667
Platelets	1.05	(0.82-1.35)	0.682	0.94	(0.73-1.20)	0.595
Ratio neutrophils / Lymphocytes	0.77	(0.60-0.98)	0.038	1.24	(0.97-1.59)	0.087
IgG	0.98	(0.76-1.25)	0.848	1.08	(0.84-1.39)	0.549
IgA	0.91	(0.71-1.17)	0.465	1.16	(0.90-1.51)	0.274
IgM	1.19	(0.93-1.54)	0.173	0.97	(0.76-1.23)	0.789
IgE	1.28	(1.00-1.63)	0.049	1.37	(1.07-1.77)	0.015

Table 3.2.1 - The longitudinal effect of inflammatory biomarkers in LMM models on asthma, multivariate

Table 3.2.2 The longitudinal effect of inflammatory biomarkers in LMM models on atopic outcomes, FEV1

Table 3.2.2						
Blood samples	Intercept			FEV1		
				Slope		
	Estimate	CI	p-value	Estimates	CI	p-value
Neutrophils	-0.02	(-0.06-0.03)	0.457	0.02	(-0.03-0.06)	0.399
Eosinophils	-0.02	(-0.06-0.03)	0.519	0.00	(-0.05-0.05)	0.997
Lymphocytes	-0.04	(-0.09-0.00)	0.077	0.05	(0.00-0.10)	0.044
Platelets	-0.07	(-0.12--0.03)	0.002	0.04	(-0.01-0.08)	0.127
Ratio neutrophils / Lymphocytes	0.01	(-0.03-0.06)	0.517	-0.02	(-0.07-0.02)	0.324
IgG	-0.00	(-0.05-0.04)	0.846	0.00	(-0.04-0.05)	0.931
IgA	0.03	(-0.02-0.08)	0.199	-0.02	(-0.07-0.03)	0.410
IgM	-0.02	(-0.07-0.02)	0.332	-0.01	(-0.06-0.03)	0.556
IgE	-0.01	(-0.05-0.04)	0.774	-0.02	(-0.06-0.03)	0.454
Table 3.2.2 - The longitudinal effect of inflammatory biomarkers in LMM models on FEV1, multivariate						

Table 3.2.3 The longitudinal effect of inflammatory biomarkers in LMM models on atopic outcomes, FeNO

Table 3.2.3 The longitudinal effect of inflammatory biomarkers in LMM models on FeNO, multivariate						
Blood samples	FeNO					
	Intercept			Slope		
	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	-0.72	(-3.02-1.59)	0.544	0.39	(-1.89-2.67)	0.737
Eosinophils	3.84	(1.53-6.15)	0.001	1.75	(-0.57-4.06)	0.139
Lymphocytes	-2.15	(-4.65-0.36)	0.093	1.54	(-0.98-4.06)	0.232
Platelets	0.49	(-1.92-2.90)	0.689	-2.30	(-4.73-0.14)	0.064
Ratio neutrophils / Lymphocytes	0.47	(-1.87-2.81)	0.694	-0.44	(-2.78-1.89)	0.710
IgG	1.85	(-0.48-4.18)	0.119	-1.31	(-3.72-1.10)	0.288
IgA	1.57	(-0.88-4.01)	0.209	-1.60	(-4.28-1.09)	0.244
IgM	-0.63	(-3.01-1.75)	0.604	-0.32	(-2.68-2.04)	0.792
IgE	3.91	(1.64-6.18)	0.001	4.84	(2.55-7.14)	<0.001

Table 3.2.3 - The longitudinal effect of inflammatory biomarkers in LMM models on FeNO, multivariate

Latent class trajectory analysis of childhood biochemistry measurements

We selected the optimal LCT model for each biomarker based on the lowest BIC value, the highest entropy, and the clinical interpretation of the trajectories (**Appendix, Paper III, Supplementary, Tables S4-S12, Figures S1-9**)

Asthma: In the covariate adjusted multivariable LCT analyses with asthma as outcome we found that a specific IgM trajectory (Latent Class Trajectory 1, LCT1, n=29) defined by an increase in IgM levels in early childhood followed by a decline was associated with a decreased risk of asthma by age 18 compared to the other three identified IgM trajectories (OR=0.16 (0.02-0.57), p=0.016) (**Figure 3.1**). There were no other significant associations between any other latent biochemistry measurement trajectories in childhood and asthma at age 18 (**Table 3.3.1**).

Airway obstruction: Similar with the results from the LMM analyses, the adjusted LCT analyses showed a significant association between a specific platelet count trajectory in childhood (LCT2, n=193) defined by a decrease in platelet count in early childhood followed by an increase and lower FEV1 at age 18 compared to the other identified platelet count trajectory (beta-estimates=-0.12 (-0.21 to -0.03), p=0.013) (**Figure 3.1-3.2**). We did not observe any other significant associations in the adjusted LCT analyses for FEV1 or sRaw (**Table 3.3.1**).

Airway inflammation: In the adjusted LCT analyses with FeNO as outcome, we found a significant association between FeNO and a specific trajectory for blood eosinophils. The specific trajectory for eosinophils (LCT3, n=142 defined by a generally elevated level of blood eosinophils and an increase in early childhood followed by a decline, compared to the other two identified blood eosinophil trajectories (beta-estimate=12.79 (7.82-17.75), p<0.001) were associated with elevated FeNO at 18 years (**Figure 3.1-3.2, Table 3.3.1**). Further, a trajectory for total-IgE level in childhood (LCT3, n=94), defined by a continuous rise during childhood and adolescence compared to the other two identified total-IgE trajectories (beta-estimate=9.67 (4.24-15.10), p<0.001) were also associated with elevated FeNO at age 18 years (**Figures 3.1-3.2, Table 3, Table 3.3.1**).

Figure 3.1 Latent class trajectories of inflammatory biomarkers

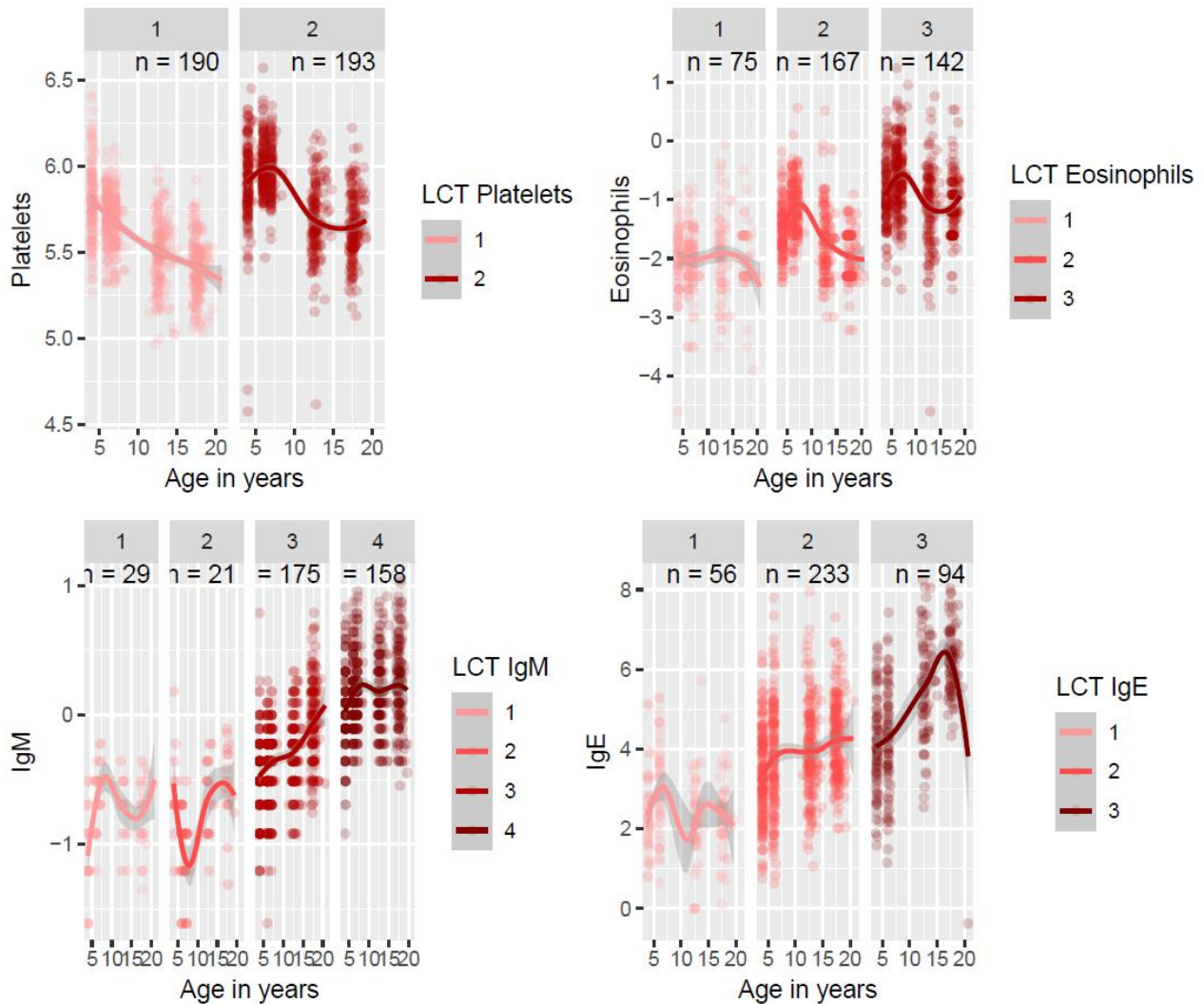


Figure 3.1. Showing specific Latent Class Trajectories for platelets, eosinophils, IgM, and IgE.

Airway hyperresponsiveness: At last, in the adjusted LCT analyses we also found significant association between the specific LCT2 (n=29) platelet count trajectory in childhood defined by a decline in early childhood and followed by an increase and lower PD20, i.e. higher AHR at age 18 years compared to the other platelet trajectory (beta-estimate, -1344 (-26235 to -65), $p=0.039$ (**Figures 3.1-3.2, Table 3.3.2**). A NLR trajectory (LCT2, n=280) defined by a higher set point and a continuous rise over time (**Appendix, Paper III, Supplementary, Figure S5**) also tended to be associated with a lower PD20 in the adjusted analyses compared to the other NLR trajectory (beta-estimate, -1336 (-2760-

87), $p=0.066$) (**Table 3.3.2**). In the unadjusted analyses, NLR trajectory LCT2 ($n=280$) was associated with a lower PD20 (beta-estimate, -1569 [-2933 to -206], $p=0.024$), and also a neutrophils trajectory (LCT1, $n=64$) defined by a generally decline was associated with a lower PD20 (beta-estimate, -1531 [-3079 to -16], $p=0.052$) (**Appendix, Paper III, Supplementary, Figure S1, Table S13.3**).

Figure 3.2 Scatterplot of significant associations between latent class trajectories of inflammatory biomarkers and outcomes

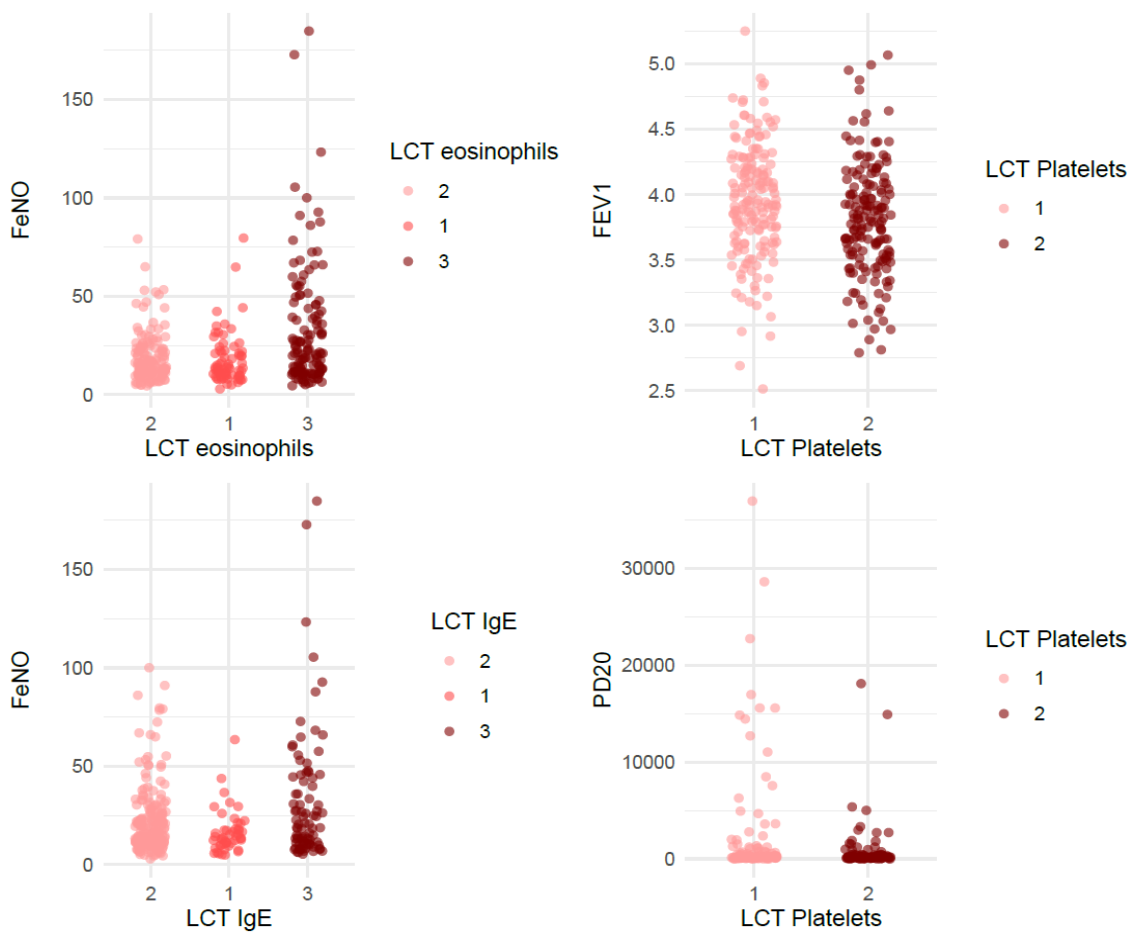


Figure 3.2. Scatterplot, showing significant associations between Latent Class Trajectories for platelets, eosinophils, IgM, IgE, and asthma-related outcomes (FeNO, FEV1, and PD20).

Table 3.3.1 The longitudinal effects of inflammatory biomarkers in LCT models on atopic outcomes

Table 3.3.1 The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes										
Blood samples	LCT	Asthma			FEV1			FeNO		
		OR	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	LCT1	1.32	(0.68-2.51)	0.395	0.01	(-0.11-0.14)	0.821	-1.76	(-8.22-4.70)	0.593
	LCT3	0.59	(0.25-1.29)	0.215	0.04	(-0.09-0.17)	0.556	0.43	(-6.29-7.16)	0.899
Eosinophils	LCT1	0.67	(0.31-1.37)	0.284	0.09	(-0.04-0.21)	0.163	-1.47	(-7.49-4.54)	0.631
	LCT3	1.29	(0.75-2.20)	0.353	0.03	(-0.08-0.13)	0.604	12.79	(7.82-17.75)	<0.001
Lymphocytes	LCT2	1.02	(0.62-1.68)	0.929	-0.03	(-0.12-0.06)	0.547	1.93	(-2.75-6.62)	0.418
Platelets	LCT2	0.93	(0.56-1.54)	0.778	-0.12	(-0.21--0.03)	0.013	1.17	(-3.54-5.88)	0.626
Ratio Neutrophils / Lymphocytes	LCT2	1.36	(0.78-2.34)	0.271	0.05	(-0.06-0.15)	0.400	0.03	(-5.33-5.40)	0.900
IgG	LCT2	0.86	(0.52-1.43)	0.566	-0.03	(-0.12-0.07)	0.559	-3.85	(-8.65-0.95)	0.116
IgA	LCT1	-	-	-	-0.05	(-0.66-0.55)	0.858	-11.70	(-42.57-9.18)	0.458
	LCT2	2.13	(0.28-11.71)	0.400	-0.05	(-0.41-0.30)	0.764	-3.29	(-21.39-14.81)	0.722
	LCT4	5.33	(0.85-41.98)	0.073	-0.22	(-0.65-0.20)	0.299	-2.70	(-24.51-19.10)	0.808

IgM	LCT1	0.16	(0.02-0.57)	0.016	0.00	(-0.17-0.17)	0.978	2.97	(-5.88-11.82)	0.510
	LCT2	1.10	(0.33-3.18)	0.870	0.10	(-0.12-0.33)	0.375	5.21	(-6.32-16.73)	0.376
	LCT4	0.88	(0.52-1.49)	0.639	-0.05	(-0.15-0.05)	0.350	1.51	(-3.54-6.56)	0.558
IgE	LCT1	0.63	(0.26-1.39)	0.282	-0.03	(-0.17-0.10)	0.642	-4.70	(-11.45-2.06)	0.173
	LCT3	1.52	(0.86-2.65)	0.142	-0.04	(-0.15-0.07)	0.447	9.67	(4.24-15.10)	<0.001

Table 3.3.1 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, multivariate

Table 3.3.2 The longitudinal effects of inflammatory biomarkers in LCT models on atopic outcomes

Table 3.3.2 The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes							
Blood samples	LCT	sRaw			PD20		
		Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	LCT1	18.60	(-6.32-43.53)	0.144	-1153.81	(-2798.02-490.40)	0.169
	LCT3	6.59	(-20.06-33.23)	0.28	239.28	(1515.68-1994.24)	0.789
Eosinophils	LCT1	0.03	(-0.06-0.12)	0.512	-882.06	(-2589.17-825.05)	0.311
	LCT3	-0.01	(-0.08-0.07)	0.814	-973.07	(-2408.27-462.12)	0.184
Lymphocytes	LCT2	0.01	(-0.05-0.08)	0.694	1159.96	(-122.38-2442.30)	0.076
Platelets	LCT2	-0.04	(-0.11-0.03)	0.229	-1343.84	(-2622.46-65.23)	0.039
Ratio Neutrophils / Lymphocytes	LCT2	0.01	(-0.07-0.09)	0.795	-1336.34	(-2759.52-86.84)	0.066
IgG	LCT2	-0.00	(-0.07-0.07)	0.975	890.24	(-404.31-2184.80)	0.178

IgA	LCT1	-0.23	(-0.76-0.30)	0.401	-546.43	(-9850.25-8757.39)	0.908
	LCT2	0.16	(-0.07-0.38)	0.167	-1017.73	(-5269.01-3233.56)	0.639
	LCT4	0.05	(-0.25-0.36)	0.735	-2117.29	(-8611.35-4376.78)	0.523
IgM	LCT1	-0.08	(-0.21-0.04)	0.191	-546.43	(-9850.25-8757.39)	0.908
	LCT2	-0.06	(-0.22-0.10)	0.469	-1017.73	(-5269.01-3233.56)	0.639
	LCT4	0.01	(-0.06-0.09)	0.688	-2117.29	(-8611.35-4376.78)	0.523
IgE	LCT1	-1.00	(-28.90-26.91)	0.944	680.75	(-1326.40-2687.89)	0.506
	LCT3	12.75	(-8.87-34.38)	0.248	282.13	(-1171.50-1735.77)	0.704

Table 3.3.2 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, multivariate

DISCUSSION

PRIMARY FINDINGS

In **Paper I**, we provided a comprehensive description of the health and habits of adolescents from a high-risk asthma cohort, focusing on the 18-year follow-up visit in the COPSAC₂₀₀₀. We gathered detailed information on risk behaviours and environmental risk factors and performed multiorgan assessments. Our investigation of adolescents showed that almost one-fourth had asthma, around one-fourth were overweight or obese, and two-thirds drank alcohol more frequently than once per month. Furthermore, 16.8% had a history of a neuropsychiatric diagnosis in childhood, and almost one-fourth had experience with drug use.

In **Paper II**, we investigated the health and habits of Danish adolescents with asthma compared with those without asthma. In this study, we found an increased risk of neuropsychiatric comorbidity and increased risk of self-destructive behaviours among adolescents with asthma. We did not see an association between asthma and any other risk behaviours, such as alcohol consumption and tobacco smoking. Additionally, among females, we observed an association between obesity and asthma.

In **Paper III**, we performed an exploratory analysis on blood biochemistry measurements collected from the participants in the COPSAC₂₀₀₀ cohort throughout childhood and adolescence. We utilized longitudinal analyses showing trajectories from specific blood measurements, including platelets, neutrophils, lymphocytes, NLR, eosinophils, total-IgE, IgG, IgA, and IgM, and the relation to asthma and asthma-related outcomes at 18 years. The childhood trajectories of platelets, eosinophils, and total-IgE were linked to asthma-related outcomes, including reduced lung function, increased AHR, and increased airway inflammation.

STRENGTHS AND LIMITATIONS

The papers included in this thesis are based on the COPSAC₂₀₀₀ cohort. A significant strength is prospective monitoring, in which participants from early childhood through adolescence up to age 18 underwent continuous clinical examinations at the COPSAC research unit ^{43,63,64}. This made them experienced in completing tests and assessments appropriately,

contributing to a high completion rate. During the visits, all objective measures were conducted by trained and qualified staff who strictly adhered to the standard operating procedures (SOP). Furthermore, all asthma diagnoses were made and followed at the clinical research unit at COPSAC, and the diagnostic procedure followed SOP, including treatment and evaluation, ensuring consistency. This is in contrast with studies focusing on asthma, where the diagnosis is made by a general practitioner, via questionnaires, or through parental reporting. Additionally, the participants included in the cohort were all born to mothers with asthma. This may have enhanced symptom recognition. The asthma diagnosis used in **Paper I**, **Paper II**, and **Paper III** was based on objective criteria.

A substantial strength of **Paper I** is the extensive amount of detailed information on environmental risk factors, risk behaviours, and a thorough multi-organ assessment, including most organ systems. Also, a broad biosample collection was conducted, enabling future analyses.

Another strength relates to the neuropsychiatric diagnoses used in **Paper I and Paper II**. The diagnoses included in the analyses were acquired from the Danish medical records, which cover all diagnoses in the national healthcare system. Risk behaviours like alcohol consumption and tobacco smoking were obtained without the presence of the participants' parents, and information on self-destructive behaviour and drug use was collected through questionnaires.

Furthermore, it is a strength that the longitudinal analyses in **Paper III** included blood measurements collected at four time points spanning 4 to 18 years during childhood and adolescence. Moreover, data were analysed using three different statistical methods: a cross-sectional approach, a repeated-measures approach, and, lastly, a latent-trajectory approach.

Although several important strengths are identified in this thesis, some important limitations also need to be added ^{64–66}. Due to the design of a high-risk cohort and participants' ethnic similarity (most are Caucasian), potential selection bias could not be eliminated. Nevertheless, in **Paper I**, a match between the participants in COPSAC₂₀₀₀ and adolescents in the population-based cohort, DNBC, was made enable a comparison of differences in risk behaviours, environmental risk factors, comorbidities, and asthma prevalence. In **Paper II**, we investigated associations between risk behaviours, neuropsychiatric comorbidity, and asthma. But the cohort was not originally designed to identify

these associations, which could have influenced our ability to detect them due to limited power or residual confounding. Furthermore, all participants diagnosed with asthma had mild to moderate disease and were well controlled. This could possibly influence the findings of associations, as more uncontrolled asthma and severe symptoms are linked to a rise in the prevalence of neuropsychiatric diseases. Also, the asthma diagnosis used in the analyses is based on cross-sectional methods focusing on current asthma at 18 years. This approach could introduce bias against the absence of associations, as participants with previously diagnosed asthma in remission are included as health controls.

Another possible limitation is the preparation of the neuropsychiatric diagnoses used in **Paper I** and **Paper II**. After collecting the medical records, we divided them into 12 groups to facilitate classification. This simplified classification could induce an inaccurate diagnosis. In **Paper III**, trajectories from blood measurements were associated with asthma and asthma-related outcomes at 18 years. But as the cohort only includes 411, and only 93 were diagnosed with asthma, it can be challenging to study different asthma phenotypes because of insufficient power. In addition, most asthma cases were mild or moderate, and the asthma-related outcomes measured during the 18-year follow-up visit were already present at the time of exposure, making it difficult to determine directionality.

INTERPRETATION

In **Paper I**, we presented a large dataset describing the health and habits of Danish adolescents from a high-risk asthma cohort, anchored on the 18-year follow-up visit. We focused on environmental exposures, risk behaviours, and other lifestyle-related conditions, such as obesity and neuropsychiatric disorders. Furthermore, these unique data include biosamples for future omics analyses and a multiorgan assessment that enables further research exploring the mechanisms underlying other non-communicable disorders and atopic diseases, and facilitates collaboration with researchers worldwide.

In **Paper II**, we investigated the association between risk behaviours and non-atopic comorbidities, such as neuropsychiatric disorders and obesity, because previous studies have found that living with a chronic disease increases risk behaviours and the prevalence of non-atopic comorbidities ^{16,17,20,67–69}. Several explanations are suggested, e.g, behaviours

among the adolescents with chronic diseases are grounded in a need for acceptance from their surroundings, a result of less restrictive rules and boundaries from the parents to adolescents with chronic diseases, or the fact that living with a chronic disease can be time-consuming, which can affect attention on risk behaviours and preventive guidance.

For the last decade, there has been a growing interest in the connection between asthma and neuropsychiatric disorders ^{11,20,69}. It is known that genetics influences the risk of both diseases, and in our high-risk cohort, we found that around one-fourth had concurrent asthma vs. 15% in the background population ¹¹. Furthermore, we observed that 16.8% of the adolescents in the cohort had a former or recent history of neuropsychiatric disorders, which is higher compared to the prevalence of mental disorders globally ^{70,71}. Moreover, when focusing on the specific neuropsychiatric diagnoses, we observed an increased rate of ADHD in the cohort, in contrast to the prevalence in the background population in Denmark ^{70,71}. This observation could be due to the structured testing in the cohort, as all participants were screened, whereas in the background population, only persons with severe or impairing symptoms are examined. However, the increased prevalence of both asthma and ADHD may be linked by a shared origin from the surrounding environment early in life and genetic influence ^{20,70-72}. In opposition, we viewed a significant relation between autism and asthma, although previous studies finds ambiguous results concerning an association ^{20,69,71,74,75}.

Finally, asthma has been associated with increased risk of self-destructive behaviour, depression, and anxiety ⁶⁷. This is due to the vulnerability that is induced when diagnosed with a chronic disease. Our result did not find an association between depression or anxiety, which could be due to insufficient power, but we did note that self-destructive behaviour through childhood or adolescents were reported by one-fifth of the adolescents in the cohort, and a significant association was seen in the unadjusted model, although this was only a trend after adjustment.

The growing curiosity about a shared origin between asthma and non-atopic comorbidities, including obesity ^{23,76-78}, has led many studies to explore the association ^{21,22,24,76,79-83}. Furthermore, obesity in childhood and adolescence is associated with a wide range of negative health consequences, such as dyslipidaemia, diabetes, hypertension, low self-esteem, and depression ^{84,85}. In the Danish background population, one-fourth of school-aged

children have a BMI>25% ⁸⁶, which aligns with our cohort's findings, but we did not find a significant association between asthma and obesity. This may be due to insufficient power. However, among females, we did find an association between asthma and obesity. This result has previously been reported, and it is supposed that it is influenced by sex hormones, particularly oestrogen ^{87,88}. The theory is that sex hormones influence the Th2 inflammatory response, leading to increased cytokine production and affecting asthma development ^{87,88}. Nevertheless, the result is arguable, as the opposite is also observed ⁸⁹.

In **Paper II**, we evaluated risk behaviours among participants with and without asthma. Alcohol consumption is defined as a risk behaviour because of its association with metabolic and cardiovascular diseases, and influences the neurodevelopment of the brain ^{90,91}. Because of these negative health consequences, alcohol consumption among adolescents has been widely debated in recent years, and initiatives have been started to reduce the use of alcohol. The alcohol habits we observed in our cohort were similar to those of other European adolescents ⁹², and compared to former generations, the consumption is decreasing ⁹³. This is also observed in other European countries ^{94,95}.

Based on previous studies, we hypothesized that living with asthma increased the tendency of alcohol and binge drink ^{17,95}. However, in our study, there were no significant associations between alcohol consumption and asthma ². Perhaps because of properly controlled socioeconomic confounding in the analyses.

We also examined tobacco consumption among participants in the cohort. In contrast to other studies, we did not find an association between smoking and asthma compared to the participants without asthma ^{18,95,96}.

When examining drug use among the adolescents in the cohort, we found that fewer compared to the background population in Denmark had experience with drugs ⁹⁸. Among the group of participants with asthma, we observed that drug use was significantly lower compared to the group of participants without asthma. A possible explanation for this could be underreporting in the questionnaires, even though we collected the information anonymously. Also, we speculated whether the observation was a result of optimised health among the adolescents living with a chronic disease by decreasing risk behaviours, such as drug use, especially inhalation of drugs, like laughing gas ⁹⁹. The observation could also be driven by fear. A fear of activating an exacerbation or a fear of an interaction with their daily

medication. In contrast, the opposite behaviour among adolescents with a chronic disease has also been found in studies. Living with a chronic disease increases the use of drugs, maybe as a coping strategy ^{18,100}.

Finally, we included screen time as a risk behaviour because a high level of daily screen time is linked to negative health consequences, such as obesity and decreased cognitive and socioemotional function ¹⁰¹. In our study, we found a median daily screen time of 6 h. There was no association between asthma and mean screen time, but we observed a tendency toward a higher risk of asthma at 18 years with >8 h of daily screen time. However, this was only significant in the unadjusted analysis.

In **Paper III**, we explored the trajectories of biochemical measurement in childhood and adolescence and their association with asthma and asthma-related outcomes at 18 years.

We found associations between platelet trajectories, AHR, and lung function. In the literature, platelets' role in the pathogenesis of chronic airway inflammation and asthma is described, which brings speculations on whether platelets could be a prominent contender in allergic diseases and asthma ^{41,42}. Especially, the migration intrapulmonary is suggested as important in the pathogenesis of allergic asthma ¹⁰². Research has shown that platelets possess the ability to express IgE receptors that can trigger histamine release, activation of eosinophils, and production of spasmogenic mediators that stimulate constriction in the bronchi ^{41,102}. In addition, examining patients with allergy in a recent study showed that platelet malfunction was more distinct in patients with severe asthma, indicating a potential role in the severity of the disease ³⁴. In our study, we found that longitudinal platelet development from early childhood until the end of adolescence was associated with reduced lung function and increased AHR at 18 years. This observation was seen in both models, the LMM and the data-driven LCT of platelet counts, and it is in line with existing literature, as mentioned above ^{34,41,42,102}. Further, we observed an association between higher platelet count and reduced FEV1 at 18 years in the cross-sectional analysis, although this observation was not significant after covariate adjustment.

These findings imply that platelets are involved in lung function development and AHR in early life, and there is also an association with platelet count development over time. Therefore, further research is needed to clarify the potential role of platelets in asthma, develop specific prevention strategies, and target treatment.

It is increasingly well established that asthma is a heterogeneous disease, with the most common endotype being allergic asthma, which is driven by ILC2 cells that produce Th2 Cells and cytokines, such as IL-4, IL-5, and IL-13^{10,29–31,33}. The result of cytokine release is eosinophilic inflammation, which induces the production of IgE and causes clinical disease manifestations^{8,29,30,33}. To measure airway inflammation, FeNO has been used as a noninvasive marker of eosinophils³³. The findings of our study were consistent with this knowledge. The LMM showed increased eosinophils and increased IgE in early childhood, and increasing IgE levels through childhood were associated with increased eosinophil count, increased IgE total, and FeNO at 18 years.

Research has also speculated whether neutrophilic inflammation is involved in the pathogenesis of asthma, mostly non-allergic asthma and wheezing in early childhood³⁵. An increased level of neutrophils in the airways is found during asthma exacerbations¹⁰³. The enlarged number of neutrophils in the airways increases inflammation in the bronchi and induces a progression of the disease, resulting in status asthmaticus compared to milder asthma¹⁰⁴. Further, patients with severe asthma are more likely to have bronchial inflammation dominated by neutrophils even after recovery³⁵. In our study, we examined adolescents with mild to moderate asthma and observed, in the LMM analysis, an association between the development of neutrophils in childhood and risk of asthma. Lower neutrophil counts at age 4 and increasing neutrophil counts during childhood and adolescence were associated with a higher risk of asthma at 18 years. However, we did not observe a significant association with FEV1, FeNO, or AHR, and in the LCT analyses, no association between blood neutrophil trajectories and asthma status was observed.

Previous studies have shown an association between NLR and asthma^{36,104–106}, finding that NLR is significantly elevated among children with asthma^{40,105}, and elevated NLR is associated with the severity of asthma during an exacerbation³⁶. Results from previous studies were consistent with those of our study. In the LMM analysis, we found a significant association between the NLR and asthma at 18 years. Furthermore, in the cross-sectional analysis, we observed that lymphocyte count was associated with reduced PD20 and, hence,

elevated NLR. In the unadjusted LCT analysis, we found that neutrophils and NLR were significantly associated with increased AHR at 18 years.

Only a few studies have explored the relation between IgG, IgM, and asthma, and the results are ambiguous. An older study from the United Kingdom observed that lower IgG levels were associated with asthma in adults ⁴³, whereas another study, investigating the association between IgG and asthma in adults, did not find any significant results, although subclasses IgG2 and IgG3 were reduced in patients with asthma ¹⁰⁷. Another study focusing specifically on the IgG4 subclass showed an association between higher IgG4 levels and allergic asthma, but included only cases with elevated levels ¹⁰⁸. In our study, we did not observe any association between IgG and asthma or asthma-related outcomes in both LMM and LCT analyses. In the LCT analysis, we observed an association between a specific IgM trajectory with higher IgM levels at age 4 and reduction through childhood and adolescence, and a lower risk of asthma at 18 years. We speculated whether this indicated a high load of infections in early childhood that declines later, as IgM is produced during acute infections. Only one study has investigated the relationship between IgM and asthma by analyzing IgM levels in bronchoalveolar lavage fluid from patients with and without asthma ¹⁰⁹. However, the study did not find a significant association between IgM and asthma ¹⁰⁹.

CONCLUSION AND PERSPECTIVES

The thesis contributes to our understanding of asthma in adolescence and the relation to comorbidities and risk behaviours. Further, we examined inflammatory biomarkers longitudinally to identify systemic inflammation and its association with asthma and asthma-related outcomes.

In **Paper I**, we outlined a comprehensive description of the health and habits of adolescents living in Denmark, focusing on the 18-year follow-up visit in the COPSAC₂₀₀₀ cohort. We gathered detailed information on risk behaviours and environmental risk factors and performed multiorgan assessments. Our investigation of Danish adolescents showed that almost one-fourth had asthma, around one-fourth were overweight or obese, and two-thirds drank alcohol more frequently than once per month. Furthermore, 16.8% had a history of a neuropsychiatric diagnosis in childhood.

In **Paper II**, we investigated Danish adolescents with asthma compared to non-asthma adolescents. We found an increased risk of neuropsychiatric comorbidity and increased risk of self-destructive behaviours among adolescents with asthma. We did not see an association between asthma and any other risk behaviours, such as alcohol consumption and tobacco smoking. Additionally, among females, we observed an association between obesity and asthma.

In **Paper III**, we used longitudinal analyses to examine trajectories of specific blood measurements, including platelets, neutrophils, lymphocytes, NLR, eosinophils, total-IgE, IgG, IgA, and IgM, and their relation to asthma and asthma-related outcomes at 18 years. The study showed that childhood trajectories of platelets, eosinophils, and total-IgE were linked to asthma-related outcomes, including reduced lung function, increased AHR, and increased airway inflammation.

The large amount of data presented in **Paper I** provides the opportunity to explore risk factors and underlying mechanisms behind asthma, but also other lifestyle-related, non-communicable disorders such as obesity and neuropsychiatric disorders. Furthermore, the data bring opportunities for collaborative efforts with other cohorts worldwide and provides a unique foundation for future research within childhood diseases.

The findings in **Paper II** contribute to the understanding of adolescents living with asthma and the challenges and vulnerability it brings when being diagnosed with a chronic disease. The increased risk of neuropsychiatric disorders and self-destructive behaviours underscores the importance of regular monitoring and treatment of asthma. In regular monitoring, we should access information on mental health and well-being, which enables early interventions in adolescents with asthma and difficulties.

Furthermore, the commonalities addressed in our study between asthma and non-atopic comorbidities are interesting. The relationship between asthma and non-atopic comorbidities is widely debated among researchers, although the mechanism underlying this association remains unclear. However, comorbidities among adolescents with asthma need attention, as a better understanding of pathophysiology is needed to improve treatment, quality of life, and, ideally, future preventive therapies. In a future study, replication of the findings in a large population-based setting with greater power will enable the identification of an association between specific neuropsychiatric disorders and specific asthma phenotypes.

In **Paper III**, we showed that inflammatory biomarker trajectories, including platelet, eosinophil, and total-IgE, in childhood were linked to asthma-related outcomes in adolescence. This corresponds well with the theory that asthma proneness is most likely established in early childhood due to an intricate interplay between genetic and environmental exposures that disrupts immune system development, leading to low-grade systemic inflammation, including airway inflammation. The study suggests that repeated measurements of blood chemistry may provide insight into asthma pathophysiology and long-term outcomes and may be useful in asthma risk stratification. Furthermore, the potential role of platelets in airway inflammation opens the possibility for targeted preventive therapies; however, further insight and knowledge are still required, but future studies can hopefully build on the results presented in this thesis.

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APPENDIX

PAPER I **Mølbæk-Engbjerg T**, Vahman N, Mikkelsen M, Fink NR, Christensen ED, Brustad N, Sass L, Løvenhøj H, Strandberg-Larsen K, Groot J, Andersen AMN, Vinding R, Schoos AMM, Stokholm J, Bønnelykke K, Chawes B

Design of the 18-year follow-up of the Danish COPSAC2000 birth cohort¹

BMJ Paediatrics Open, 2024, vol. 8 (1), DOI: 10.1136/bmjpo-2024-002634, PMID: 39214547

PAPER II **Mølbæk-Engbjerg T**, Vahman N, Ali M, Skov F, Vinding R, Horner D, Brustad N, Thorsen J, Schoos AMM, Stokholm J, Bønnelykke K, Chawes B

Risk behaviours and non-atopic comorbidities of adolescents with asthma²

The World Allergy Organization journal, 2025-08, Vol.18 (8), p.101093-101093, Article 101093
DOI: 10.1016/j.waojou.2025.101093, PMID: 40718156

PAPER III **Mølbæk-Engbjerg T**, Ali M, Horner D, Brustad N, Sultan T, Jensen SK, Thorsen J, Vahman N, Brix S, Schoos AMM, Stokholm J, Bønnelykke K, Chawes B

Longitudinal trajectories of inflammatory biomarkers and their association with asthma

Manuscript prepared for submission

PAPER I

Mølbaek-Engbjerg T et al.

Design of the 18-year follow-up of the Danish COPSAC2000 birth cohort
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Design of the 18-year follow-up of the Danish COPSAC₂₀₀₀ birth cohort

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ABSTRACT

Background Atopic diseases, obesity and neuropsychiatric disorders are lifestyle-related and environmental-related chronic inflammatory disorders, and the incidences have increased in the last years.

Objective To outline the design of the 18-year follow-up of the Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₀₀) birth cohort, where risk factors of atopic diseases, obesity and neuropsychiatric disorders are identified through extensive characterisation of the environment, along with deep clinical phenotyping and biosampling for omics profiling.

Methods COPSAC₂₀₀₀ is a Danish prospective clinical birth cohort study of 411 children born to mothers with asthma who were enrolled at 1 month of age and closely followed at the COPSAC clinical research unit through childhood for the development of atopic diseases. At the 18-year follow-up visit, biomaterial (hair, blood, urine, faeces, throat, and skin swabs, nasal lining fluid and scraping, and hypopharyngeal aspirates) and extensive information on environmental exposures and risk behaviours were collected along with deep metabolic characterisation and multiorgan investigations including anthropometrics, heart, lungs, kidneys, intestines, bones, muscles and skin. Neuropsychiatric diagnoses were captured from medical records and registers accompanied by electronic questionnaires on behavioural traits and psychopathology.

Results A total of 370 (90%) of the 411 cohort participants completed the 18-year visit. Of these, 25.1% had asthma, 23.4% had a body mass index >25 kg/m² and 16.8% had a psychiatric diagnosis in childhood. A total of 68.7% drank alcohol monthly, and when drinking, 22.2% drank >10 units. Of the participants, 31.4% were currently smoking, and of these, 24.1% smoked daily. A total of 23.8% had tried taking drugs, and 19.7% reported having done self-destructive behaviour. The mean screen time per day was 6.0 hours.

Conclusion This huge dataset on health and habits, exposures, metabolism, multiorgan assessments and biosamples from COPSAC₂₀₀₀ by age 18 provides a unique opportunity to explore risk factors and underlying mechanisms of atopic disease and other lifestyle-related, non-communicable diseases such as obesity and neuropsychiatric disorders, which are highly prevalent in the community and our cohort.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Atopic diseases, obesity and neuropsychiatric disorders have increased in incidence in recent years.

WHAT THIS STUDY ADDS

⇒ Detailed data on health and habits, exposures, metabolism, multiorgan assessments and biosamples from Copenhagen Prospective Study on Asthma in Childhood by age 18.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The data provide a unique opportunity to explore risk factors and underlying mechanisms of atopic disease and other lifestyle-related, non-communicable diseases.

INTRODUCTION

Atopic diseases such as asthma, atopic dermatitis and allergic rhinitis are some of the most common chronic, inflammatory diseases in children and show an increasing prevalence.^{1,2} At the same time, an overlap between atopic and other non-communicable, lifestyle-related diseases, such as obesity and neuropsychiatric disorders, has been observed, proposing environment or lifestyle-induced mechanisms in early life as a common denominator.^{3–8} Children with asthma have an increased risk of developing depression and anxiety,⁹ and more recently, a link to attention deficit hyperactivity disorder (ADHD) was observed.¹⁰ Also, asthma is associated with metabolic syndrome and obesity, and speculations on a link between multiple prenatal exposures causing low-grade systemic inflammation have been proposed.^{11–13} Our main hypotheses are as follows: (1) Asthma and other chronic non-communicable inflammatory disorders such as obesity and neuropsychiatric disorders are programmed in early life and may share environmental risk factors; (2) Dysregulation of the immune system in early life can lead to chronic inflammatory diseases such as asthma, obesity

and neuropsychiatric disorders; (3) There is an overlap between 18 years with asthma, obesity and neuropsychiatric disorders and (4) An individual's capacity to metabolise nutrients is associated with a risk of asthma, obesity and neuropsychiatric disorders.

The Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₀₀) is a birth cohort study of children at risk of asthma designed to assess gene–environment interactions and early-life exposures that can be modified to prevent the development of atopic diseases. This paper aims to outline the hypotheses and the planned analyses and provide details of the 18-year follow-up visit of the COPSAC₂₀₀₀ cohort, including an extensive metabolic and multiorgan assessment and biobanking for omics analyses. This valuable information and material provide a unique possibility for studying risk factors and underlying mechanisms in the origins of atopic disease, obesity, neurodevelopmental disorders and their overlap.

METHODS

Study population

The COPSAC₂₀₀₀ is a single-centre clinical, prospective, mother–child cohort study of 411 individuals born to mothers with asthma who were recruited during

pregnancy from the Danish National Birth Cohort (DNBC). The children were included at one month of age and visited the COPSAC research unit at scheduled visits every 6 months until age 7 and again at ages 12 and 18. Further, the children visited the research unit on the occurrence of any acute airway or skin symptoms. The recruitment and baseline characteristics of COPSAC₂₀₀₀ are previously described in detail¹ (see figures 1–3).

Initial recruitment into and participation in DNBC, which is a large general population birth cohort, has been previously described in detail.¹⁴ In this study, we compare available and comparable baseline characteristics from a large sample of complete observations from the 18-year follow-up of DNBC with the baseline characteristics of COPSAC₂₀₀₀ at age 18.

Environmental exposures and habits

Information on educational level, other children living in the home, exposure to pets, passive smoking and fireplaces was collected. We registered personal patterns of substance use, such as alcohol, tobacco and drug use. The participants were also asked about self-destructive behaviour. Further, the participants were interviewed about screen time habits, characterised as time spent on

Flowchart COPSAC₂₀₀₀

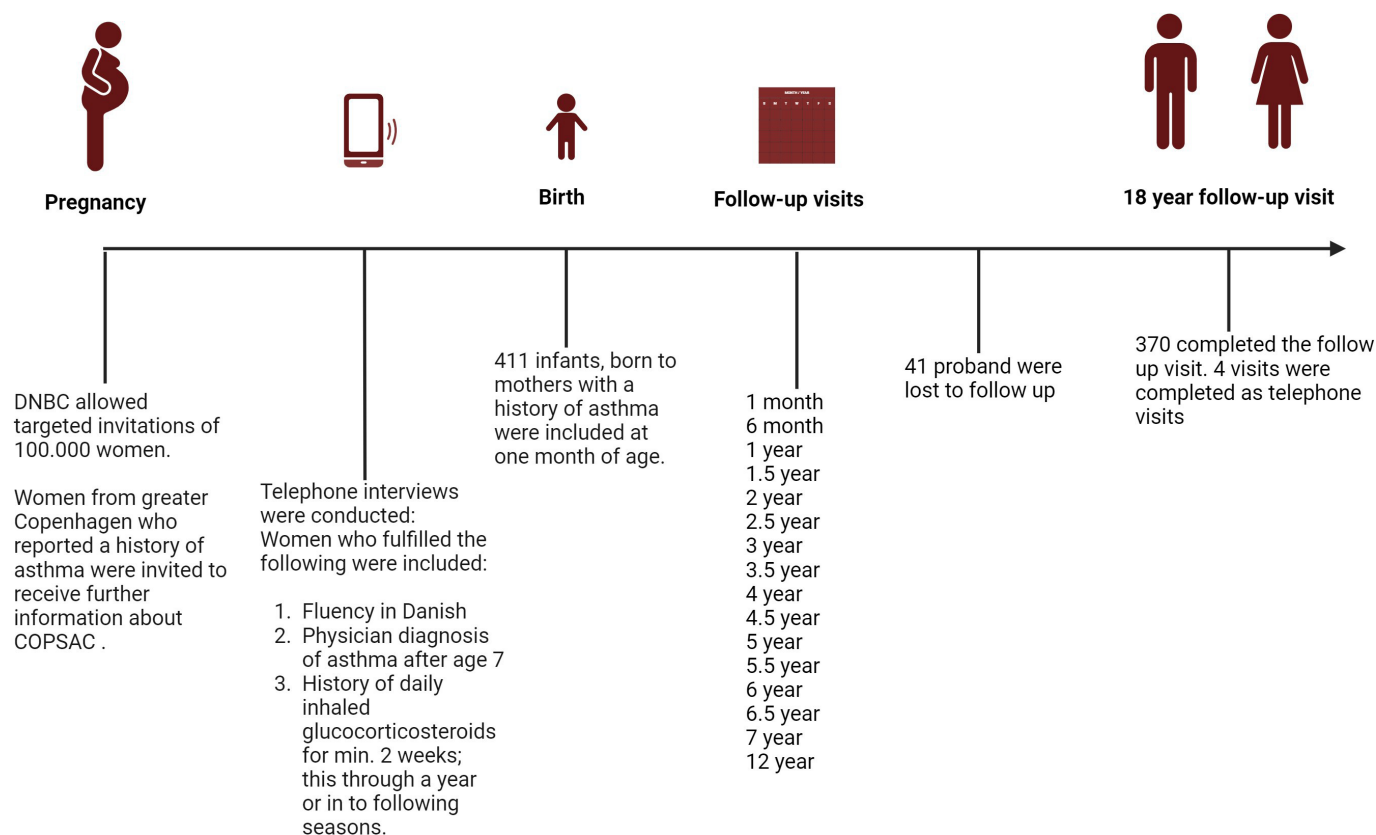


Figure 1 Flowchart illustration of the inclusion process of the COPSAC₂₀₀₀ cohort, and the follow-up visits until the 18-year follow-up visit. Created in Biorender.

18-year follow-up visit

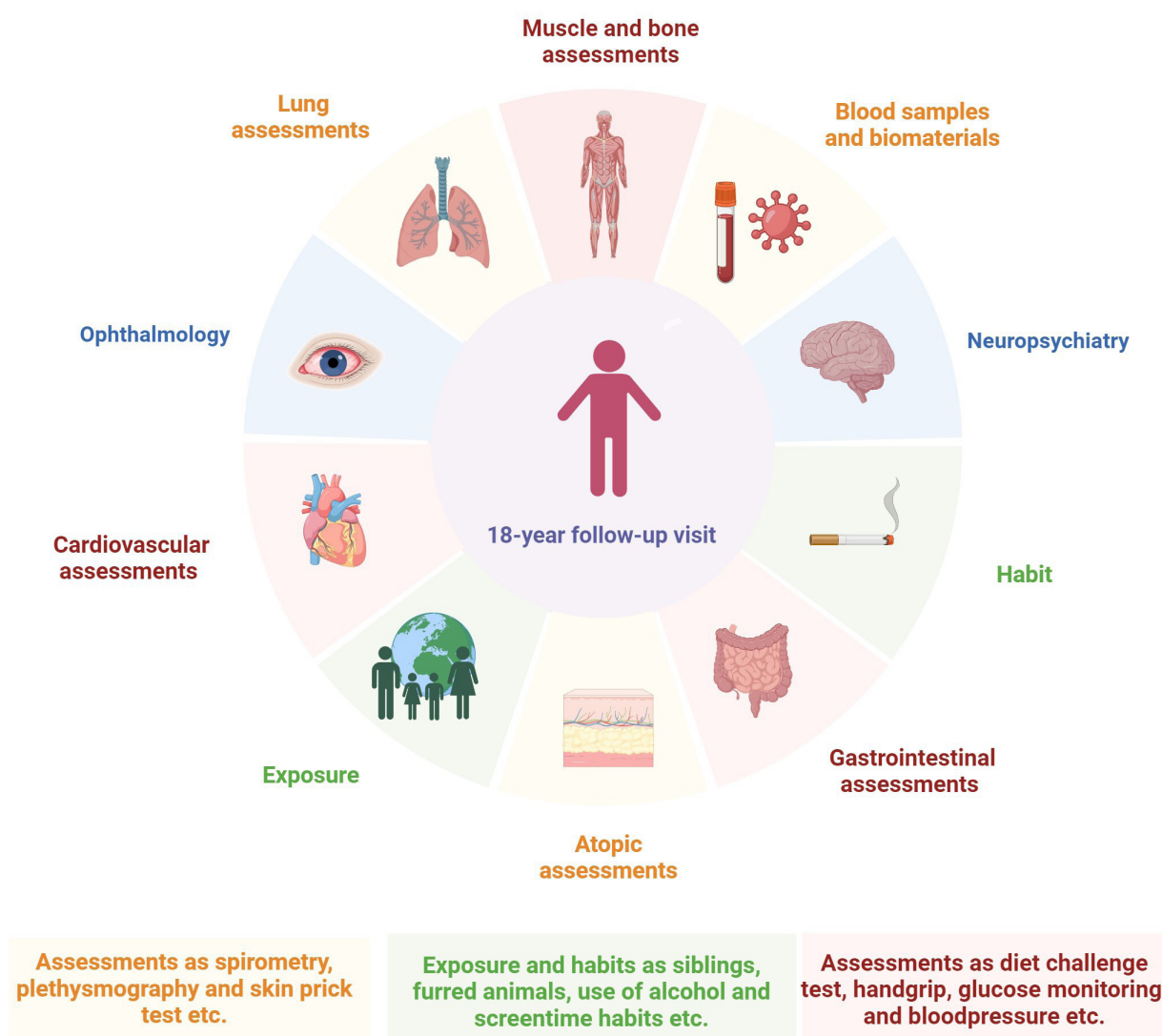


Figure 2 Illustration of the assessments described at the 18-year visit. Created in Biorender.

a screen in their spare time during the week (Monday to Thursday) and on weekends (Friday to Sunday).

Medicine and general medical history

The National Medicine Registry was checked for prescriptions redeemed up to 2 years before the visit date, compliance and duration of intake. We registered all medical diagnoses at the visit through interviews with the families. Hospital records were checked, and all contacts with the healthcare system were registered in the database. Any missed medical diagnoses were appended retrospectively at the visit and confirmed through the interview with the participant.

Atopic disease assessments

A physical examination was conducted at the visit, primarily focused on skin details, diagnosing eczema in subjects with skin issues following Hanifin and Rajka

criteria.¹⁵ Asthma was solely diagnosed by the COPSAC physicians. Children with typical symptoms of asthma were defined as having asthma if at least two of the following criteria were fulfilled: (1) Obstructive lung function with forced expiratory volume in 1s (FEV1) <80% and/or FEV1/forced vital capacity <80% measured by spirometry and/or specific airway resistance (sRaw) >1.6 kPa/s; (2) Bronchodilator reversibility: change of FEV1 $\geq$ 12% or sRaw $\geq$ 30% from baseline after inhalation of short-acting beta-2-agonist; (3) Airway inflammation: fractional exhaled nitric oxide (FeNO) $\geq$ 25 ppb and/or (4) Bronchial hyper-responsiveness to methacholine (drop in FEV1 $\geq$ 20% from baseline by a dose of methacholine $\leq$ 8 mg/mL) and/or exercise ($\geq$ 10% decrease in FEV1 from baseline). Remission was defined as no symptoms or asthma treatment for 1 year.^{16 17}

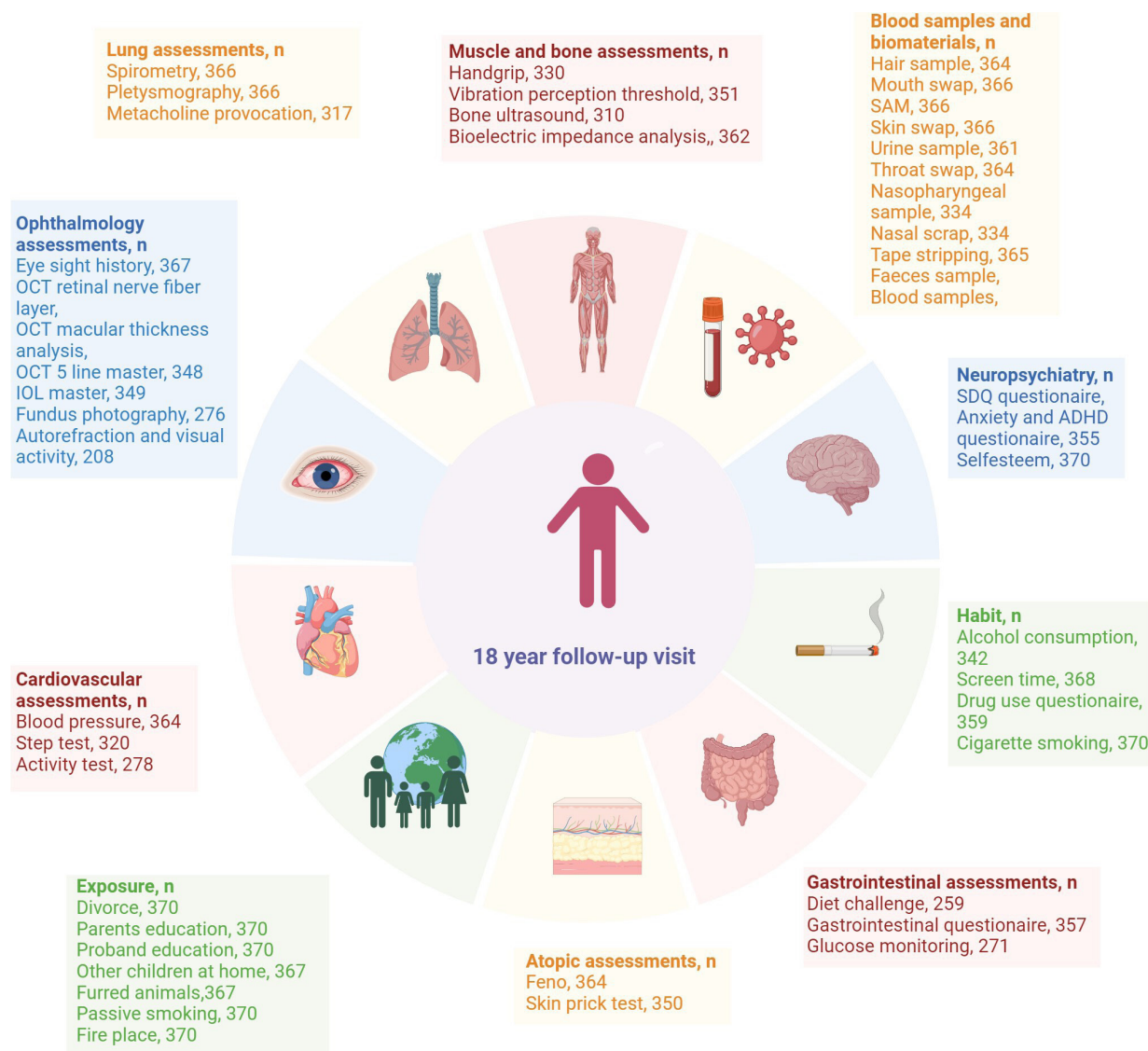


Figure 3 Illustration of the assessments described at the 18-year visit. Created in Biorender. ADHD, attention deficit hyperactivity disorder; SDQ, Strengths and Difficulties Questionnaire; OCT, optical coherence tomography; IOL, Intraocular Lens.

Allergic rhinitis diagnosis was based on allergic sensitisation and a corresponding history of sneezing, blocked, runny or itching nose on relevant exposure in the past 12 months in periods without a concurrent cold.¹⁸

Lung function tests and allergic sensitisation

Lung function was measured by spirometry (MasterScope Pneumoscreen, Erich Jäeger, Würzburg, Germany),¹⁹ and sRaw was assessed by whole-body plethysmography (MasterScope Bodybox, Erich Jäeger, Würzburg, Germany).²⁰ FeNO was measured using the Denox 88 version 88 DX0256, Switzerland.²¹ Bronchial reactivity was measured by methacholine provocation using the APS Pro, CareFusion 234, Germany.²² Allergic sensitisation to common inhalants and food allergens was diagnosed based on a skin prick test and measurements of specific IgE¹⁸.

Neuropsychiatric diagnosis and mental health

We collected psychiatric diagnoses from the medical records, including any psychiatric diagnosis, former and present. The diagnoses were divided into subgroups: ADHD, autism spectrum disorder, anxiety, depression, eating disorder, mental retardation, developmental disorder, personality disorder, schizophrenia, post-traumatic stress disorder, obsessive-compulsive disorder and others.

During the visit, three electronic questionnaires on behavioural traits and psychopathology were completed: the Strengths and Difficulties Questionnaire, the Adult ADHD Self Report Scale and the Depression, Anxiety and Stress Scale-21.

Self-rated social position, self-esteem, was registered on a scale from 1 to 5, where 1 represented a subjective feeling of being unpopular or very low in the social

hierarchy and 5 represented a subjective feeling of being the most popular and socially accepted person in the group of peers.

Metabolic assessments

We performed a standardised metabolic meal challenge after fasting. First, we took blood and urine samples at baseline; whereafter, the participant drank a smoothie made of palm oil, dextrose, skimmed milk powder and water. Continuously with the meal challenge test, we took blood and urine samples consecutively to observe the decomposition of the products using metabolomics assessments. A flash glucose monitoring (Freestyle Libre) was performed by applying a subcutaneous sensor to the posterolateral part of the non-dominant upper arm. The sensor measured glucose levels every minute, summarising measurements every 15 min for 2 weeks. We simultaneously made a food photo diary for 14 days, where the participants took pictures of all intakes of food and beverages except for water. We measured height and weight,¹ and blood pressure was assessed in a relaxed sitting position, registering three consecutive measurements. We assessed spontaneous physical activity using an omnidirectional accelerometer (ActiGraph wGT3X+Actilife) on the non-dominant wrist continuously for the following 2 weeks, assessing activity during the day and sleep.^{23 24}

Blood samples

At the beginning of the visit, we collected fasting blood samples, which were analysed for haematology, infection status, immunology, endocrinology/metabolic panel, inflammation markers, coagulation, proteins, vitamins, kidney and liver function, and sex hormones.

Immunological assessments

Blood samples were analysed for multiple immunological outcomes such as C reactive protein (CRP), IgA, IgG, IgM, IgE, transglutaminase IgA, and IgG, and a broad selection of antibodies such as ANA, ANCA, SR and CCP.

We also assessed airway immune profiles by quantifying 18 cytokines in upper airway fluid collected with filter paper strips as previously detailed.²⁴

Ophthalmological outcomes

We measured uncorrected visual acuity, refractive error and best-corrected visual acuity using an automated refractor (ARK-1s, NIDEK CO. LTD, Hiroishi-cho, Japan). The axial length of the eyes was measured with an optical biometer (IOLMaster, Carl Zeiss Meditec AG, Jena, Germany). We obtained a three-dimensional image of the macula and the optic disk in both eyes through optical coherence tomography (Cirrus HD-OCT Model 4000 (Carl Zeiss Meditec AG, Jena, Germany)).²⁵ Finally, a colour fundus photography was performed using a non-mydiatic retinal camera (TRC-NW7SF Mark II, Topcon, Tokyo, Japan). We recorded any parent-reported ophthalmological diagnoses during childhood. Additionally, the participant was interviewed regarding glasses or contact

lenses, prescribed power and a history of squinting at any time during childhood.

Bone and muscle assessments

We measured body Composition Analysis by Bioelectrical Impedance Analysis using a Tanita scale (TANITA MC-780MA) to analyse height-dependent muscle mass rating, skeletal muscle mass, fat percentage, and distribution, body mass index (BMI), body water distribution, and basal metabolic rate. A Saehan DHD-1 digital hand dynamometer was used to measure handgrip strength.²⁶ We measured the density of the bones with an ultrasound of the non-dominant hand (IGEA DBM Sonic BP, Bone Profiler, Model: BP01). The vibration perception threshold was measured under the feet using a handheld biothesiometer (G&G MediCare). We assessed the participant's maximum oxygen consumption (VO₂max in L/min) using The Danish Steptest^{25 27} to estimate cardiorespiratory fitness.

Gastrointestinal assessment

During the visit, the participants completed electronic questionnaires regarding gastrointestinal symptoms and the Bristol stool chart.²⁷

Biobank materials

At the visit, we collected multiple biosamples. These included a throat swab for virus detection, a mouth swab for bacteria, a nasopharyngeal sample, a nasal scrap for mRNA, upper airway lining fluid collected using a synthetic absorptive matrix for cytokines, tape stripping for collecting skin layer cells, a skin swab from the axil and antebrachium, a hair sample, a urine sample and a faecal sample for 16s rRNA sequencing and gut metabolomics.

Omics analysis

We are currently planning the following omics analysis on the biobank materials collected at the 18-year follow-up visit: (1) Plasma metabolomics, 0–4 hours, evaluated before and after a nutritional challenge test including 1H-NMR metabolomics measured at eight different times over 4 hours; (2) Plasma hormones (GIP, GLP1, Insulin, C peptide and Glucagon), 0–4 hours, evaluated before and after a nutritional challenge test measured at eight different times over 4 hours; (3) Stool metagenomics (microbiome); (4) Stool metabolomics (Based on NMR) and (5) Stool viromics.

Data management and statistics

We made data collection following Good Clinical Practice guidelines. We obtained all information at the 18-year follow-up visit through personal interviews done by medical doctors and research assistants with paediatric training, using predefined questions and closed questions response categories. Standard operating procedures were predefined for all study and database registration procedures. We collected data online during the visit. We saved any editing and changes of the data in an audit trail

in the database documents. We double-checked the database against source data by an external person. We used data from participants who completed the 18-year visit, and missing values were treated as missing observations.

Data were analysed descriptively with counts N (%), mean ($\pm$ SD) and median (IQR). Additionally, we conducted a t-test or X^2 test to assess differences between genders. In cases without normal distribution, the Wilcoxon test was used. Data processing was conducted with R (R Foundation for Statistical Computing, R V.4.0.3 (2020-10-10), Vienna, Austria).

Planned analyses

First, we plan to investigate the association between environmental risk factors and risk behaviours and asthma, obesity, and neuropsychiatric disorders at age 18 using univariate and multivariate logistic regression models. The overlap between asthma, obesity and neuropsychiatric disorders will be analysed using logistic regression and visualised with Venn diagrams.

Second, we plan to investigate the relationship between the immunological assessments cross-sectionally at age 18 years in relation to asthma, obesity, and neuropsychiatric disorders and longitudinally through childhood using latent class trajectories with multiple assessments from early childhood with random effects incorporated.

Third, we plan to analyse the association between the functional metabolism captured by changes in plasma metabolite levels during the standardised meal challenge using data-driven principal component analysis (PCA), time series analysis and supervised PLS-DA models (Partial Least Squares Discriminant Analysis). In these models, we will integrate plasma hormone levels, gut metagenomics and stool metabolomics.

RESULTS

Demographics

A total of 370 (90%) of the 411 children in the cohort completed the 18-year visit. The mean age was 17.7 years, with a maximum of 20.7 years and a minimum of 17.0 years; 49.4% were males and 96.6% were Caucasians. A total of 80% had started high school or similar. At home, 58.9% had younger children, 23.5% had older children and 38.4% had divorced parents. The cohort exhibited an equal distribution between rural and urban living environments, with 53% residing in rural areas and 47% in urban areas (table 1).

Environmental exposures and habits

In their homes, 27.0% lived with a cat and 48.9% with a dog. A total of 37.0% were exposed to a fireplace at home 1–100 days per year, and 10.3% were exposed >100 days per year. A total of 61.1% were exposed to passive smoking, whereas 29.7% were exposed >100 days per year. At age 18 years, 31.4% of the participants were currently smoking, and of these, 24.1% smoked daily. Among smokers, the distribution of how many cigarettes they

smoked per day was: 1–4/day, 80.4%; 5–9/day, 10.8% and >9/day, 8.8%. Only 1% had never drunk alcohol, whereas 68.4% drank more than once per month. When drinking, 68.7% drank >4 units per event, and 22.2% drank >10 units per event. A total of 23.8% had tried taking drugs. Of those, 70% had tried hash, 25.3% cocaine, 20.7% laughing gas (nitric oxide), 13.9% had tried ecstasy (MDMA, 3,4-methylenedioxy-methamphetamine) and 39.1% had tried other types of drugs such as amphetamine, sedatives, LSD (Lyserg-syre-Diethylamid), opiates, euphoric mushrooms and lighter gas. There were only gender differences with respect to laughing gas ($p=0.01$) and other drugs ($p=0.04$), which had been tried by males more often than females.

The mean screen time per day was 6.0 hours and was higher for males vs females: 6.7 vs 5.9 hours ($p<0.01$). This assessment included using television, games and phones in leisure time (table 1).

Atopic diagnosis

A total of 44.3% had an asthma diagnosis at some point in the first 18 years of life. By age 18, 25.1% were diagnosed with asthma, allergic rhinitis was diagnosed in 38.6% of the probands, and atopic dermatitis was diagnosed in 10.3% (table 2). More females than males had atopic dermatitis ($p=0.05$), whereas there was a trend of more allergic rhinitis among males ($p=0.06$) and no gender differences in the presence of asthma by age 18.

Lung function and allergy tests

The lung function tests showed that 9.5% had an $FEV_1<80\%$, 46.8% had an $FEV_1<90\%$ and 7.6% had increased airway resistance, that is, $sRaw>1.6$ kPa/s. An increased FENO>25 ppb was seen in 28.1% of the probands. The methacholine provocation test was positive in 4.6%, defined as a 20% reduction in FEV_1 caused by a dose <8 μ mol/mL.

The median (IQR) total IgE level was 80.0×10^3 IU/L (28.0–238.1); 19.5% had elevated specific IgE (>0.35 ku/L) towards foods and 50.8% had elevated specific IgE towards aeroallergens. The skin prick test was positive for foods in 7.6% and aeroallergens in 47.3% of the participants (table 2). In general, males more often had adverse lung function and allergy tests compared with females (table 2).

Anthropometrics, bone and muscle assessments

The mean (SD) BMI was 22.8 kg/m² (0.39) for males and 23.3 kg/m² (0.42) for females. Males and females had an equal BMI distribution, and there was no significant difference between the genders when testing for this ($p>0.51$) (table 1). At the visit, 97.8% completed the body composition analysis, and 89.2% had handgrip strength measured. Bone density was measured by ultrasound in 83.8% of the probands and vibration tests were performed in 94.9%.

Metabolic assessments

At the visit, 70.0% completed the meal challenge, 73% had their blood glucose monitored and 74.1% fulfilled

Table 1 Demographics

Cohort	DNBC*	COPSAC2000			P value†
	All N (%)	All N (%)	Female N (%)	Male N (%)	
Gender	44 485 (58.6 female)	370 (90)	188 (50.8)	182 (49.2)	0.35
Parents' divorce	13 394 (30.1)	142 (38.4)	74 (39.4)	68 (37.4)	0.77
Race (Caucasian)	N/A	355 (96.0)	183 (97.3)	172 (95.0)	0.34
Proband education					
Elementary school, completed	N/A	357 (96.5)	182 (96.8)	175 (96.2)	1.00
College, started	35 280 (83.6)	288 (77.8)	155 (82.4)	133 (73.1)	0.07
Tradesman, started	4331 (10.3)	38 (10.3)	9 (4.8)	29 (15.9)	<0.01
University, started	361 (0.9)	1 (0.3)	–	1 (0.51)	–
Parents education, completed					
Elementary school, mother	N/A	13 (3.51)	8 (4.26)	5 (2.74)	0.41
Elementary school, father	N/A	17 (4.60)	11 (5.85)	6 (3.30)	0.23
High school, mother	N/A	6 (1.62)	6 (3.19)	–	–
High school, father	N/A	7 (1.89)	5 (2.66)	2 (1.10)	0.26
Tradesman, mother	N/A	94 (25.4)	39 (20.7)	55 (30.2)	0.10
Tradesman, father	N/A	102 (27.6)	47 (25.0)	55 (30.2)	0.43
University, medium long, mother	N/A	125 (33.8)	62 (33.0)	63 (34.6)	0.93
University, medium long, father	N/A	70 (18.9)	35 (18.6)	35 (19.2)	1.00
University, long, mother	N/A	69 (18.6)	41 (21.8)	28 (15.4)	0.12
University, long, father	N/A	82 (22.2)	40 (21.3)	42 (23.1)	0.83
Fireplace					
1–100 days	N/A	137 (37)	63 (33.5)	74 (40.7)	0.20
>100 days	N/A	38 (10.3)	18 (9.6)	20 (11.0)	0.53
Furred animals					
Cats, yes	N/A	100 (27.0)	52 (27.7)	48 (26.4)	0.87
Dogs, yes	N/A	181 (48.9)	96 (51.1)	85 (46.7)	0.46
Other children younger, yes	N/A	218 (58.9)	105 (55.9)	113 (62.1)	0.47
Other children older, yes	N/A	87 (23.5)	46 (24.5)	41 (22.5)	0.89
Current smoking	9639 (21.7)	116 (31.4)	58 (30.9)	58 (31.9)	0.38
Every day‡	3335 (34.6)	28 (24.1)	15 (25.9)	13 (22.4)	0.71
Min ones every week‡	2193 (22.8)	37 (31.9)	15 (25.9)	22 (37.9)	0.25
Min ones every month‡	4111 (42.7)	51 (44.0)	28 (48.2)	23 (39.7)	0.48
Number of cigarettes smoked in the last 4 weeks§					
1–4 per day	2879 (52.1)	82 (80.4)	36 (73.5)	46 (86.8)	0.35
5–9 per day	1326 (24)	11 (10.8)	9 (18.4)	2 (3.8)	0.08
>9 per day	1189 (21.5)	9 (8.8)	4 (8.1)	5 (9.4)	0.74
Passive smoking					
1–100 days per year	N/A	116 (31.4)	58 (30.9)	58 (31.9)	0.92
>100 days per year	N/A	110 (29.7)	61 (32.4)	49 (26.9)	0.29
Drug use ever	14 673 (33)	87 (23.8)	31 (16.8)	56 (30.8)	0.69
Hash/pot	13 907 (94.8)	60 (70.0)	23 (74.2)	37 (66.1)	0.06
Cocaine	1804 (12.3)	22 (25.3)	8 (25.8)	14 (25.0)	0.26
Laughing gas (NO)	1464 (10)	18 (20.7)	3 (9.7)	15 (26.8)	0.01
Ecstasy/MDMA/fantasy	1317 (9)	12 (13.9)	5 (16.1)	7 (12.5)	0.75
Others¶	2979 (20.3)	34 (39.1)	11 (35.5)	23 (41.1)	0.04

Continued

Table 1 Continued

	DNBC*	COPSAC2000			
Cohort	All N (%)	All N (%)	Female N (%)	Male N (%)	P value†
Alcohol; drinking frequency					
Never	3273 (7.4)	3 (1.0)	–	3 (1.6)	0.14
Max once per month	10017 (22.5)	89 (24.1)	49 (26.1)	40 (22.0)	0.46
>1 per month	21 195 (70.1)	253 (68.4)	124 (66.0)	129 (70.9)	0.46
Alcohol; units					
1–4	11 853 (28.8)	88 (23.8)	51 (27.1)	37 (20.3)	0.26
5–9	21 952 (53.3)	172 (46.5)	93 (49.5)	79 (43.4)	0.41
>10	7407 (18)	82 (22.2)	29 (15.4)	53 (29.1)	0.91
BMI<18.5 (%) underweight	4395 (9.9)	32 (8.7)	17 (9.20)	15 (8.2)	0.72
BMI 18.5–24.99 (%) normal weight	32 336 (72.7)	249 (67.8)	121 (65.4)	128 (70.3)	0.66
BMI 25–30 (%) overweight	5770 (13)	57 (15.5)	31 (16.8)	26 (14.3)	0.51
BMI>30 (%) obese	1984 (4.5)	29 (7.9)	16 (8.7)	13 (7.1)	0.58
Screen time					
Total screen time hours, median (IQR)	N/A	6.0 (4.5–7.0)	5.90 (4.5–6.5)	6.7 (5.0–7.9)	<0.01
Asthma, current	N/A	93 (25.1)	46 (24.4)	47 (25.8)	0.85
Self-injury	N/A	73 (21)	56 (31.1)	17 (9.9)	0.92
Self-esteem all (mean/SD)	N/A	3.58/0.80			
1		5 (1.35)	3 (3.23)	2 (1.10)	1.00
2		18 (4.86)	12 (12.9)	6 (3.30)	0.26
3		142 (38.4)	81 (87.1)	61 (33.5)	0.08
4		162 (43.8)	77 (82.80)	85 (46.7)	0.29
5		40 (10.8)	14 (15.1)	26 (14.3)	0.05
Neuropsychiatric disorder ever	N/A	62 (16.8)	30 (16.0)	32 (17.6)	0.80
Bold values represent p-values at or below the significance level of 0.05.					
*Absolute and relative frequencies for a sample of complete response observations from the 18-year DNBC follow-up.					
†t-test comparing significance between the gender.					
‡Only for current smokers.					
§Only for current smokers. Data are available from 102 probands.					
¶Amphetamine, sedatives, LSD, opiates, euphoric mushrooms, lighter gas.					
BMI, body mass index; COPSAC2000, Copenhagen Prospective Study on Asthma in Childhood; DNBC, Danish National Birth Cohort; LSD, Lysergic Acid Diethylamide; MDMA, 3,4-Methylenedioxy-methamphetamine; N/A, not available.					

the activity test. Blood pressure was mostly measured in the normal range; only 0.5% of the males and 0.5% of the girls had abnormally high blood pressure (males: MAP>103 mm Hg; females: MAP>99 mm Hg.).

Blood samples

Blood sample results are presented with their mean or median, normal reference interval and percentage of participants with values outside the normal range in online supplemental appendix, for example, mean plasma 25-hydroxyvitamin D was 64 nmol/L with 31.4% of the participants having a value below the normal range (<50 nmol/L) and mean LDL (low-density lipoprotein) cholesterol was 2.0 with 7% having values above the upper range (>3 mmol/L) (online supplemental tables S1–S3).

Immunological outcomes

The mean plasma IgA was 1.49 g/L, with 3.1% of females placed below the normal range vs 4.5% of males and 2.5% of females placed above the normal range vs 2.6% for males. IgG had a mean value of 10, with 3.8% of females placed below the normal range vs 5.8% of males and 0.6% of females placed above the normal range vs 2.6% of males. The median CRP was 0.74, with an IQR of 1.7 (online supplemental tables S1 and S3).

Ophthalmological outcomes

Out of 366 children, eyesight history was available for 99.2% and 38% had used glasses or contact lenses at some point, with a myopia/hyperopia ratio of 3:1.

Table 2 Atopic assessments and diagnoses

	All N (%)	Females N (%)	Males N (%)	P value*
Spirometry, FEV1†				
FEV1, L<80%	35 (9.5)	11 (5.9)	24 (13.2)	0.03
FEV1, L<90%	173 (46.8)	93 (49.5)	80 (44.0)	0.31
sRaw				
sRaw kPa/s>1.6 (%)	28 (7.6)	12 (6.4)	16 (8.8)	0.52
Methacholine provocation, median PD20/μmol‡	225.40	186.2	298.6	0.01
% <cut-off off	17 (4.6)	8 (4.3)	9 (5.0)	
Feno>25 ppb§ (%)	104 (28.1)	40 (21.3)	64 (35.2)	<0.01
IgE total x 103IU/L, median (IQR)§	80.0 (28.0–238.1)	69.4 (25.0–216.5)	103.9 (32.4–262.6)	0.06
Positive specific IgE any kU/L, N (%)§	192 (51.9)	80 (42.6)	112 (61.5)	<0.01
Positive specific IgE food kU/L, N (%)§	72 (19.5)	34 (18.1)	38 (20.9)	0.6
Positive specific IgE inhalation kU/L, N (%)§	188 (50.8)	78 (41.5)	110 (60.4)	<0.01
Positive SPT any (%)	176 (47.6)	75 (39.9)	101 (55.5)	<0.01
Positive SPT food (%)	28 (7.6)	11 (5.9)	17 (9.3)	0.23
Positive SPT inhalation (%)	175 (47.3)	74 (39.4)	101 (55.5)	<0.01
Asthma, current (%)	93 (25.1)	46 (24.4)	47 (25.8)	0.86
Asthma, ever (%)	164 (44.3)	77 (41.0)	87 (47.8)	0.44
Rhinitis, current (%)	143 (38.6)	62 (33.0)	81 (44.5)	0.06
Atopic dermatitis, current (%)	38 (10.3)	25 (13.3)	13 (7.14)	0.05

Bold values represent p-values at or below the significance level of 0.05.

*Significance between the genders is calculated with t-test. In causes of not normal distributed data, Wilcoxon test has been used.

†Calibrated for sex, height and age.

‡PD20 is the median dose of methacholine that causes a 20% reduction in FEV1. The test is positive when a 20% reduction in FEV1 is caused by a PD20 <8 μmol.

§Normal range FeNO <25 ppb.

FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; SPT, skin prick test; sRaw, specific airway resistance.

Biobank material

For 98.9% of the probands, we collected mouth swabs for bacteria and skin swabs. Throat swabs for viruses, SAM, tape stripping and a hair sample were collected in 98.6% of the probands. In 97.3% of the cases, we collected a urine sample, nasopharyngeal samples and nasal scrap for mRNA in 90.3% of the proband. Only 58.1% delivered a faeces sample.

DISCUSSION

Primary findings

In this extensive investigation of environmental risk factors, risk behaviours and multiorgan assessments, we obtained highly granulated information about the health and habits of Danish adolescents. We found that approximately one-fourth had asthma, one-fourth had a BMI>25 kg/m² and 16.8% had a neuropsychiatric diagnosis in childhood. In addition, more than two-thirds of the 18-year-old Danish adolescents drank alcohol more than once per month, and when drinking, more than one-fifth drank >10 units of alcohol. Further, almost one-fourth had tried taking drugs.

Perspective

This huge dataset on health and habits, exposures, metabolism, multiorgan assessments and biosamples for omics profiling from the COPSAC₂₀₀₀ birth cohort by age 18 years provides strong data to explore risk factors and metabolic mechanisms behind atopic diseases and other lifestyle-related, non-communicable disorders such as obesity and neuropsychiatric diseases and the commonality between these disorders. The standardised meal challenge is a unique opportunity to study the relationship between an individual's metabolic capacity and the risk of asthma, obesity and neuropsychiatric disorders. This could pave the path for dietary intervention studies to prevent and treat these common disorders. The presentation of this rich data source, biobank material and methods is also an invitation for collaborative efforts with other cohorts worldwide.

Strengths and limitations

The major strength of this study is the multiple assessments from most organ systems and the excessive amount of information describing the environment and risk behaviours of the participants. Furthermore, multiple

biosamples are collected and stored for future omics analysis. All objective measurements were done by trained research personnel strictly following standard operating procedures. The participants of the COPSAC₂₀₀₀ cohort have participated in such examinations repeatedly from birth and are therefore highly competent in performing tests and examinations, which assure a high completion rate.

It is also a major strength that all diagnoses of asthma, atopic dermatitis and allergic rhinitis were diagnosed and monitored at the COPSAC research unit and not by general practitioners or based on parent interviews or questionnaires. All diagnostic procedures followed standard operating procedures, ensuring standardised diagnosis, treatment and regular evaluation. Furthermore, all participants were born to mothers with asthma, which improves symptom recognition.

A limitation of the study is its external validity because of the high-risk nature of the cohort, which was also predominantly Caucasians. However, comparing the 18 years in the DNBC allows us to compare differences in environmental factors, risk behaviours, obesity and asthma prevalence in a population-based cohort. Furthermore, we collected psychiatric diagnoses from the medical records, including any psychiatric diagnosis, former and present. The diagnoses were divided into 12 subgroups to simplify the classification. This simplified classification could cause a less precise diagnosis.

CONCLUSION

This huge dataset on health and habits, exposures, metabolism, multiorgan assessments and biosamples from COPSAC₂₀₀₀ by age 18 years provides a unique opportunity to explore risk factors and metabolic mechanisms underlying atopic disease and the commonality with other lifestyle-related, non-communicable disorders such as obesity, dyslipidaemia and neuropsychiatric diseases, which are highly prevalent in the community and our cohort.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval The study was conducted under the guiding principles of the Declaration of Helsinki and was approved by the Copenhagen Ethics Committee (COPSAC2000: KF 01-289/96, COPSAC2000 18 and 24 years follow up: H-16039498), and the Danish Data Protection Agency (COPSAC2000). Both parents gave oral and written informed consent before enrolment. At the COPSAC2000 18-year visit, the study participants also consented. Participants gave informed consent to participate in the study before taking part. Governance: We know and comply with recognised codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) Good Clinical Practice guidelines and the Helsinki Declaration. Privacy is important to us, so we follow national and international legislation such as the General Data Protection Regulation (GDPR), the Danish Act on Processing of Personal Data, and the practice of the Danish Data Inspectorate.

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SUPPLEMENTARY

TABLE S1

Table S1: Blood samples		
Lever	Median (IQR)	% placed outside normal reference interval
ALAT F:10–45 U/L M:10-70 U/L	25 (10.0)	F: Low: 0.6; High: 4.4 M: Low: 0.6; High: 4.6
ASAT F: 15-35 U/L M: 15-45 U/L	28 (9.0)	F: Low: 1.9; High: 10.8 M: High: 7.8
Amylase 10-65 U/L	78.5 (17.5)	Low: 2.35 High: 11.1
Alkaline phosphatase 35-105 U/L	74 (31.0)	Low: 10.4 High: 6.5
Bilirubin 5-25 µmol/L/ L	10 (6.0)	Low: 1.17 High: 19.1
GGT F: 10-45 U/L M: 10-80 U/L	17 (10.0)	F: Low: 3.2; High: 1.9 M: Low: 0.7; High 0.7
LDH 105 - 205 U/L	146 (28.2)	Low: 19.9 High: 3.2
Haptoglobin 0.35 – 1.85 g/L	0.97 (0.6)	Low: 4.2 High: 5.8
	Mean (SD)	% placed outside normal reference interval
Albumin 36-48 g/L	43.3 (2.9)	Low: 10.6 High: 13.3
Antitrypsin 0.97-1.68 g/L	1.35 (0.3)	Low: 5.11 High: 13.7

Coagulation	Median (IQR)	% placed outside normal reference interval
Antithrombin 0.80-1.20 × 10 ³ int.enh. / L	1.0 (0.1)	Low: 2.1 High: 3.3
INR <1,2	1.1 (0.1)	High: 0.6
Homocystein < 10.0 µmol/L	10.1 (4.7)	High: 2.4
	Mean (SD)	% placed outside normal reference interval
APTT 20-29 s	30.5 (5.2)	Low: 5.9 High: 17.8
Nephrology	Median (IQR)	% placed outside normal reference interval
Creatinine kinase, CK F: 50-150 U/L M: 50-270 U/L	93 (82.0)	F: Low: 18.4; High: 12.0 M: Low: 0.6; High 16.1
Creatinine F: 45-90 µmol/L M: 60-105 µmol/L	68 (15.0)	F: High: 0.6 M: Low: 6.5; High 0.6
Carbamide F: 2.6-6.4 mmol/L M: 3.2 – 8.1 mmol/L	4.5 (1.7)	F: Low: 3.2; High: 1.3 M: Low: 5.8; High: 0.6
Phosphate F: 0.76 – 1.41 mmol/L M: 0.71 – 1.53 mmol/L	1.19 (0.2)	F: High: 6.3 M: High: 3.9
PSA < 3,0 µg/L	0.4 (0.4)	_***
	Mean (SD)	% placed outside normal reference interval
Kalium 3.5 – 4.6 mmol/L	141 (1.7)	Low: 10.9 High: 0.3
Calcium-ion frit 1.18-1.32 mmol/L	1.24 (0.0)	Low: 1.0 High: 0.3

Sodium 137 - 145 mmol/L	1.24 (0.0)	Low: 4.4 High: 20.9
Urate F: 0.16-0.35 mmol/L M: 0.23-0.48 mmol/L	0.311 (0.1)	F: Low: 2.5; High: 4.4 M: Low: 0.6; High: 0.6
Coronary enzymes	Median (IQR)	% placed outside normal reference interval
Troponin ITNI < 24 ng/L	7 (11.5)	High: 1.5
ProBNP No reference level	56.5 (35.8)	-
Vitamins	Median (IQR)	% placed outside normal reference interval
B12 vitamin 200 - 600 pmol/L	351 (162.0)	Low: 5.8 High: 5.1
Folate >8.6 nmol/L	11 (7.5)	Low: 29.7
	Mean (SD)	% placed outside normal reference interval
D-vitamin, P-25-OH-Vitamin D(D3+D2) 50 - 160 nmol/L	64 (42.0)	Low: 31.4 High: 0.3
Jern 9 - 34 µmol/L	16.6 (6.8)	Low: 8.6 High: 1.2
Magnesium 0.70 – 1.10 mmol/L	0.822 (0.1)	Low: 1.6
Haematology	Median (IQR)	% placed outside normal reference interval
Platelets F: 165 - 400 x 10 ⁹ /L M: 145 - 350 x 10 ⁹ /L	250 (68.0)	F: Low: 1.2; High: 1.9 M: High: 4.6
Ferritin 13-152 µg/L	38 (41.0)	Low: 11.9 High: 2.3
Erythrocyte vol. Spred. No reference levels.	13 (0.0)	-

Reticulocytes F: 39-139 x 10 ⁹ /L M: 39-139 x 110 ⁹ /L	58 (22.0)	F: Low: 12.4 M: Low: 7.1; High 0.6
Eosinophilic >0.5 x 10 ⁹ /L	0.2 (0.2)	High: 5.6
Basophilic >0.2 x 10 ⁹ /L	0 (0.1)	High: 33.8
Atypical cells No reference levels	0.1 (0.0)	-
Monocytes 0.2-0.8 x 10 ⁹ /L	0.4 (0.2)	Low: 0.3 High: 1.2
Neutrophiles 1.6-5.9 x 10 ⁹ /L	2.99 (1.5)	Low: 1.5 High: 25.4
Transferrin saturation 0.2-0.5	0.24 (0.1)	Low: 33.1 High: 3.2
Transferritin, massek. F: 2.12-3.91 g/L M: 2.12-3.3 g/L	2.66 (0.6)	F: Low: 3.2; High: 3.2 M: Low: 7.1; High: 3.9
CRP < 9 mg/L	0.74 (1.7)	High: 3.7
	Mean (SD)	% placed outside normal reference interval
Haemoglobin F: 7.3 – 9.5 mmol/L M: 8.3 – 10.5 mmol/L	8.62 (0.8)	F: Low: 5.7 M: Low: 3.3
MCV 82-98 fL	85.9 (3.6)	Low: 10.9
Erytrocytter F: 3.94-5.16 x 10 ¹² /L M: 4.25-5.71 x 10 ¹² /L	4.73 (0.4)	F: Low: 2.5 M: Low: 1.3; High: 0.6
Erytrocytter vol.fr F: 0.35-0.46 M: 0.4-0.5	0.41 (0.1)	F: Low: 3.8 M: Low: 7.8
Lymphocytes 1.0-3.5 x 10 ⁹ /L	1.91 (0.5)	Low: 0.9 High: 0.3

MCH 1.7-2.1 fmol	1.83 (0.1)	Low: 2.9 High: 0.3
MCHC 19.7-22.2 mmol /L	21.3 (0.6)	Low: 0.3 High: 5.1
Leucocytes 3.5-8.8 x 10 ⁹ /L	5.8 (2.1)	Low: 1.0 High: 6.1
Sex hormones	Mean (SD)	% placed outside normal reference interval
Prolactin F: 25-600 x 10 ⁻³ IU/L M: 40-400 x 10 ⁻³ IU/L	196 (91.5)	F: High: 1.9 M: High: 2.0
Estradiol F: Not normal references for this age group M: > 0.3 nmol/L	0.15 (0.1)	-.***
FSH F: Not normal references for this age group M: 1.4-7.5 IU/L	4.05 (3.5)	F: -*** M: Low: 3.3; High: 6.6
Progesteron F: Not normal references for this age group M: 0.7-4.3 nmol/L	1.8 (1.1)	-.***
Lutropin, LH F: 1-53 IU/L M: 1-7.5 IU/L	3.9 (2.6)	F: High: 0.6 M: -***
HCG** < 3 IU/L	1 (2.0)	-.***
Metabolism	Mean (SD)	% placed outside normal reference interval
Proinsulin 336-4113 pmol/L	427 (222.0)	Low: 27.4
Insulin 20.8 – 173.6 pmol/L	71 (56.0)	Low: 0.6 High: 7.4

Hemoglobin A1c, IFCC < 48 mmol/mol	5.4 (0.6)	High: 0.3
HbA1C, middle glucose < 7.7 mmol/L	32 (3.0)	High: 0.3
Glucose 4.2-6.3 mmol/L	5.1 (0.5)	High: 1.6
Cholesterol, VLDL > 0.9 mmol/L	0.4 (0.2)	High: 2.6
Cholesterol, HDL < 1 mmol/L	1.18 (0.3)	***_
Cholesterol, LDL >3 mmol/L	2 (0.7)	High: 7.0
Cholesterol, total > 5 mmol/L	3.7 (0.8)	High: 2.9
Triglyceride > 2 mmol/L	0.88 (0.5)	High: 3.2
PTH, parathyrin 2-8.5 pmol/L	4.84 (2.5)	Low: 1.0 High: 5.1
TSH, Thyrotropin 0.4-4.8 x 10 ⁻³ IU/L	1.82 (1.2)	High: 3.6
Immunology and antibodies	Mean (SD)	% placed outside normal reference interval
IgA F: 0.65-3.06 g/L M: 0.63-3.21 g/L	1.49 (1.0)	F: Low: 3.1; High: 2.5 M: Low: 4.5; High: 2.6
IgG F: 7.4-15.6 g/L M: 7.1-15.3 g/L	10 (2.6)	F: Low: 3.8; High: 0.6 M: Low: 5.8; High: 2.6
IgM F: 0.66-2.61 g/L M: 0.5-1.94 g/L	1 (0.6)	F: Low: 8.2; High: 1.3 M: Low: 8.4; High: 2.3

TS1 1: Blood samples: Presentation of blood samples with mean values and number in % of values placed outside normal reference interval.

:

M=Male, F=Female

*Samples not collected, haemolysed, or too little material to run the analysis.

**24 probands with positive values.

*** All values are placed in the normal interval.

12 **TABLE S2**
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Table S2: Faeces	Median (IQR)	Negative (%)	% placed outside normal reference interval
F-Calprotectin <50: IBD** implausible 50 – 200: Elevated, repetition recommended >200: Elevated, IBD** likely	34 (48.0)	109 (27)	>200: 2.1 50-200: 9.6 <50: 22.0

14 **Table S2:**
15 *Samples not collected or too little material to run the analysis.
16 **Inflammatory bowel disease
17 or too little material to run the analysis.

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20 **TABLE S3**
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Table S3: Immunology and antibodies	Median (IQR)
Smith’s Antibody	2.0 (2.0)
Sedimentations-reaction, SR	7.0 (3.3)
Cykl.citrul.peptid antibody, CCP	1.0 (1.0)
Sjögrens syndrome A IgG	32.0 (0.0)
Sjögrens syndrome B IgG	1.0 (0.5)
Transglutaminase IgA	2.0 (7.5)
Transglutaminase IgG	1.0 (1.0)
rRNA 2'O-methyltransferase fibrillarin-anti (IgG)	1.3 (1.0)
Centromer B antibody IgG – CBAIgG	3.2 (1.0)
P-Histidin--tRNA-ligase(Jo 1) -antistof(IgG)	-
P-DNA (dobbel-stranded) -antibody(IgG)	4.9 (4.5)
P-Chromodomain-helicase-DNA-binding protein 4-antibody (IgG)	7.2 (9.0)
P-proliferating nucleus-antibody (IgG)	2.0 (0.0)
P-ACE (Peptidyl dipeptidase A)	51.2 (22.0)
P-Exosome component 10-antibody (IgG)	1.3 (0.3)
P-Connective tissue related disease antibody	0.3 (0.2)
Scleroderma IgG	1.7 (1.0)

P-Ribonucleo protein U1 (70 kDA+A+C) - antibody (IgG)	2.7 (1.0)
P-DNA-directed RNA polymerase III subunit RPC1-antibody (IgG)	3.0 (2.0)
P-Acidic ribosomal protein antibody (IgG)	1.4 (0.3)
Rheuma-factor IgM	20.0 (2.0)

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PAPER II

Mølbæk-Engbjerg T et al.

Risk behaviours and non-atopic comorbidities of adolescents with asthma
The World Allergy Organization journal, 2025



Risk behaviours and non-atopic comorbidities of adolescents with asthma

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ABSTRACT

Background: Risk behaviours, obesity, and neuropsychiatric comorbidity have been demonstrated in various chronic diseases but are less well described among adolescents with asthma.

Methods: We clinically assessed asthma status at the 18-year follow-up visit of the Danish Copenhagen Prospective Studies on Asthma in Childhood (COPSAC₂₀₀₀) birth cohort born to mothers with asthma, and we investigated risk behaviours and non-atopic comorbidities. We included obesity and neuropsychiatric diseases captured from medical records and electronic questionnaires on behavioural traits and psychopathology. Associations between asthma status, risk behaviours, and non-atopic comorbidity were analysed using logistic regression models.

Results: A total of 370 individuals (90%) completed the 18-year visit, and 93 of these (25.1%) had current asthma. Comparing adolescents with and without asthma, binge drinking was reported in 75.2% vs 66.0%, current smoking in 26.9% vs 32.9%, and drug use in 16.1% vs 26.0%. High daily screen use was reported in 25.8% vs 16.6%; 26.9% vs 17.3% reported self-destructive behaviour; 24.7% vs 14.1% had neuropsychiatric comorbidity, and 10.8% vs 6.9% had obesity. In univariate analyses, asthma was associated with self-destructive behaviour, OR = 1.84 (1.03–3.21), $p = 0.035$, and neuropsychiatric comorbidity, OR = 2.01 (1.11–3.56), $p = 0.019$. In multivariable analysis with backward selection, asthma was associated with neuropsychiatric comorbidity, OR = 2.04 (1.004–4.12), $p = 0.049$, a trend of increased self-destructive behaviour, OR = 1.76 (0.93–3.29), $p = 0.079$, and less drug use, OR = 0.59 (0.29–0.96), $p = 0.045$.

Conclusion: Asthma was associated with neuropsychiatric comorbidity and self-destructive behaviour, but less drug use. There were no consistent associations with other risk behaviours or obesity.

Keywords: Asthma, Obesity, Neuropsychiatric diagnosis, Self-destructive behaviour, Screen time

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Key message

Risk behaviours, obesity, and neuropsychiatric comorbidity have been demonstrated in chronic diseases but are less well described among adolescents with asthma. In our cohort at risk of asthma, adolescents with asthma had a higher risk of neuropsychiatric comorbidity and a trend of more self-destructive behaviour. These findings underscore that adolescents with asthma have an increased risk of chronic non-atopic disease and self-harm, which clinicians should be aware of in the regular monitoring and treatment of asthma.

INTRODUCTION

Risk behaviours are conscious actions that increase the risk of harmful health consequences, a well-known adolescent phenomenon, to promote independence and help establish autonomy.¹ Existing literature shows that young adults living with a chronic disease behave in a more risk-taking manner than their healthy peers.^{2,3} An American study, for example, has shown that adolescents with asthma were more likely to report cigarette smoking, drug use, and binge drinking as compared to their healthy peers.³

Adolescents with asthma also have an increased risk of developing depression and anxiety compared to adolescents without asthma,⁴ and more recently, a link between asthma and neurodevelopmental disorders, such as attention deficit hyperactivity disorder (ADHD), has been shown.⁵ Asthma has also been associated with dyslipidemia,^{6,7} and it is hypothesised that obesity and asthma are associated because of shared genetics, environmental risk factors, and underlying low-grade systemic inflammation.⁸

Using descriptive analysis, this study aims to investigate risk behaviours, obesity, and neuropsychiatric comorbidity in a well-characterised cohort of 18-year-olds with and without asthma.

METHODS

Study population

The COPSAC₂₀₀₀ cohort is a single-centre, prospective birth cohort study consisting of 411

children born to mothers with asthma. The children were included at 1 month of age and visited the COPSAC research unit at scheduled visits every 6 months until age 7 and again at ages 12 and 18. Furthermore, the children visited the research unit upon the occurrence of any acute airway or skin symptoms. Recruitment and baseline characteristics have previously been described in detail.⁹

Risk behaviours

At the 18-year follow-up visit, the participants were interviewed about their risk behaviours, including alcohol consumption, binge drinking, tobacco smoking, drug use, screen time use, and self-destructive behaviour. The participants were interviewed without their parents being present.

Alcohol consumption

The participants were asked if they had ever consumed more than 1 unit of alcohol and at what age this first occurred. They were then asked about their alcohol consumption in the past year, including frequency of drinking (never, max once per month, 2–4 times per month, 2–3 times per week, or ≥ 4 times per week), and numbers of units per occasion (0 units, 1–2 units, 3–4 units, 5–6 units, 7–9 units or ≥ 10 units). Finally, they were asked about the number of episodes in which they consumed 5 or more units of alcohol on a single occasion, such as binge drinking.

Tobacco smoking

The participants were interviewed about the age at which they first smoked a cigarette, their current smoking status, frequency of smoking (daily, weekly, less than weekly, or never), and estimated daily cigarette consumption over the past 4 weeks. Additionally, they were asked if they had experience with e-cigarettes. Current smoking was defined as smoking within the last week, including daily smoking, and occasional smoking was defined as smoking less than weekly but within the previous 4 weeks.

Drug use

The participants were asked about their prior experience with drug use, answering an online questionnaire (part of the Depression, Anxiety, and Stress Scale [DASS21]), and, if applicable, which substances they had tried, including cannabinoids,

amphetamine, ecstasy/3,4-methylenedioxy-N-methylamphetamine (MDMA)/fantasy, cocaine, opiates (such as heroin, morphine, or methadone), Lysergic Acid Diethylamide (LSD), sedatives, inhalation of lighter gas, inhalation of nitrous oxide ("laughing gas"), hallucinogenic mushrooms, or others. They were then asked to specify the frequency of their drug use, which was categorised as never, once, less than once per month, monthly, weekly, daily, or almost daily.

Self-destructive behaviour

The frequency of self-destructive behaviours including cutting, scratching, hitting, and over-eating, was captured as part of the DASS21 online questionnaire. The participants were asked whether they had ever done self-destructive behaviour (yes/no). If they answered yes, they were asked how often they had done that in the previous year and they were given the possibility to specify the type of self-destructive behaviour. After completing the online questionnaire, the participants returned to the research clinic staff. If any concerns or questions were raised as a result of the questionnaire, they were addressed directly by the staff. In cases where further support was needed, the participants and/or their parents were guided on how to access appropriate services within the Danish healthcare system. In the analyses, we used any self-destructive behaviour (yes/no).

Screen time

The participants were interviewed about their screen time habits over the past year, distinguishing between screen time on a television (TV) screen, a telephone, tablet, or computer. Screen time was categorised based on use during weekdays (Monday to Thursday) and weekends (Friday to Sunday) as the average number of hours spent on screen during leisure time. The total screen time was taken as an addition of TV screen, telephone, tablet, or computer. For this study, weekday and weekend screen time were combined. High daily screen time use was defined as exceeding 8 h per day.

Lung function tests and asthma diagnosis

Lung function was measured by spirometry (MasterScope Pneumoscreen, Erich Jäeger,

Würzburg, Germany),¹⁰ and specific airway resistance (sRaw) was assessed by whole-body plethysmography (MasterScope Bodybox, Erich Jäeger, Würzburg, Germany).¹¹ Fractional exhaled nitric oxide (FeNO) was measured using the Denox 88 version 88 DX0256, Switzerland.¹²

Asthma was solely diagnosed by the COPSAC physicians.¹³ Based on the European Respiratory Society (ERS) recommendations,¹⁴ an asthma diagnosis was confirmed if at least 2 of the following criteria were fulfilled: 1) Obstructive lung function ($FEV_1 < 80\%$ and/or $FEV_1/FVC < 80\%$ and/or $sRaw > 1.6$ kPa/s); 2) Bronchodilator reversibility (change of $FEV_1 \geq 12\%$ and/or $sRaw \geq 30\%$ from baseline after inhalation of short-acting beta-2-agonist); 3) Airway inflammation ($FeNO \geq 25$ ppb); and/or 4) Bronchial hyper-responsiveness as measured by methacholine challenge test (drop in $FEV_1 \geq 20\%$ from baseline by a dose of methacholine ≤ 8 mg/ml) and/or exercise test ($\geq 10\%$ decrease in FEV_1 from baseline). Remission was defined as no symptoms or asthma treatment for 1 year.

Non-atopic comorbidity

Neuropsychiatric disorders

The diagnoses were obtained from the medical records based on ICD-10 criteria, including any neuropsychiatric diagnosis, former and present. The diagnoses were grouped into the following groups: ADHD, autism spectrum disorder, anxiety, depression, eating disorder, mental retardation, personality disorder, schizophrenia, post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), and others (Supplemental Appendix for details).

During the 18-year visit, 3 electronic questionnaires on behavioural traits and psychopathology were completed: 1. The strengths and difficulties questionnaire (SDQ),¹⁵ 2. The Adult ADHD Self-Report Scale (ASRS),¹⁶ and 3. The DASS21¹⁷ (Supplemental Appendix for details).

BMI and obesity

Weight was measured under standard conditions without clothing but in underwear, using a calibrated digital scale. Height was measured by a Harpenden stadiometer (Holtain Ltd, Crymch, Dyfed, Wales) as previously described.⁹ Body

mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and grouped according to the World Health Organisation (WHO) definition as underweight: BMI < 18.5; normal weight: 18.5–25; overweight: 25–30, and obesity: > 30.¹⁸

Statistical analysis

The associations between asthma and risk behaviours, obesity, and neuropsychiatric disorders were analysed using logistic regression models. Data were analysed in a univariate- and multivariable regression model adjusted for sex, gestational age (GA), mother's age, mother's education, household income, breastfeeding, and smoking during pregnancy. The multivariable model also included all risk behaviours and non-atopic comorbidities: binge drinking, current and occasional smoking, drug use, self-destructive behaviour, high daily screen time use, neuropsychiatric disorder, and obesity. To simplify the model and clarify which variables explain the most, a backward selection procedure was applied by successively dropping the parameter with the highest p-value, keeping only variables with $p < 0.10$ in the final model. Finally, interaction with sex was explored, and post hoc analyses were stratified for sex. Data processing was conducted with R (R Foundation for Statistical Computing, R version 4.0.3 (2020-10-10), Vienna, Austria). Due to the exploratory nature of the study, we did not adjust for multiple comparisons.

Ethics

The study was conducted following the guiding principles of the Declaration of Helsinki. It was approved by the Local Ethics Committee (COPSAC₂₀₀₀: H-B-2008-093, COPSAC₂₀₀₀ 18- & 24-years follow-up: H-16039498) and the Danish Data Protection Agency (COPSAC₂₀₀₀: 2015-41-3696). Both parents gave written informed consent before enrolment. At the COPSAC₂₀₀₀ 18-year visit, the study participants also gave written consent.

RESULTS

Demographics

At the 18-year visit, follow-up was completed for 370 (90%) of the 411 participants in the cohort, where 93 participants (25.1%) had concurrent

asthma, and (44.3%) had an asthma diagnosis at any time earlier in childhood. Demographics are detailed in [Table 1](#), showing that among adolescents with vs without asthma there was an increased prevalence of allergic rhinitis (66.7% vs 29.2%, $p < 0.001$) and more days with passive tobacco smoke exposure ($p = 0.03$).

Risk behaviours

As shown in [Supplemental Fig. 1](#), certain risk behaviours were inter-correlated, such as smoking and drug use ($r = 0.52$) and alcohol use ($r = 0.21$) and self-esteem and smoking ($r = -0.18$), drug ($r = -0.16$) and alcohol use ($r = -0.21$), which showed the strongest positive and negative correlations, respectively.

Alcohol consumption

Among adolescents with asthma, 64.5% reported drinking more than once per month, and when drinking, 75.2% drank ≥ 5 units per occasion. Of those who drank ≥ 5 units per event, 20.4% drank > 10 units of alcohol per event. Among adolescents without asthma, 69.7% reported drinking more than once per month, and when drinking, 66.0% drank ≥ 5 units per event. Of those adolescents without asthma who drank ≥ 5 units per event, 22.7% drank > 10 units ([Table 2](#)). No significant associations existed between asthma status and binge drinking in the univariate- or multivariable models ([Table 3](#)).

Tobacco smoking

At age 18 years, 26.9% of the adolescents with asthma were smokers (daily, weekly, or occasionally) vs 32.9% of adolescents without asthma ([Table 2](#), [Fig. 1](#)). There was no significant association between tobacco smoking and asthma status ([Table 3](#)).

Drug use

Experience with any drug use tended to be less frequent among adolescents with vs without asthma in the univariate analysis: 16.1% vs 26.0%, OR = 0.57 (95% CI, 0.30–1.05), $p = 0.08$ ([Table 3](#), [Fig. 1](#)). Among adolescents with vs without asthma, 60.0% vs 70.8% had tried cannabinoids, 33.3% vs 23.6% had tried cocaine, 6.7% vs 23.6% had inhaled laughing gas, and 26.7% vs 11.1% had tried MDMA. After the backward selection

Demographics, N (%)	All N (%) N = 370	Current asthma N = 93	Not current asthma N = 277	p-value
Allergic rhinitis, current	143 (38.6)	62 (66.7)	81 (29.2)	<0.001
Atopic dermatitis, current	38 (10.3)	15 (16.1)	23 (8.3)	0.19
Parents' divorce	142 (38.4)	38 (40.9)	104 (37.5)	0.66
Race (Caucasian)	355 (96.7)	89 (95.7)	266 (96.0)	1.00
Fireplace				0.92
1-100 days	137 (37.0)	36 (38.7)	101 (36.5)	
>100 days	38 (10.3)	9 (9.7)	29 (10.5)	
Furred animals				
Cats, yes	98 (26.5)	21 (22.6)	77 (27.8)	0.40
Dogs, yes	179 (48.4)	43 (46.2)	136 (49.1)	0.74
Passive smoking				0.03
1-100 days per years	116 (31.4)	36 (38.7)	80 (28.9)	
>100 days per year	110 (29.7)	18 (19.4)	92 (33.2)	
Other children younger, yes	217 (58.6)	46 (49.5)	171 (61.7)	0.12
Other children older, yes	87 (23.5)	26 (28.0)	61 (22.0)	0.45
Proband education				0.38
Elementary school, completed	357 (96.5)	91 (97.8)	266 (96.0)	
College, started	288 (77.8)	72 (77.4)	216 (78.0)	
Tradesman, started	38 (10.3)	5 (7.94)	33 (12.0)	
University, started	1 (0.3)	1 (1.1)	-	
Parents education, completed				0.33
Elementary school, mother	13 (3.5)	5 (5.4)	6 (2.2)	
Elementary school, father	17 (4.6)	4 (4.3)	13 (4.7)	
High school, mother	6 (1.6)	1 (1.1)	5 (1.8)	
High school, father	7 (1.9)	4 (4.3)	3 (1.1)	
Tradesman, mother	94 (25.4)	33 (35.5)	61 (22.0)	
Tradesman, father	102 (27.6)	23 (24.7)	79 (28.5)	
University, medium long, mother	125 (33.8)	29 (31.2)	96 (34.7)	
University, medium long, father	70 (18.9)	12 (12.9)	58 (20.9)	
University, long, mother	69 (18.6)	16 (17.2)	53 (19.1)	
University, long, father	82 (22.2)	25 (26.9)	57 (20.6)	

Table 1. Demographic information of the probands included in the COPSAC₂₀₀₀ cohort at the 18-year visit. Bold indicates p-values < 0.05. The table presents demographic information of the probands included in the COPSAC₂₀₀₀ cohort at the 18-year visit

Risk behaviours	All N = 370	Current asthma N = 93	Not current asthma N = 277	p- value ^c
Alcohol; drinking frequency, N = 342 (92.4)				0.46
Never	3 (1.0)	-	3 (1.0)	
Max once per month	89 (24.1)	26 (28.0)	61 (22.0)	
>1 per month	253 (68.4)	60 (64.5)	193 (69.7)	
Alcohol; units				0.28
1-4	88 (23.8)	16 (17.2)	71 (25.6)	
5-9	172 (46.5)	51 (54.8)	120 (43.3)	
>10	82 (22.2)	19 (20.4)	63 (22.7)	
Smoking, N = 189 (51.1)	116 (31.4)	25 (26.9)	91 (32.9)	0.34
Current smoking ^a	65 (17.6)	16 (17.2)	49 (17.7)	
Occasional smoking ^a	51 (13.8)	9 (9.7)	42 (15.2)	
Former smoker	73 (19.7)	18 (19.4)	55 (19.9)	
Daily	28 (7.6)	5 (5.4)	23 (8.3)	0.35
1-4 cigarettes per day	11 (39.3)	3 (60.0)	8 (34.7)	
5-9 cigarettes per day	8 (28.6)	2 (40.0)	6 (26.1)	
>9 cigarettes per day	9 (32.1)	-	9 (39.1)	
Drug use ever	87 (23.8)	15 (16.1)	72 (26.0)	<0.01
Cannabinoids	60 (70.0)	9 (60.0)	51 (70.8)	
Cocaine	22 (25.3)	5 (33.3)	17 (23.6)	
Laughing gas (NO)	18 (20.7)	1 (6.7)	17 (23.6)	
Ecstasy/MDMA/Fantasy	12 (13.9)	4 (26.7)	8 (11.1)	
Others ^b	34 (39.1)	7 (46.7)	27 (37.5)	
Screen time, N = 367 (99.2)				
Total screen time hours, median (IQR)	6.0 (4.5-7.0)	6.0 (4.5-8.0)	6.0 (4.5-7.0)	0.93
High daily screen time use	70 (18.9)	24 (25.8)	46 (16.6)	0.01
Self-destructive behaviour	73 (19.7)	25 (26.9)	48 (17.3)	<0.01

Table 2. Descriptive information on risk behaviours from probands in the cohort. *Bold indicates p-values < 0.05. The table presents descriptive information on risk behaviour from probands in the cohort. The information is presented in groups of asthma yes/no. ^aCurrent smoking was defined as daily or weekly smoking, and occasional smoking was defined as smoking less than weekly. ^bEtc. Amphetamine, sedatives, LSD, opiates, euphoric mushrooms, lighter gas. ^cSignificance between the genders is calculated with a t-test. In cases of not normal distributed data Wilcoxon test has been used*

Asthma associations <i>Predictors</i>	OR	Univariate model <i>CI</i>	<i>p</i>	Fully adjusted model			Backward selection model		
				OR	CI	<i>p</i>	OR	CI	<i>p</i>
Binge drinking ^a	1.66	(0.93–3.08)	0.10	1.70	(0.88–3.43)	0.12			
Current smoking ^b	0.86	(0.44–1.62)	0.64	0.50	(0.17–1.29)	0.17	–	–	–
Occasional smoking ^b	0.56	(0.24–1.19)	0.15	0.55	(0.21–1.33)	0.20	–	–	–
Drug use (yes)	0.57	(0.30–1.05)	0.081	0.73	(0.32–1.62)	0.44	0.59	(0.29–0.96)	0.045
High daily screen time use ^c	1.74	(0.98–3.04)	0.053	1.68	(0.83–3.35)	0.15	–	–	–
Self-destructive behaviour (yes)	1.84	(1.03–3.21)	0.035	1.65	(0.81–3.30)	0.16	1.76	(0.93–3.29)	0.079
Neuropsychiatric disorder ^d	2.01	(1.11–3.56)	0.019	2.17	(1.04–4.48)	0.037	2.04	(1.004–4.12)	0.049
Obesity	1.55	(0.66–3.44)	0.30	1.67	(0.61–4.36)	0.30	–	–	–

Table 3. Association between risk behaviours, obesity, and neuropsychiatric disorders. Bold indicates *p*-values < 0.05. The adjusted model is adjusted for sex, GA, mother's age, educational level of the mother, household income, smoking during pregnancy, and breastfeeding. All risk behaviours are included in the multivariable model: binge drinking, smoking, drug use, high daily screen time use, self-destructive behaviour, neuropsychiatric disorder, and obesity. ^aBinge drinking refers to alcohol units ≥ 5 . ^bCurrent smoking refers to smoking daily or weekly, and occasional smoking refers to smoking less than weekly. ^cHigh daily screen time use >8 h/day. ^dNeuropsychiatric disorder ever through childhood

procedure, the multivariable model showed a significant inverse association between lower drug use and risk of asthma: adjusted OR = 0.59 (0.29–0.96), *p* = 0.045 (Table 3, Fig. 1).

Screen time

The median screen time per day was 6.0 h in both adolescents with and without asthma, whereas there was a trend of more adolescents with vs without asthma who had a high daily screen time use, ie, screen time >8h/day: 25.8% vs 16.6%, OR = 1.74 (0.98–3.04), *p* = 0.053 (Table 2). However, this association was not significant in the multivariable and backward selection models (Table 3).

Self-destructive behaviour

Self-destructive behaviour was reported in 26.9% of adolescents with asthma compared to 17.3% of adolescents without asthma (Table 2). In the univariate analysis, asthma was associated with significantly more self-destructive behaviour, OR = 1.84 (1.03–3.21), *p* = 0.035, but the

multivariable model after backward selection only showed a trend of increased risk among adolescents with asthma, OR = 1.76 (0.93–3.29), *p* = 0.08 (Table 3).

Non-atopic comorbidity

Neuropsychiatric disorders

A total of 16.8% of the participants had been diagnosed with a neuropsychiatric diagnosis at some point in childhood. The distribution of specific diagnosis among children with any diagnosis is visualised in Supplemental Fig. 2. Adolescents with vs without asthma had a doubled risk of neuropsychiatric comorbidity, 24.7% vs 14.1%, OR = 2.01 (1.11–3.56), *p* = 0.019, which was confirmed in the multivariable analysis, OR = 2.17 (1.04–4.48), *p* = 0.037, and in backward selection procedure OR = 2.04 (1.004–4.12), *p* = 0.049 (Table 3).

The most frequent neuropsychiatric diagnosis was ADHD, which was diagnosed in 10.8% of adolescents with asthma compared to 5.8% without

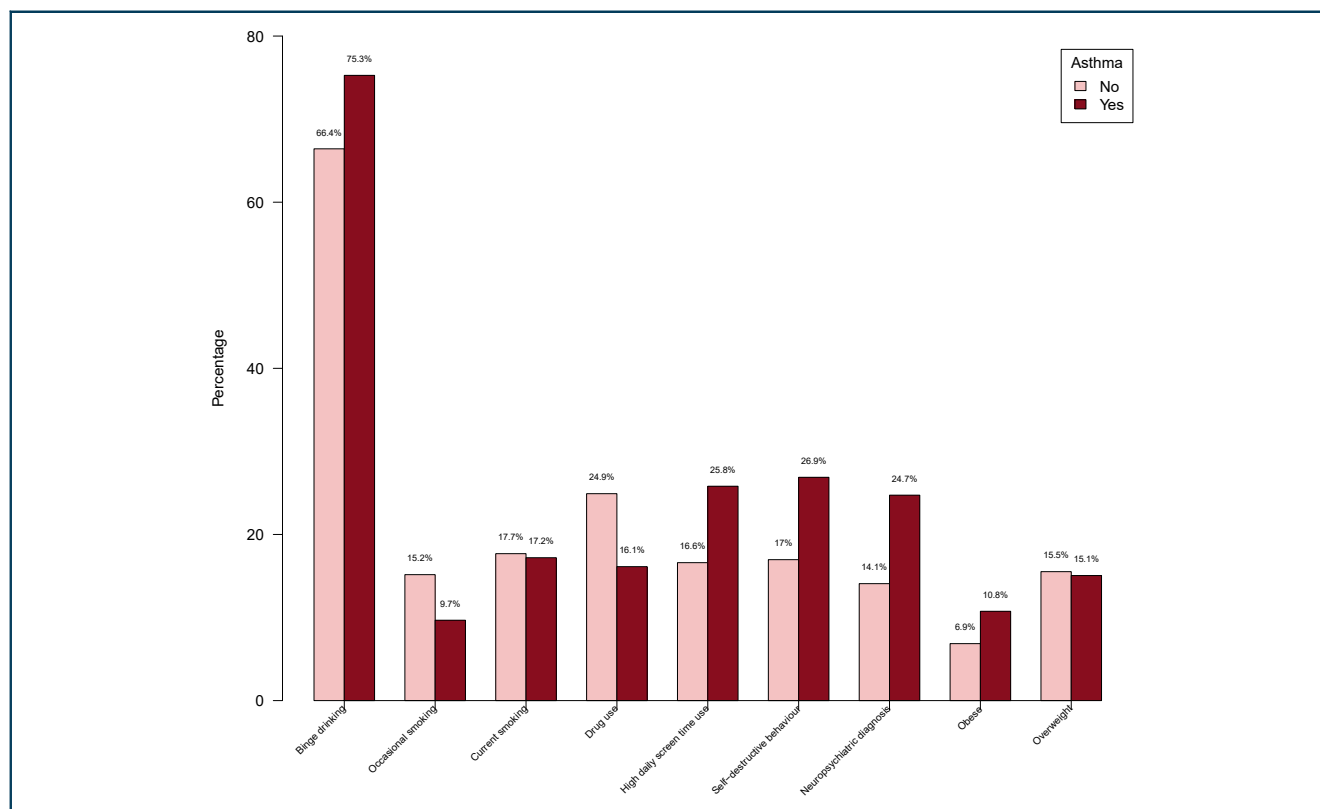


Fig. 1 Distribution of risk behaviours, BMI, and neuropsychiatric disorders in adolescents with vs without asthma

asthma. Autism was diagnosed in 7.5% of adolescents with asthma vs 1.8% without asthma, depression in 4.3% vs 1.2%, eating disorders in 3.2% vs 2.9%, OCD in 3.2% vs 2.2%, and anxiety in 2.2% vs 3.6%. There was a significant difference in autism between adolescents with vs without asthma ($p = 0.01$) but not for the other specific neuropsychiatric diagnoses (Table 4).

Data from the ASRS questionnaire were available from 348 children. Of those, the total score median (IQR) was 4 (2.0–7.0) in adolescents with asthma vs 4.5 (2.0–7.0) in adolescents without asthma ($p = 0.97$) (Table 4).

Data from the SDQ questionnaire were available from 347 children. Looking into the total difficulty score, 74 (21.3%) adolescents meet the criteria for an abnormal score. Of those, 22 (25.0%) were adolescents with asthma vs 52 (20.1%) without asthma, $p = 0.59$. In the impact score, 95 (27.4%) fulfilled the criteria for an abnormal score, and of those, 23 (26.1%) were adolescents with asthma vs 72 (27.8%) without asthma, $p = 0.94$ (Table 4).

Obesity

Among adolescents with asthma, 15.1% were categorised as having overweight (BMI 25.1–30.0 kg/m²) and 10.8% as having obesity (BMI >30 kg/m²), which was 15.5% and 6.9% among adolescents without asthma (Table 4). No significant associations existed between BMI categories and asthma status (Table 3, Fig. 2).

Post hoc analysis of gender differences

Asthma was associated with obesity among females, OR = 3.48 (1.18–10.33), $p = 0.02$, but not among males, OR = 1.55 (0.66–3.44), $p = 0.30$, which showed a significant gender difference (p -interaction = 0.04) (Supplemental Table 1). In stratified analyses, it appeared that asthma was more strongly associated with self-destructive behaviour among females, OR = 2.24 (1.10–4.56, $p = 0.03$), than males, OR = 1.31 (0.39–3.79), $p = 0.64$. However, there were no significant gender differences in the association between asthma and self-destructive behaviour or any other risk behaviours or neuropsychiatric comorbidity (p -interactions > 0.05) (Supplemental Table 1).

BMI and neuropsychiatric disorders, N (%)	All N = 370	Current asthma N = 93	Not current asthma N = 277	p-value ^d
BMI, N = 367 (99.1)				0.58
BMI <18.5	32 (8.7)	6 (6.45)	26 (9.39)	
BMI 18.5-24.9	249 (67.8)	64 (68.8)	185 (66.8)	
BMI 25-30	57 (15.5)	14 (15.1)	43 (15.5)	
BMI >30	29 (7.90)	10 (10.8)	19 (6.9)	
ASRS questionnaire, N = 348 (94.0) ^a	4.0 (2.0-7.0)	4.5 (2.0-7.0)	4.0 (2.0-7.0)	0.97
SDQ - total difficulty score, N = 347 (93.7) ^b	74 (21.3)	22 (25.0)	52 (20.1)	0.59
SDQ - daily functioning score (impact score) ^b	95 (27.4)	23 (26.1)	72 (27.8)	0.94
Neuropsychiatric disorder ^c	62 (16.8)	23 (24.7)	39 (14.1)	0.02
ADHD	26 (7.0)	10 (10.8)	16 (5.8)	0.16
Depression	8 (2.2)	4 (4.3)	4 (1.4)	0.11
Anxiety	12 (3.2)	2 (2.2)	10 (3.6)	0.74
Autism	13 (3.1)	7 (7.5)	5 (1.8)	0.01
Eating disorder	11 (3.0)	3 (3.2)	8 (2.9)	1.00
Personality disorder	2 (0.5)	-	2 (0.7)	-
Mental retardation	3 (0.8)	1 (1.1)	2 (0.7)	1.00
PTSD	5 (1.4)	2 (2.2)	3 (1.1)	0.60
Schizophrenia	2 (0.5)	1 (1.1)	1 (0.4)	0.44
OCD	9 (2.4)	3 (3.2)	6 (2.2)	0.70

Table 4. Association between BMI categories, specific neuropsychiatric disorders, and asthma. *Bold indicates p-values < 0.05.* ^aASRS (Adult ADHD self reported scale), data from 348 proband, 88 with asthma and 260 without asthmatic disease. ^bSDQ-questionnaire. Data available from 347 probands. ^cNeuropsychiatric disorders include a group of other diagnoses. This group includes 7 individuals. Some of the probands have more than 1 neuropsychiatric diagnosis, which is why the number of specific categories does not sum to 62. ^dFishers test

DISCUSSION

Primary findings

In our high-risk asthma cohort, we found that adolescents with asthma had a higher risk of neuropsychiatric comorbidity and a trend of a higher risk of self-destructive behaviour. In contrast, the risk of drug use was lower compared to adolescents without asthma. Other risk behaviours, such as tobacco smoking and alcohol consumption, were not associated with asthma, whereas obesity was only associated with asthma among females.

Interpretation

The previously observed associations between risk behaviours and chronic diseases¹ have been suggested to arise due to the following: First, adolescents living with a chronic disease have a higher need for acceptance from other adolescents. Second, parents of adolescents who live with a chronic disease may be less restrictive regarding rules and boundaries that do not directly involve the chronic disease. Third, chronic disease can cause less focus on risk behaviours and reduce preventive counselling.¹

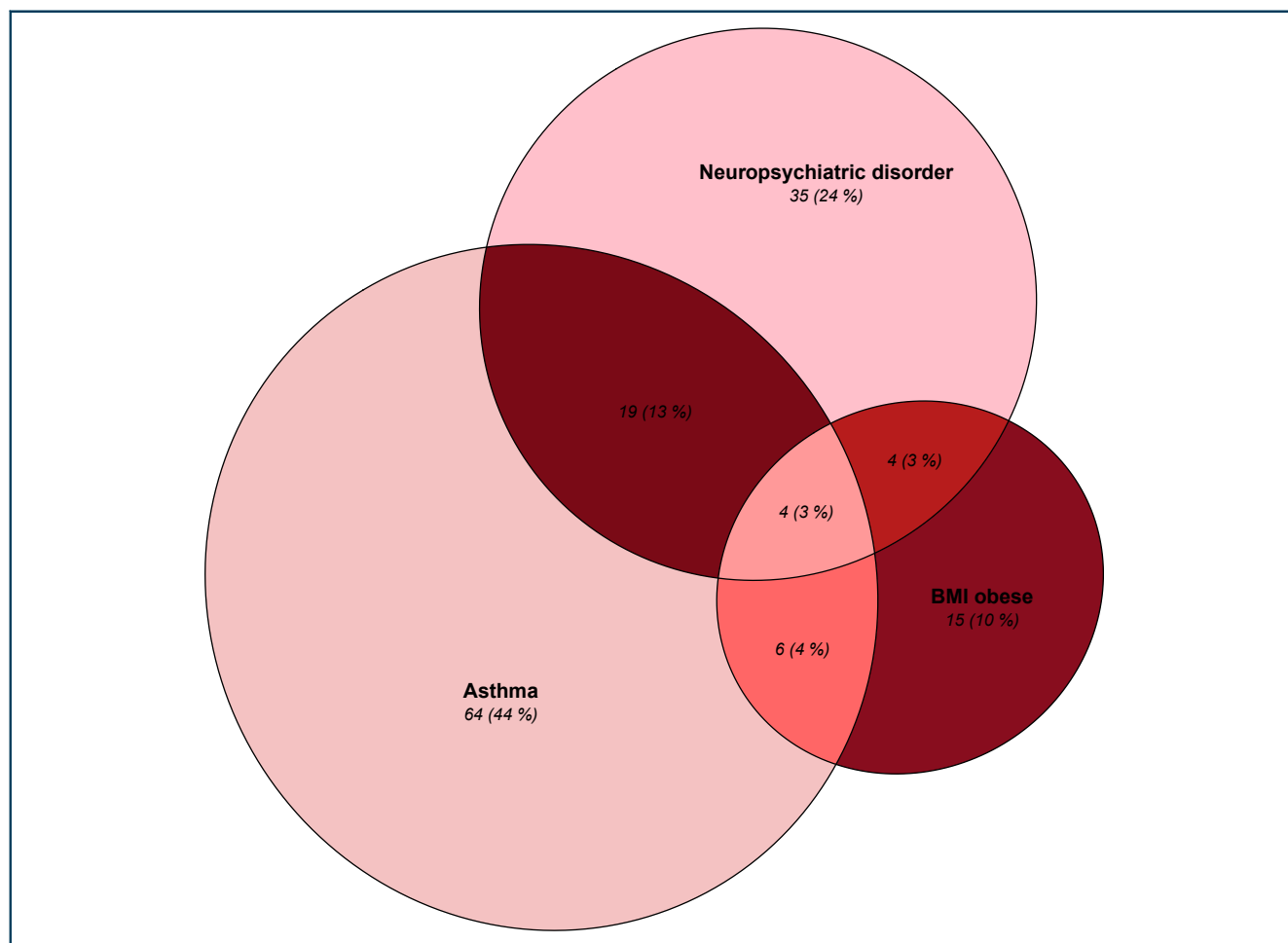


Fig. 2 Venn diagram showing the relationship between the 147 adolescents with either asthma and/or neuropsychiatric disorders and/or BMI ≥ 30 . The number in each category and the percentage of the 147 children is given in the figure as N (%)

Genetic predisposition increases the risk of developing asthma,¹⁹ which concurs with the high number of participants with asthma in our cohort, where one-fourth had asthma compared to around 15% in the background population.⁹ In recent years, there has been an increased focus on the relationship between asthma and other non-communicable, lifestyle-related disorders such as obesity²⁰⁻²² and neuropsychiatric disorders.^{8,19} We observed a prevalence of current or past neuropsychiatric comorbidity of 16.8%, which is a higher rate than the worldwide prevalence of mental disorders of 13% reported among adolescents.^{23,24} In our cohort, 7.0% of the participants were diagnosed with ADHD compared to 1-3% in the Danish background population.^{23,24} One explanation could be that we tested all children in the cohort, including

those with mild symptoms or minimal impact from the disease, which differs from those in the background population that gets tested. Another hypothesis is that asthma and ADHD in childhood are associated traits, and the observed parallel increase in incidence may be due to shared environmental risk factors.^{5,24} In our study, we found that children with asthma in childhood tended to have a double risk of neuropsychiatric disorder at 18 years. The most prevalent neuropsychiatric disorder in our cohort was ADHD, but there was no significant association between ADHD and asthma. In contrast, we observed a significant association between autism and asthma, which has also been demonstrated in some previous studies but not others.^{5,24-26} Interestingly, a study by Qu et al conducted in a US urban cohort of 2580 children

showed a significant association between autism and asthma, which diminished after adjusting for maternal factors such as maternal age and weight and child factors such as birth weight and gestational age at birth.²⁷ In our study, the association between autism and asthma was still significant after adjusting for maternal age and child gestational age at birth, but we did not adjust for maternal weight or birth weight, which could be possible confounders.

Almost one-fourth of the participants in our cohort reported that they had tried using drugs, which is low compared to the Danish youth population, where nearly 40% have tried cannabinoids, and 10% have tried other types of drugs,²⁸ which aligns with what is seen in a study of young Swiss men.²⁹ Surprisingly, we found that drug use was lower in adolescents with asthma at age 18 years, where the data was collected anonymously in questionnaires with the involvement of the parents, which could have biased the answers. We speculate if this could be caused by a disease-specific behaviour, i.e., that having a chronic disease such as asthma causes a lower rate of certain risk behaviours,³⁰ especially inhaling drugs as adolescents with asthma compared to adolescents without asthma reported less use of cannabinoids (60.0% vs 70.8%) and laughing gas (6.7% vs 23.6%), whereas there were higher reporting of non-inhalant drugs such as MDMA (26.7% vs 11.1%). Other possible reasons for a lower drug use among adolescents with asthma could also be related to fear of drug interactions with asthma medication, concerns about triggering asthma attacks, and/or increased health awareness/parental supervision in this cohort, where all mothers have a history of asthma. However, previous studies have shown the opposite, i.e., living with a chronic disease does not cause less risky behaviour.³¹ In contrast to our findings of less drug use, other studies have shown that adolescents with asthma are more likely to report any drug use compared to healthy adolescents, and it is suggested that this could be a coping mechanism for living with a chronic disease.³

We observed that one-fifth reported self-destructive behaviour at some point in their lives. Self-destructive behaviour was associated with asthma in the univariate analyses, and a similar trend was observed in the multivariable models.

This corresponds to what has been observed previously, i.e., chronic diseases, such as asthma, may increase the risk of self-harm because of an increased vulnerability from strain and stress and an overrepresentation of depression and anxiety.³² However, in our study we did not observe a significant association between either depression or anxiety and asthma although a higher percentage of adolescents with asthma had depression compared to adolescents without asthma (4.3% vs 1.4%, $p = 0.11$).

We did not find an association between asthma status and alcohol consumption in our cohort. However, an American study found that binge drinking was associated with less asthma control and nonadherence among adolescents with asthma² and another American study showed that adolescents with asthma were more prone to binge drinking as compared to their healthy peers.³ Previous studies have found cardiovascular and metabolic side effects related to large alcohol consumption as well as an affected brain development.^{33,34} In our cohort, two-thirds of the adolescents drank at least 1 time per month, and of these, more than one-fifth drank more than 10 units of alcohol. Of note, only 1% of the participants had never drunk alcohol. However, the alcohol consumption patterns in our adolescents are like that of adolescents from other European countries.³⁵ Many initiatives have been initiated to reduce the use of alcohol, and even though consumption is high in our cohort, it is lower compared to previous generations.³⁶ A decrease in alcohol consumption has also been observed in countries like Austria and Sweden.^{37,38} The lack of association with alcohol consumption in our study could be due to the well-controlled analyses, including socioeconomic confounding.

We observed that one-fourth of adolescents with asthma were smokers compared to one-third of adolescents without asthma. There was no association between smoking habits and asthma status in our cohort, which contrasts previous findings in another study showing a significant association between smoking and asthmatic disease, suggesting that adolescents with asthma are more likely to smoke, which could be a coping mechanism for their disease.³

We found that the cohort's median daily screen time was 6 h. Previous studies have shown that a high level of time spent on screens per day is associated with adverse health effects such as obesity and impaired cognitive and socioemotional development.³⁹ We did not find an association when analysing the correlation between asthma and mean screen time per day. However, daily screen time of at least 8 h per day showed a trend of association with an increased risk of asthma in the univariate analysis, but this was not significant in the multivariable models.

We found that almost one-fourth had a BMI > 25, and of those, around 8% were obese. This is consistent with the Danish school-aged children in the background population.⁴⁰ Obesity in childhood and adolescence can negatively affect almost every organ system, thereby causing a broad range of consequences, including hypertension, dyslipidemia, insulin resistance, diabetes, fatty liver disease, as well as psychosocial complications such as low self-esteem and depression.^{41,42} Many studies have shown an association between asthma and obesity.^{43–45} However, we did not observe this in our cohort, which could be due to a lack of power, as the study was not initially designed for this analysis. Nevertheless, we observed a significant association between asthma and obesity among females. This sex-specific association has been observed previously, and it has been hypothesised that sex hormones, especially oestrogen, affect the modulation of Th2 cytokine production, influencing the inflammatory process in the airways and increasing the risk of asthma.^{46,47} In contrast, other studies have not shown sex differences in the association between asthma and obesity.⁴⁸

Strengths and limitations

All objective measurements were done by trained research personnel strictly following standard operating procedures. The participants of the COPSAC₂₀₀₀ cohort have participated in such examinations repeatedly from birth and are therefore highly competent in performing tests and assessments, which assure a high completion rate. Asthma was diagnosed and monitored at the COPSAC research unit and not by general

practitioners or based on parent interviews or questionnaires. The asthma diagnoses were based on objective criteria and treatment, and regular evaluation followed standard operating procedures. Furthermore, all participants were born to mothers with asthma, which may improve symptom recognition. All neuropsychiatric diagnoses were obtained from Danish medical records, encompassing all diagnoses in the national healthcare system. Finally, risk behaviours such as drug use and self-destructive behaviours were assessed in questionnaires, and risk behaviours such as alcohol consumption and smoking were evaluated in the absence of the parents.

A limitation of the study is the external validity because of the high-risk cohort design with all children were born to mothers with asthma, which could have affected our ability to find associations between risk behaviours, neuropsychiatric comorbidity and asthma. Further, all adolescents with asthma in our study had mild to moderate, well-controlled disease, which may have hampered our ability to detect an increased prevalence of risk behaviours and subgroups of neuropsychiatric comorbidity such as ADHD as it is well-known that more severe asthma symptoms and poor control are associated with higher prevalences of neuropsychiatric disorders. In addition, this study is based on a cross-sectional analysis focusing on current asthma at age 18 years, where we labelled children who previously had asthma (i.e., asthma in remission) as healthy controls, which may have introduced a bias towards null findings for association with risk behaviours. Importantly, the birth cohort was not designed to study these associations and null findings in our study concerning risk behaviours could also be due to insufficient power in the analyses and/or residual confounding. All risk behaviours regarding personal integrity were collected; however, underreporting from the participants cannot be excluded and the definition of some risk behaviours such as a high screen time is somewhat arbitrary, whereas other risk behaviours such as self-destructive behaviour and drug use are dichotomized as yes/no to obtain sufficient numbers in the analyses. We collected psychiatric diagnoses from the medical records, including any psychiatric diagnosis, former and present. The diagnoses were divided into 12 sub-groups to

simplify the classification and increase the power, which could cause a less precise diagnosis.

CONCLUSION

In our cohort at risk of asthma, adolescents with asthma had a higher risk of neuropsychiatric comorbidity and a trend of more self-destructive behaviour. In contrast, drug use was lower compared to adolescents without asthma, whereas other risk behaviours were not associated with asthma. We did not observe an association between obesity and asthma, although sex-stratified analysis did show an association among females. These findings underscore that adolescents with asthma have an increased risk of chronic non-atopic disease and self-harm, which clinicians should be aware of in the regular monitoring and treatment of asthma.

Abbreviations

ADHD, Attention deficit hyperactivity disorder; ASRS, The Adult ADHD Self-Report Scale; BMI, Body mass index; COPSAC₂₀₀₀, Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀; DASS21, Depression, Anxiety, and Stress Scale –21 Items; FeNO, Fractional exhaled nitric oxide; FEV₁, Forced Expiratory Volume at 1 second.; GA, Gestational age; GLM, Generalised linear model; LSD, Lysergic Acid Diethylamide; MAP, Median arterial pressure; MDMA, 3,4-methylenedioxy-N-methylamphetamine; OCD, obsessive-compulsive disorder; PTSD, post-traumatic stress disorder; sRaw, Specific airway resistance; SDQ, the strengths and difficulties questionnaire

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Authors contributions

The guarantor of the study is BC, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. TME has written the first

draft of the manuscript. All co-authors have provided important intellectual input and contributed considerably to the analyses and interpretation of the data. All authors guarantee that the accuracy and integrity of any part of the work have been appropriately investigated and resolved, and all have approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit it for publication. No honorarium, grant, or other forms of payment were given to any of the authors to produce this manuscript.

Ethics statement

The study was conducted following the guiding principles of the Declaration of Helsinki. It was approved by the Local Ethics Committee (COPSAC₂₀₀₀: H-B-2008-093, COPSAC₂₀₀₀ 18- & 24-years follow-up: H-16039498) and the Danish Data Protection Agency (COPSAC₂₀₀₀: 2015-41-3696). Both parents gave written informed consent before enrolment. At the COPSAC₂₀₀₀ 18-year visit, the study participants also gave written consent.

Declaration of competing interest

All authors declare no potential, perceived, or actual conflict of interest regarding the content of this manuscript. The funding agencies did not have any role in the design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.waojou.2025.101093>.

Governance

We know and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) Good Clinical Practice guidelines and the Helsinki Declaration. Privacy is important to us, so we follow national and international legislation such as the General Data Protection Regulation (GDPR), the Danish Act on Processing Personal Data, and the practice of the Danish Data Inspectorate.

Submission declaration

The manuscript represents our original work, and it has not been published before.

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Supplemental Appendix

METHODS

Data collection followed Good Clinical Practice guidelines. Standard operating procedures were predefined for all study and database registration procedures. We collected data online during the visit. We saved any editing and changes of the data in an audit trail in the database documents. We double-checked the database against source data by an external person.

Non-atopic comorbidity

Neuropsychiatric disorders: The diagnoses were obtained from the medical records based on ICD-10 criteria, including any neuropsychiatric diagnosis, former and present. The diagnoses were grouped into the following groups; ADHD (including the following ICD-10 codes: F.919, F.901, F.900, and F.90), autism spectrum disorder (including the following ICD-10 codes: F.840, F.841, F.8410, F.8412, and F.845), anxiety (including the following ICD-10 codes: F.419, F.41, F.409, F.401, F.410, F.411), depression (including the following ICD-10 codes: F.32, F.321, F.313, F.329, F.339, F.33), eating disorder (including the following ICD-10 codes: F.982 and F.509), mental retardation (including the following ICD-10 codes: F.799, F.79, F.700, F.710, F.849), personality disorder (including the following ICD-10 codes: F.609, F.607, F.606), schizophrenia (including the following ICD-10 code: F.209, F.200), post-traumatic stress disorder (PTSD) (including the following ICD-10 codes: F.439, F.431, F.4302) , obsessive-compulsive disorder (OCD) (including the following ICD-10 codes: F.42, F.429, F.422, F.428, F.959, F.951, F.952), and others (including the following ICD-10 codes: F.941, F.981, F.100).

The strengths and difficulties questionnaire (SDQ) is a tool designed to assess mental health and evaluate an individual's ability to function in daily life. Data from the SDQ questionnaire are presented as the total difficulty/impact scores. We utilised the total difficulty score ranges from 0 to 40, and values above 14 are abnormal ^{1,2}. The impact score evaluates the impact of difficulties on daily life, is a marker for daily functioning, and varies from 0 to 10. Values of one or above are defined as abnormal ³.

DASS21 is a questionnaire designed to address symptoms of depression, anxiety, and stress. In our study, we used an extended version that also gathered information on drug use and experience with self-destructive behaviour ⁴.

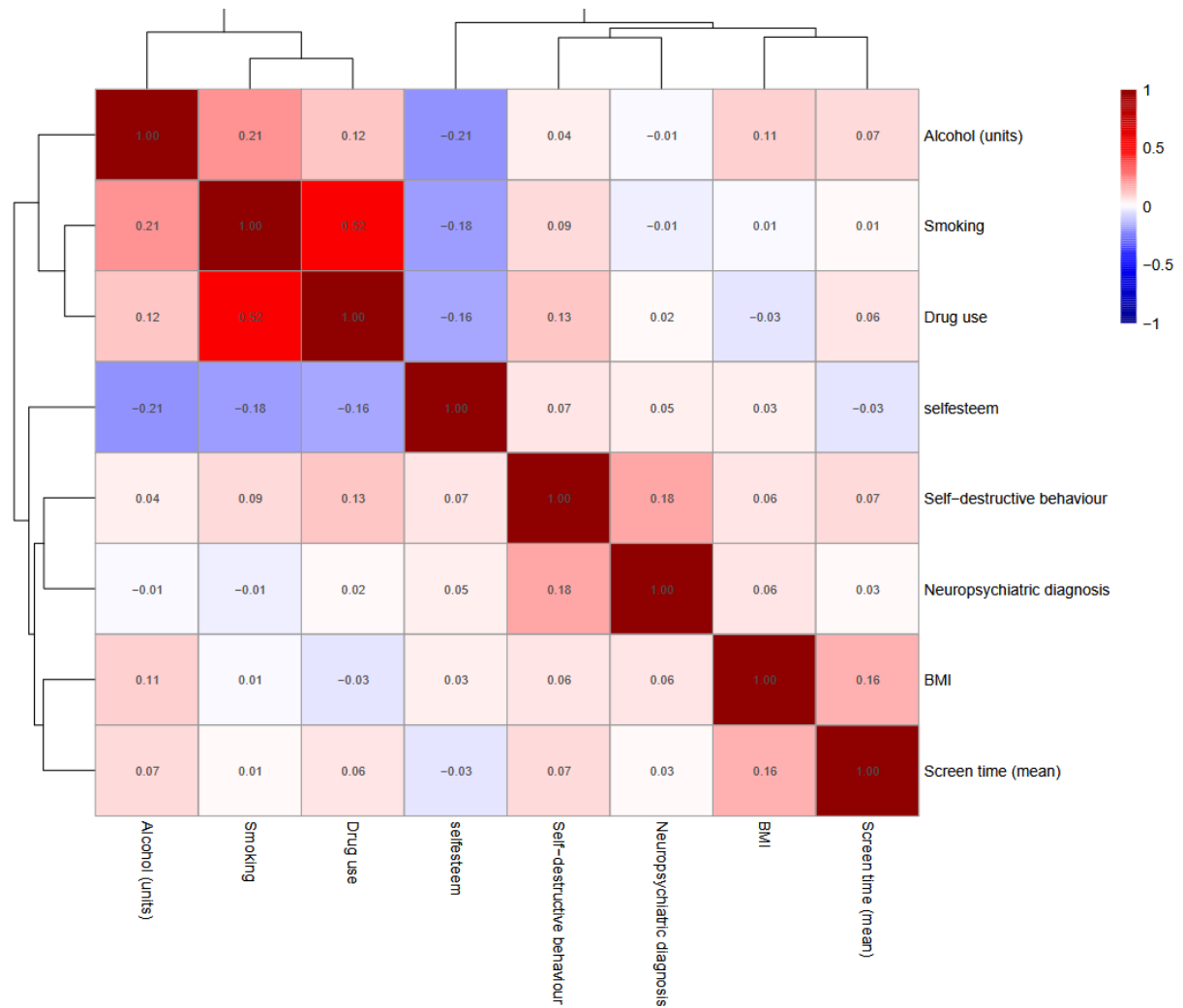
The Adult ADHD Self-Report Scale (ASRS) is a screening tool for attention and hyperactivity issues. It comprises 18 items, including six main core questions and 12 supplementary questions for preliminary assessment before further evaluation ⁵.

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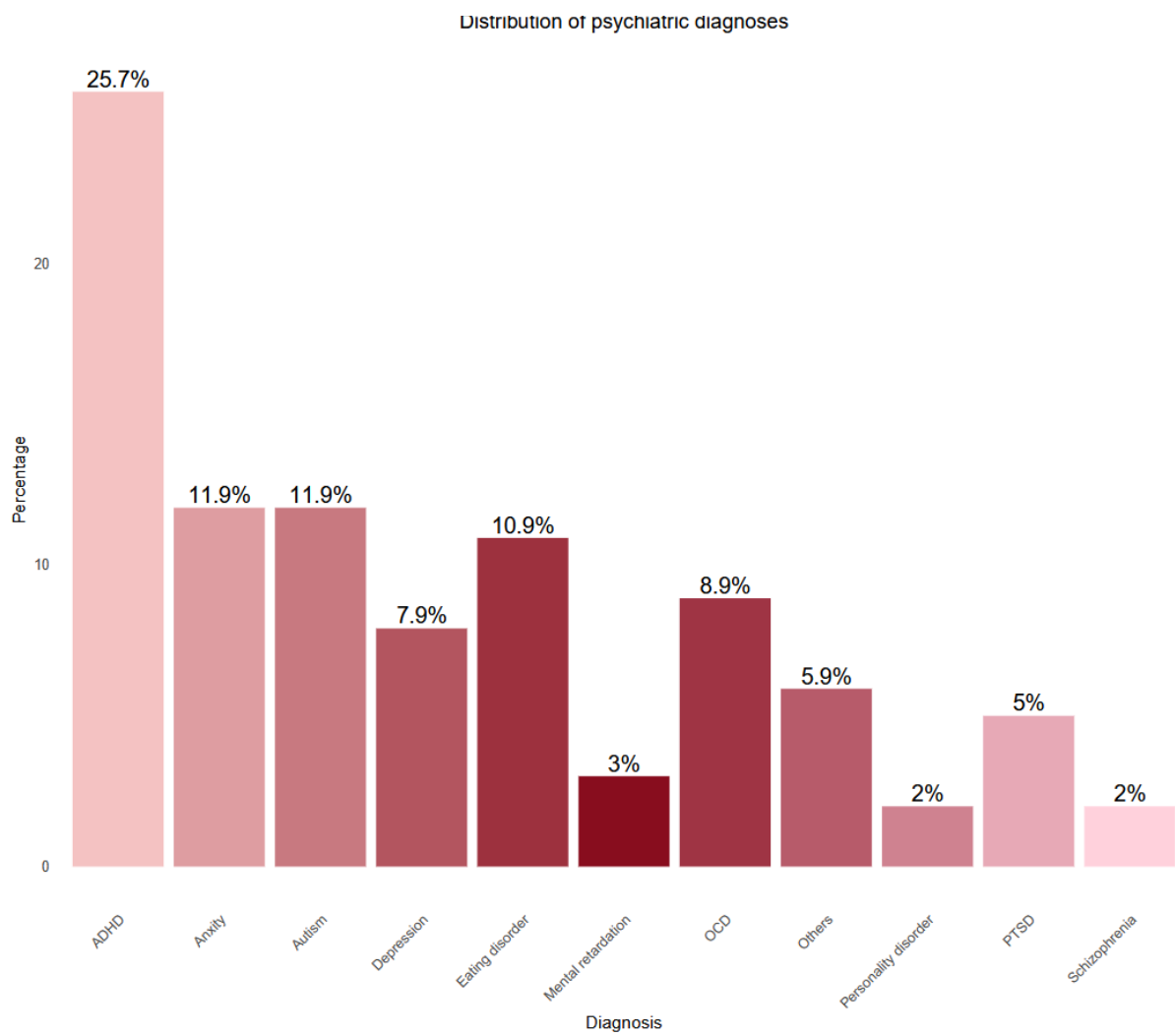
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Supplemental Figures

Supplemental Figure 1. Heatmap showing correlations between risk behaviours included in the study.



Supplemental Figure 2. Barplot showing the distribution of psychiatric disorders in the cohort through childhood.



Supplemental Table

Table ST1. Asthma associations stratified for gender

Asthma associations stratified for genders	Univariate model			Univariate model			^aAdjusted model			^aAdjusted model			^a Backward selection			^a Backward selection		
	females			males			females			males			females			males		
	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p
Binge drinking ^b	1.52	(0.70 – 3.52)	0.30	1.84	(0.78 – 4.85)	0.19	1.31	(0.51 – 3.52)	0.58	2.09	(0.75 – 6.70)	0.18	-	-	-	-	-	-
Current smoking ^c	0.75	(0.25 – 1.96)	0.58	0.91	(0.37 – 2.12)	0.83	0.62	(0.12 – 2.64)	0.54	0.46	(0.10 – 1.82)	0.29	-	-	-	-	-	-
Occasional smoking ^c	0.82	(0.28 – 2.16)	0.70	0.34	(0.08 – 1.10)	0.10	0.82	(0.21 – 2.95)	0.76	0.30	(0.06 – 1.18)	0.11	-	-	-	-	-	-
Drug use	0.53	(0.17 – 1.39)	0.23	0.59	(0.26 – 1.29)	0.20	0.36	(0.07 – 1.47)	0.18	1.18	(0.38 – 3.64)	0.78	0.27	(0.06 – 0.88)	0.046	-	-	-
High daily screen time use ^d	1.90	(0.75 – 4.58)	0.16	1.65	(0.77 – 3.42)	0.19	2.19	(0.65 – 7.33)	0.20	1.71	(0.63 – 4.57)	0.28	2.79	(0.92 – 8.38)	0.065	-	-	-
Self-destructive behaviour	2.24	(1.10 – 4.56)	0.03	1.31	(0.39 – 3.79)	0.64	1.62	(0.61 – 4.24)	0.32	1.74	(0.47 – 5.85)	0.38	-	-	-	-	-	-
Neuropsychiatric disorder ^e	2.90	(1.26 – 6.56)	0.01	1.39	(0.58 – 3.14)	0.44	4.55	(1.40 – 15.78)	0.01	1.30	(0.40 – 3.88)	0.65	6.00	(2.03 – 18.74)	0.001	-	-	-

Obesity	3.48	(1.18 – 10.33)	0.02	1.55	(0.66 – 3.44)	0.30	3.49	(0.81 – 15.75)	0.09	0.42	(0.06 – 2.01)	0.32	-	-	-	-	-	-
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Table ST1. Asthma associations stratified for gender

The table shows the association between asthmatics disease at 18 years and predictors. Analyses are made with GLM. Interaction ns are controlled and included if significant.

^a Adjusted for sex, GA, mother's age, educational level of the mother, household income, smoking during pregnancy, and breastfe eding. All risk behaviours are included in the multivariable analysis.

^b Binge drinking refers to alcohol units ≥ 5.

^c Current smoking refers to smoking weekly, and occasional smoking refers to smoking less than weekly.

^d High daily screen time use > 8 hours/day.

^e Neuropsychiatric disorder ever through childhood.

PAPER III

Mølbaek-Engbjerg T et al.

Longitudinal trajectories of inflammatory biomarkers and their association with
asthma

Manuscript prepared for submission

Longitudinal trajectories of inflammatory biomarkers and their association with asthma

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Short title: Biochemistry trajectories and risk of asthma

Authors' Contributions: The guarantor of the study is BC, from conception and design to conduct of the study and acquisition of data, data analysis, and interpretation of data. TME has written the first draft of the manuscript and performed the data analysis. MA and DH contributed to the data analysis and manuscript writing. All co-authors have provided important intellectual input and contributed considerably to the interpretation of the results. All authors guarantee that the accuracy and integrity of any part of the work have been appropriately investigated and resolved, and all have approved the final version of the manuscript. The corresponding author had full access to the data and had final responsibility for the decision to submit it for publication. No honorarium, grant, or other forms of payment were given to any of the authors to produce this manuscript.

Source of Funding: The Lundbeck Foundation (Grant no R16-A1694), the Ministry of Health (Grant no 903516), the Danish Council for Strategic Research (Grant no 0603-00280B), and The Capital Region have provided core support to the COPSAC Research Center. Greater Copenhagen Health Science Partners and the Children's Lung Foundation also funded this project.

Conflict of interest: All authors declare no potential, perceived, or actual conflict of interest regarding the content of this manuscript. The funding agencies did not have any role in the design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

Governance: We know and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) Good Clinical Practice guidelines, and the Helsinki Declaration. Privacy is important to us, so we follow national and international legislation such as the General Data Protection Regulation (GDPR), the Danish Act on Processing Personal Data, and the practice of the Danish Data Inspectorate.

Abbreviations:

AHR = Airway Hyperresponsiveness

AIC = Akaike Information Criterion

BIC = Bayesian Information Criterion

COPSAC₂₀₀₀₀ = Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀

CRP = C-Reactive Protein

FeNO = Fractional exhaled Nitric Oxide

FEV₁ = Forced Expiratory Volume at 1 second.

FVC = Forced Vital Capacity

GA = Gestational Age

HsCRP = High-sensitive C-Reactive Protein

IgA = Immunoglobulin A

IgE = Immunoglobulin E

IgG = Immunoglobulin G

IgM = Immunoglobulin M

LCT = Latent Class Trajectories

LMM = Linear Mixed Models

MMEF = Maximal Mid-Expiratory Flow

NLR = Neutrophils – Lymphocytes Ratio

PD20 = Methacholine dose causing a 20% drop in FEV₁

SD = Standard deviation

sRaw = Specific airway resistance

Th2 cell = T helper 2 cell

ABSTRACT

Background

Asthma is an inflammatory airway disease originating in early life, but it is understudied whether childhood trajectories of standard blood biochemistry measures are associated with the risk of developing asthma.

Methods

We modelled trajectories of blood neutrophils, lymphocytes, eosinophils, platelets, total-IgE, IgA, IgM, and IgG measured at ages 4, 5, 6, 7, 12 and 18 years in the COPSAC₂₀₀₀ birth cohort (n=411), and investigated their association with asthma diagnosis, airway obstruction using spirometry (FEV1) and plethysmography (sRaw), Type-2 airway inflammation (FeNO), and airway hyperresponsiveness (AHR) at age 18 years. We employed linear regression, linear mixed model (LMM) with variable slopes and intercepts, and latent class trajectory (LCT) analysis, adjusting for sex, gestational age, socioeconomics, smoking and antibiotic use during pregnancy, and childhood passive smoke exposure.

Results

In the LMM analyses, increasing blood neutrophils through childhood, higher eosinophils, higher total-IgE and increasing total-IgE were significantly associated with asthma at age 18. Further, higher eosinophils, higher total-IgE and increasing total-IgE were associated with higher FeNO, whereas higher platelet count was associated with lower FEV1 at age 18. The LCT models confirmed that specific childhood trajectories for eosinophils and total-IgE associated with higher FeNO level and that a particular platelet count trajectory associated with lower FEV1 and

increasing AHR. There were no consistent associations with trajectories of lymphocytes, IgG, IgA, or IgM, and no associations with sRaw.

Conclusion

Trajectories of standard blood biochemistry measures throughout childhood, particularly eosinophils, total-IgE, and platelet count, were associated with airway inflammation, reduced lung function, and increased AHR at age 18.

Keywords:

Asthma, Blood biochemistry, Inflammation, Platelets, Eosinophils, IgE, Neutrophils.

INTRODUCTION

Asthma is a chronic, inflammatory airway disease often occurring in early childhood or during adolescence¹. Traditionally, asthma is thought to be driven by Type 2 airway inflammation characterized by T helper 2 (Th2) and type 2 innate lymphoid cell (ILC2) cell-mediated responses²⁻⁴. Type 2-cell-mediated responses involve the release of cytokines that drive the production of Immunoglobulin E (IgE) and upregulation of eosinophils, resulting in increased blood levels of these biochemistry measures²⁻⁴. However, asthmatic airway inflammation is highly heterogeneous, involving several non-Type 2 cell types, such as neutrophils, lymphocytes, and platelets^{2,5-7}. In particular, neutrophil-associated asthma is associated with Type 17 cells such as Th17 and ILC3. Studies of patients with asthma show that neutrophilic airway inflammation dominates in certain asthma endotypes, distinguishing them from eosinophil-dominated endotypes⁵. Moreover, an increased blood Neutrophil–Lymphocyte Ratio (NLR) has been associated with systemic inflammation and severe asthma⁶. Whereas platelets have a well-established role in thrombosis and hemostasis⁸, they have also been shown to have a role in inflammatory responses⁸. Interestingly, platelets express high-affinity receptors for IgE, and up-regulation has been associated with an increased risk of severe allergic asthma^{7,9}. It has also been suggested that other immunoglobulins beyond IgE play a role in asthma pathogenesis with low IgG levels associated with severe asthma¹⁰.

Asthma susceptibility is most likely programmed in utero and early childhood, driven by complex gene-environment interactions resulting in abnormal immune development and subsequent airway inflammation¹³. Therefore, we hypothesized that abnormal immune development, which leads to asthmatic airway inflammation, may be captured by analyzing childhood trajectories of standard blood biochemistry measures. To investigate this, we analyzed trajectories of blood neutrophils,

lymphocytes, NLR, eosinophils, platelets, total-IgE, IgA, IgM, and IgG measured at ages 4, 5, 6, 7, 12 and 18 years in the Copenhagen Prospective Studies on Asthma in Childhood 2000 (COPSAC₂₀₀₀) birth cohort and their association with asthma diagnosis, airway obstruction, Type 2 airway inflammation, and airway hyperresponsiveness (AHR) at age 18 years.

METHODS

Study Population

COPSAC₂₀₀₀ is a single-center, prospective, mother-child cohort of 411 individuals born to mothers with asthma. The mothers were recruited during pregnancy, and the children were enrolled at one month of age and followed at the COPSAC research unit, with scheduled visits every six months until age seven and again at ages 12 and 18. The recruitment, baseline characteristics, and 18-year follow-up visits of COPSAC₂₀₀₀ have been described in detail previously^{1,14}.

Asthma diagnosis

Asthma at age 18 years was diagnosed by COPSAC physicians based on a previously validated symptom algorithm and response to inhaled corticosteroid treatment^{1,14}. Further, based on the European Respiratory Society recommendations¹⁵, an asthma diagnosis was objectively confirmed if at least two of the following criteria were fulfilled: 1) Obstructive lung function (Forced Expiratory Volume at 1 second (FEV1)<80% and/or FEV1/Forced Vital Capacity (FVC)<80% and/or specific airway resistance (sRaw)>1.6kPa/s); 2) Bronchodilator reversibility (change of FEV1≥12% and/or sRaw≥30% from baseline after inhalation of short-acting beta-2-agonist); 3) Airway inflammation (fractional exhaled nitric oxide (FeNO)≥25 ppb); and/or 4) AHR measured by a methacholine challenge test (drop in FEV1≥20% from baseline by a dose of methacholine ≤8 mg/ml) and/or an exercise challenge test (≥10% decrease in FEV1 from baseline). Remission was defined as having no symptoms or asthma treatment for one year.

Objective assessments

Airway obstruction: Lung function was measured by spirometry from age 5, using the MasterScope Pneumscreen (Erich Jäeger, Würzburg, Germany)^{16,17}, providing FEV1, FVC, and maximal mid-expiratory flow (MMEF). All measurements were conducted in triplicate. sRaw was assessed from age 2, by whole-body plethysmography using the MasterScope Bodybox (Erich Jäeger, Würzburg, Germany), as previously described¹⁸.

Airway inflammation: FeNO was measured from age 5 using the NIOX Flex (Aerocrine, Solna, Sweden)¹⁹. All measurements were conducted as duplicates.

Airway hyperresponsiveness was assessed at age 6 and 18 years by a bronchial challenge test using increasing doses of inhaled methacholine (APS Pro, CareFusion, 234 GmbH, Germany) and by a standardized exercise challenge test on a treadmill at age 7. By fitting a logistic function to the dose-response curves, the AHR was assessed as the provoking dose of methacholine that caused a 20% decrease in FEV1 compared to baseline (PD20)¹⁹. Bronchial hyperresponsiveness to exercise was defined as the maximum percentage decline in FEV1 within 10 minutes compared to the baseline value.

Blood biochemistry measures

Venous blood samples were collected from the children at the scheduled visits at ages 4, 5, 6, 7, 12 and 18 years. For the current study, we utilized the longitudinal assessments of blood neutrophil-, lymphocyte-, eosinophil-, and platelet counts, NLR, and total IgE, IgA, IgM, and IgG levels. Hematological biomarkers were measured in blood samples collected in Vacuette tubes containing K₃ EDTA, and cell concentrations were measured as 10⁹ cells/L using the ADVIA 2120i Hematology system from Siemens Healthcare Diagnostics. Immunoglobulins were measured in blood samples collected in Vacuette tubes containing a Lithium-Heparin

anticoagulant mixture. The material was mixed with antisera to produce immune complexes, which were available for measurements, and the concentration of immunoglobulins was measured as g/L using Vitros 5.1 F. s from Ortho-Clinical Diagnostics. All participants with at least two blood biochemistry measurements for any of the collected biomarkers during scheduled visits were included in the analysis.

Statistical analysis

First, we used linear regression models to examine the cross-sectional associations at age 18 between the selected blood biochemistry measures and asthma diagnosis, airway obstruction, FeNO, and AHR. Second, we examined the longitudinal associations of inflammatory biomarkers on asthma outcomes at age 18 using linear mixed models (LMM). Third, we analyzed the same associations after applying latent class trajectory (LCT) models.

In the LMM analyses, we modelled the blood biochemistry measures using fixed effects with a variable intercept, and random effects with both variable intercepts and variable slopes, with participant ID included to account for repeated measurements. We used the baseline level at age 4 years (intercept) and the age-related change from 4 to 18 years (slope) for each biomarker, allowing each child to have their own rate of change over time. We then extracted the subject-specific intercepts and slopes from the model and examined their associations with asthma outcomes using linear regression. The estimated intercepts and slopes were scaled, and the subsequent model estimates were therefore interpreted per 1 standard deviation (SD) change.

We further analyzed the longitudinal blood biochemistry data using LCT modeling to identify distinct developmental patterns over time. As a first step, we compared alternative polynomial age functions (linear, quadratic, and cubic) and evaluated model performance using the Bayesian

Information Criterion (BIC). For each selected growth function, we then fitted models with two to four latent classes and assessed model fit using a combination of goodness-of-fit metrics, including BIC, entropy, and class size. The optimal model for each biomarker was selected based on the lowest BIC values, highest entropy, reasonable class sizes, and the clinical interpretability of the resulting trajectories²¹.

The cross-sectional analysis, LMM, and LCT models were first done unadjusted and thereafter adjusted for sex, gestational age (GA), mother's age at delivery, mother's highest completed education and household income at the child's second birthday, smoking and antibiotic use during pregnancy, and passive smoke exposure in childhood.

Data processing was conducted with the statistical software R (R Foundation for Statistical Computing, R version 4.4.0 (2024-04), Vienna, Austria) using a significance cut-off of p-values of 0.05. Other R-packages utilized included tidyverse (2.0.0), dplyr (2.5.0), broom (1.0.6), lubridate (1.9.3), ggpubr (0.6.0), gridExtra (2.3), lamm (2.1.0), lme4 (1.1-35.5)²⁰, and scales (1.3.0).

RESULTS

Cohort characteristics

Of the 411 children in the COPSAC₂₀₀₀ cohort, 370 (90%) completed the 18-year full follow-up visit, where all had data on either asthma diagnosis, airway obstruction, airway inflammation, or AHR. Baseline characteristics for participants with follow-up and those without follow-up are presented in **Table S1**, showing significant differences between the two groups in terms of socioeconomic status, such as educational level and income, but also related risk factors such as solely breastfeed at age 3 months and maternal smoking during pregnancy.

Of the 411 children in the COPSAC₂₀₀₀ cohort, 385 (93.7%) participants had blood samples available. Among those participants, 217 (56.4%) had biochemistry assessments at all four time points, whereas 85 (22.1%) had only three timepoints, 53 (13.8%) had only two timepoints, and 30 (7.8%) had only one blood biochemistry analysis done at age 4, 5, 6, 7, 12 or 18 years.

Cross-sectional analysis at 18 years

In neither unadjusted nor covariate adjusted multivariable analyses comparing blood biochemistry measurements at 18 years with concurrent clinical outcomes, did we observe any significant associations between blood biochemistry measurements and an asthma diagnosis (**Table S2, Table 1**). The unadjusted analysis showed a significant association between higher platelet count and lower FEV1 (**Table S2**), but there were no significant associations between the biochemistry measurements and airway obstruction as measured by either FEV1 or sRaw in the adjusted analyses (**Table 1**). For airway inflammation assessed by FeNO, the adjusted analysis showed a significant association with higher eosinophil count (beta-estimate=22 ppb FeNO per 10^9 cells/L

(95% CI, 9-36), $p=0.001$) and higher IgE level (beta-estimate=0.01 ppb FeNO per IgE g/L (0.01-0.02), $p<0.001$) (**Table 1**). Finally, for AHR there was a significant association between a higher lymphocyte count and a lower PD20 (estimate=-1422 (-2809 to -36), $p=0.044$), i.e., greater AHR, (**Table 1**).

Linear mixed model analysis of childhood biochemistry measurements

Asthma: In the covariate adjusted LMM analyses, the development in blood neutrophil count over time was associated with asthma at age 18 years, showing that lower neutrophils at baseline, i.e., age 4 years (intercept OR=0.77 per 10^9 cells/L (0.59-0.98), $p=0.036$) and increasing neutrophils over time, i.e., from age 4 to 18 years (slope, OR=1.30 per 10^9 cells/L (1.01-1.68), $p=0.040$) were associated with an increased risk of asthma (**Table 2.1**). NLR was also associated with asthma, showing that lower NLR at baseline (intercept OR=0.77 (0.60-0.98), $p=0.038$) was associated with an increased risk of asthma. Further, the analysis revealed that a higher blood eosinophil count at baseline (intercept OR=1.51 per 10^9 cells/L (1.17-1.98), $p=0.002$) was associated with an increased risk of asthma at 18 years (**Table 2.1**). Finally, an increased level of total-IgE both at baseline and over time was associated with asthma in the adjusted LMM analysis (intercept OR=1.28 per IgE g/L (1.00-1.63), $p=0.049$, and slope OR=1.37 per IgE g/L (1.07-1.77), $p=0.015$) (**Table 2.1**).

Airway obstruction: The analysis of FEV1 showed a significant association between the platelet count at baseline and FEV1 at 18 years, where a higher platelet count at baseline was associated with lower FEV1 (intercept beta-estimate=-0.07 L per 10^9 cells/L (-0.12 to -0.03), $p=0.002$) (**Table 2.2**). Further, there was a trend of association between a higher lymphocyte count at baseline and lower FEV1 (intercept beta-estimate=-0.04 L per 10^9 cells/L (-0.09-0.00), $p=0.077$)

and a significant association between an increasing lymphocyte count over time and higher FEV1 at 18 years (slope beta-estimate=0.05 L per 10^9 cells/L (0.00-0.10), $p=0.044$) (**Table 2.2**). The adjusted LMM analysis of sRaw showed no significant associations (**Table 2.4**).

Airway inflammation: A higher blood eosinophil count at baseline was associated with higher FeNO level at 18 years (intercept beta-estimate=3.84 ppb per 10^9 cells/L (1.53-6.15), $p=0.001$), whereas the development in eosinophils over time, i.e. the slope, was not significant (slope beta-estimate=1.75 ppb per 10^9 cells/L (-0.57-4.06), $p=0.139$) (**Table 2.3**). Further, both a higher level of total-IgE at baseline and increasing total-IgE levels over time were associated with higher FeNO at 18 years (intercept beta-estimate=3.91 ppb per g/L (1.64-6.18), $p=0.001$, and slope beta-estimate=4.84 ppb per g/L (2.55-7.14), $p<0.001$), (**Table 2.3**).

Airway hyperresponsiveness: There were no significant associations between the development of the selected blood biochemistry measures and AHR, as indicated by PD20 at 18 years (**Table 2.5**). Results from the univariate unadjusted analyses are presented in the supplementary appendix (**Table S3.5**).

Latent class trajectory analysis of childhood biochemistry measurements

The optimal model for each biomarker was selected based on the lowest BIC values and highest entropy and clinical interpretability of the resulting trajectories (**Tables S4-S12, Figures S1-9**).

Asthma: The covariate adjusted multivariable LCT analyses with asthma as outcome showed that a specific IgM trajectory (latent class trajectory 1, LCT1, $n=29$) characterized by an increase in IgM levels in early childhood followed by a decline (**Figure 1**) was associated with a decreased risk of asthma by age 18 compared to the other three identified IgM trajectories (OR=0.16 (0.02-

0.57), $p=0.016$) (**Figure 2**). There were no significant associations between any other latent biochemistry measurement trajectories in childhood and asthma at age 18 (**Table 3.1**).

Airway obstruction: In line with the LMM results, the adjusted LCT analyses showed a significant association between a specific platelet count trajectory in childhood (LCT2, $n=193$) characterized by a decrease in platelet count in early childhood followed by an increase (**Figure 1**) and lower FEV1 at age 18 compared to the other identified platelet count trajectory (beta-estimates=-0.12 (-0.21 to -0.03), $p=0.013$) (**Figure 2**). There were no other significant findings in the adjusted LCT analyses for FEV1 or sRaw (**Table 3.1**).

Airway inflammation: The adjusted LCT analyses with FeNO as outcome showed that both a specific trajectory for blood eosinophils (LCT3, $n=142$) characterized by a general higher level of blood eosinophils and an increase in early childhood followed by a decline compared to the other two identified blood eosinophil trajectories (beta-estimate=12.79 (7.82-17.75), $p<0.001$), and a trajectory for total-IgE level in childhood (LCT3, $n=94$) characterized by a continuous rise during childhood and adolescence compared to the other two identified total-IgE trajectories (beta-estimate=9.67 (4.24-15.10), $p<0.001$) were associated with higher FeNO at age 18 years (**Figures 1-2, Table 3.2**).

Airway hyperresponsiveness: Finally, the adjusted LCT analyses also showed a significant association between the specific LCT2 ($n=29$) platelet count trajectory in childhood characterized by a decrease in early childhood and followed by an increase and lower PD20, i.e. higher AHR at age 18 years compared to the other platelet trajectory (beta-estimate, -1344 (-26235 to -65), $p=0.039$) (**Figures 1-2, Table 3.3**). A specific NLR trajectory (LCT2, $n=280$) characterized by a higher set point and a continuous rise over time (**Figure S5**) also tended to be associated with a lower PD20 in the adjusted analyses compared to the other NLR trajectory (beta-estimate, -1336

(-2760-87), $p=0.066$) (**Table 3.3**). In the unadjusted analyses, NLR trajectory LCT2 ($n=280$) was associated with a lower PD20 (beta-estimate, -1569 (-2933 to -206), $p=0.024$), and also a neutrophils trajectory (LCT1, $n=64$) characterized by a generally decreased level was associated with a lower PD20 (beta-estimate, -1531 (-3079 to -16), $p=0.052$) (**Table S13.5**).

DISCUSSION

Primary findings

In this study, we explored trajectories of standard blood biochemistry measures, including blood platelets, neutrophils, lymphocytes, NLR, eosinophils, total-IgE, IgG, IgA, and IgM through childhood and adolescence, in relation to the development of asthma and related traits at age 18 years. The longitudinal analyses showed that childhood trajectories of blood platelets, eosinophil counts, and total-IgE levels were associated with key asthma-related traits at age 18 years, including airway inflammation, reduced lung function, and increased AHR. These findings suggest that repeated assessments of standard blood biochemistry may provide early insight into asthma pathophysiology and long-term outcomes.

Interpretation

Our findings that platelet trajectories were associated with lung function and AHR is interesting in light of the role of platelets in the pathogenesis of chronic airway inflammation and asthma, positioning them as potential active participants rather than passive bystanders in allergic disease and asthma^{8,9}. In particular, their intrapulmonary migration has been implicated in the pathogenesis of allergic asthma²². Platelets express IgE receptors and can trigger the release of histamine and activation of eosinophils, while also synthesizing spasmogenic mediators that promote bronchoconstriction^{8,22}. Moreover, recent findings in individuals with allergy have shown that platelet dysfunction is more pronounced in patients with severe asthma, suggesting a potential role in disease severity⁷. The findings from these previous studies correspond with our study, where we found that the longitudinal development of platelet counts in childhood and adolescence

was associated with lower lung function and increasing AHR at age 18 years, observed in both linear mixed models and by data-driven modeling of latent trajectories of platelet counts in childhood. Furthermore, the cross-sectional analysis showed an association between higher platelet count and lower FEV1 at age 18, but this was not significant after covariate adjustments. Together, these findings suggest both an early role of platelets in lung function development and AHR and an association of the longitudinal development of platelet counts. This warrants further research into a possible role of measuring the development of platelet counts for asthma risk assessment, prevention strategies, and targeted treatment.

It is well-described that there are different endotypes of asthma, and that the most common in school age is allergic asthma driven by ILC2 and Th2-cells, producing e.g. IL-4, IL-5, and IL-13 cytokines²⁻⁴. These cytokines can cause eosinophilic inflammation and initiate the production of IgE, followed by a clinical manifestation of the disease^{2-4,23}. FeNO is a noninvasive assessment used to measure the level of Type 2 inflammation in the airways, reflecting the level of eosinophils⁴. In line with this, our LMM analysis showed a positive association between increased number of eosinophils at age 4 years, increased total-IgE levels at age 4 years and increasing total-IgE levels during childhood and risk of asthma at 18 years. In the LCT analysis, we further found a positive association between increased number of eosinophils and total-IgE and FeNO at age 18 years.

Neutrophilic inflammation may also affect asthma pathogenesis, and has mainly been associated with non-allergic asthma, including wheezing in early childhood⁵. Especially, an increased number of neutrophils in the airways has been demonstrated during exacerbations²⁴ with increasing neutrophil bronchial inflammation in patients with exacerbations progressing into status asthmaticus compared to milder asthma exacerbations²⁵. Furthermore, in patients with severe

asthma, bronchial inflammation is dominated by an increased number of neutrophils even after recovery⁵. In our study of adolescents with mild to moderate asthma, the LMM analysis showed an association between the development of neutrophil count in childhood and risk of asthma, where lower neutrophils at the age 4 years and an increasing neutrophil count during childhood and adolescence was associated with increased risk of asthma at age 18 years. However, there were no significant associations with either airway obstruction, airway inflammation, or AHR, and the LCT analyses did not reveal any associations between trajectories of blood neutrophil counts and asthma status.

Our LMM analysis also showed a significant association between the NLR and asthma at age 18 years. The role of neutrophils and lymphocytes in asthma pathogenesis was further supported by the cross-sectional analysis, showing an association between higher lymphocyte count and reduced PD20, i.e., increasing AHR, and by the LCT analysis, where the unadjusted analysis showed that neutrophils and NLR were significantly associated with increasing AHR at age 18 years. Our findings among adolescents with mild to moderate asthma align with previous studies demonstrating an association between NLR and asthma^{6,26,27}. An American study of 469 children with asthma demonstrated significantly elevated NLR compared with 170 children without asthma²⁷. A second study of 482 children with asthma and 107 controls confirmed these results, again showing markedly increased NLR among children with asthma²⁶. Finally, a cross-sectional study showed that increased NLR was associated with severity of asthma among children with asthma exacerbations seen in the emergency department⁶.

Only a few studies have explored the association between total-IgG, IgM, and asthma. Some studies have focused on total-IgG and asthma, but the results are inconsistent. A small older study from the United Kingdom showed that a reduced level of total-IgG was associated with severe

asthma in adults¹⁰. In contrast, another case-control study investigating the relation between total-IgG and asthma in adults did not find significant associations for total-IgG, but showed reduced levels of subclasses IgG2 and IgG3 among patients with asthma¹¹. Finally, a study focusing specifically on the IgG4 subclass showed an association between elevated IgG4 and allergic asthma¹² but only investigated cases with elevated levels. In our study, we did not observe any associations between total-IgG and asthma or related traits, which was consistent in both LMM and LCT analyses.

In the LCT analysis, we found that the specific IgM trajectory with increased IgM levels at age 4 years and a decline from age 4 to 18 years was associated with reduced risk of asthma at age 18 years. IgM is produced during acute infections, and this trajectory could illustrate an increase in infections during early childhood that decline later. In literature, only one study has explored levels of IgM and association with asthma. The study analyzed IgM in bronchoalveolar lavage fluid in different groups, including patients with allergic asthma, and did not find a significant association between IgM and asthma²⁸.

Strengths and Limitations

The asthma diagnoses in this study were based on objective criteria and treatment responses following standard operating procedures. Furthermore, asthma was diagnosed and monitored solely at the COPSAC research unit, assuring consistency in diagnostic procedures and specificity compared to other studies using parental reporting of diagnoses made by general practitioners or questionnaire surveys. In addition, all participants were born to mothers with asthma, which may have improved symptom recognition. Objective assessments of airway obstruction, airway inflammation, and AHR were done by trained research personnel who strictly followed standard

operating procedures. The 18-year-olds from the COPSAC₂₀₀₀ cohort have participated in such examinations repeatedly from birth and were, therefore, highly competent in performing tests and assessments, which assured a high completion rate. Finally, the longitudinal analyses of standard blood biochemistry measurements were based on data from four time points during childhood and adolescence, and the data were modelled using a cross-sectional approach, a traditional repeated-measurements approach, and an approach exploring latent trajectories.

However, the study also has some limitations. The cohort only includes 411 children, of whom 93 (22.6%) were diagnosed with asthma at age 18 years. This makes it challenging to investigate different asthma phenotypes and gain enough power for statistical analysis. Furthermore, most asthma cases were mild or moderate, and many of the disease traits measured at age 18 years may have been present during the time of exposure measurements, making the directionality of the findings difficult to disentangle. Finally, the cohort was initially designed as a high-risk cohort, which can lead to selection bias compared to a population-based cohort.

Conclusion

In this study, we explored standard blood biochemistry measures, including neutrophils, lymphocytes, NLR, eosinophils, platelets, total-IgE, IgA, IgM, and IgG, longitudinally through childhood and adolescence in relation to the development of asthma and related traits at age 18. Trajectories of blood eosinophil counts, total-IgE levels, and platelet counts were associated with asthma pathophysiology, including airway inflammation, lower lung function, and increased AHR, suggesting that repetitive assessments of standard blood biochemistry measurements may be useful for asthma risk stratification.

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TABLES AND FIGURES

Table 1. Covariate adjusted analyses of asthma outcomes and biochemistry measures at 18 years

Blood biochemistry	Asthma			FEV1			FeNO			sRaw			PD20		
	OR	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	0.91	(0.70-1.17)	0.489	0.01	(-0.04-0.05)	0.740	0.09	(-2.25-2.43)	0.938	-0.02	(-0.04-0.01)	0.262	121.41	(-415-658)	0.658
Eosinophils	0.83	(0.15-3.43)	0.816	0.07	(-0.20-0.34)	0.621	22.46	(9.22-35.70)	0.001	-0.04	(-0.21-0.12)	0.622	-1388.57	(-4457-1680)	0.375
Lymphocytes	0.61	(0.30-1.22)	0.176	0.10	(-0.02-0.21)	0.119	-2.13	(-8.23-3.96)	0.493	-0.03	(-0.11-0.04)	0.393	-1422.13	(-2809 to -36)	0.044
Platelets	1.00	(0.99-1.00)	0.159	-0.00	(-0.00-0.00)	0.472	-0.04	(-0.09-0.01)	0.140	0.00	(-0.00-0.00)	0.835	-6.71	(-19-6)	0.281
Ratio Neutrophils / Lymphocytes	1.01	(0.68-1.47)	0.945	-0.01	(-0.08-0.06)	0.755	0.04	(-3.47-3.56)	0.981	-0.01	(-0.06-0.03)	0.553	496.65	(-300-1293)	0.222
IgG	1.03	(0.87-1.23)	0.705	0.00	(-0.03-0.03)	0.887	0.52	(-1.05-2.09)	0.517	0.01	(-0.01-0.02)	0.590	-179.84	(-553-194)	0.345
IgA	0.76	(0.46-1.20)	0.252	0.03	(-0.05-0.12)	0.445	2.03	(-2.14-6.21)	0.340	-0.01	(-0.06-0.04)	0.688	-154.47	(-1140-831)	0.759
IgM	1.82	(0.93-3.55)	0.079	-0.08	(-0.21-0.04)	0.186	-2.65	(-8.94-3.65)	0.410	0.07	(-0.00-0.15)	0.056	-537.68	(-2075-1000)	0.493
Total-IgE	1.00	(1.00-1.00)	0.674	-0.00	(-0.00-0.00)	0.257	0.01	(0.01-0.02)	<0.001	0.00	(-0.00-0.00)	0.920	-0.20	(-1.8-1.4)	0.812

Table 1. Cross-sectional 18 years - multivariable. The table shows the cross-sectional analysis between biochemistry measurements and atopic outcomes at 18 years.

Table 2. The longitudinal effect of inflammatory biomarkers in LMM models on atopic outcomes

Table 2.1						
The longitudinal effect of inflammatory biomarkers in LMM models on asthma, multivariate				Asthma		
Blood samples	OR	Intercept CI	p-value	OR	Slope CI	p-value
Neutrophils	0.77	(0.59-0.98)	0.036	1.30	(1.01-1.68)	0.040
Eosinophils	1.51	(1.17-1.98)	0.002	0.78	(0.61-1.01)	0.059
Lymphocytes	10.99	(0.78-1.27)	0.961	0.95	0.73-1.21	0.667
Platelets	1.05	(0.82-1.35)	0.682	0.94	(0.73-1.20)	0.595
Ratio neutrophils / Lymphocytes	0.77	(0.60-0.98)	0.038	1.24	(0.97-1.59)	0.087
IgG	0.98	(0.76-1.25)	0.848	1.08	(0.84-1.39)	0.549
IgA	0.91	(0.71-1.17)	0.465	1.16	(0.90-1.51)	0.274
IgM	1.19	(0.93-1.54)	0.173	0.97	(0.76-1.23)	0.789
IgE	1.28	(1.00-1.63)	0.049	1.37	(1.07-1.77)	0.015

Table 2.1 - The longitudinal effect of inflammatory biomarkers in LMM models on asthma, multivariate

Table 2.2						
The longitudinal effect of inflammatory biomarkers in LMM models on FEV1, multivariate				FEV1		
Blood samples	Intercept			Slope		
	Estimate	CI	p-value	Estimates	CI	p-value
Neutrophils	-0.02	(-0.06-0.03)	0.457	0.02	(-0.03-0.06)	0.399
Eosinophils	-0.02	(-0.06-0.03)	0.519	0.00	(-0.05-0.05)	0.997
Lymphocytes	-0.04	(-0.09-0.00)	0.077	0.05	(0.00-0.10)	0.044
Platelets	-0.07	(-0.12--0.03)	0.002	0.04	(-0.01-0.08)	0.127
Ratio neutrophils / Lymphocytes	0.01	(-0.03-0.06)	0.517	-0.02	(-0.07-0.02)	0.324
IgG	-0.00	(-0.05-0.04)	0.846	0.00	(-0.04-0.05)	0.931
IgA	0.03	(-0.02-0.08)	0.199	-0.02	(-0.07-0.03)	0.410
IgM	-0.02	(-0.07-0.02)	0.332	-0.01	(-0.06-0.03)	0.556
IgE	-0.01	(-0.05-0.04)	0.774	-0.02	(-0.06-0.03)	0.454

Table 2.2 - The longitudinal effect of inflammatory biomarkers in LMM models on FEV1, multivariate

Table 2.3 The longitudinal effect of inflammatory biomarkers in LMM models on FeNO, multivariate						
Blood samples	FeNO					
	Intercept			Slope		
	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	-0.72	(-3.02-1.59)	0.544	0.39	(-1.89-2.67)	0.737
Eosinophils	3.84	(1.53-6.15)	0.001	1.75	(-0.57-4.06)	0.139
Lymphocytes	-2.15	(-4.65-0.36)	0.093	1.54	(-0.98-4.06)	0.232
Platelets	0.49	(-1.92-2.90)	0.689	-2.30	(-4.73-0.14)	0.064
Ratio neutrophils / Lymphocytes	0.47	(-1.87-2.81)	0.694	-0.44	(-2.78-1.89)	0.710
IgG	1.85	(-0.48-4.18)	0.119	-1.31	(-3.72-1.10)	0.288
IgA	1.57	(-0.88-4.01)	0.209	-1.60	(-4.28-1.09)	0.244
IgM	-0.63	(-3.01-1.75)	0.604	-0.32	(-2.68-2.04)	0.792
IgE	3.91	(1.64-6.18)	0.001	4.84	(2.55-7.14)	<0.001

Table 2.3 - The longitudinal effect of inflammatory biomarkers in LMM models on FeNO, multivariate

Table 2.4 The longitudinal effect of inflammatory biomarkers in LMM models on sRaw, multivariate						
Blood samples	sRaw					
	Intercept			Slope		
	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	-3.13	(-12.00-5.75)	0.490	1.63	(-7.29-10.56)	0.720
Eosinophils	-0.01	(-0.04-0.03)	0.730	0.01	(-0.02-0.04)	0.556
Lymphocytes	-4.96	(-14.46-4.54)	0.306	1.41	(-8.50-11.32)	0.781
Platelets	-1.52	(-10.95-7.92)	0.753	2.38	(-7.31-12.08)	0.630
Ratio neutrophils / Lymphocytes	1.03	(-7.87-9.93)	0.821	-0.17	(-9.19-8.86)	0.971
IgG	-0.22	(-9.50-9.06)	0.963	2.46	(-7.04-11.96)	0.612
IgA	2.00	(-7.48-11.48)	0.679	-1.22	(-12.16-9.73)	0.828
IgM	8.50	(-0.59-17.58)	0.067	-2.08	(-11.29-7.14)	0.659
IgE	2.42	(-6.49-11.33)	0.594	3.41	(-5.86-12.69)	0.471

Table 2.4 - The longitudinal effect of inflammatory biomarkers in LMM models on sRaw, multivariate

Table 2.5**The longitudinal effect of
inflammatory biomarkers in
LMM models on PD20,
multivariate**

Blood samples	PD20					
	Intercept			Slope		
	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	242.89	(-347.25-833.04)	0.420	-171.19	(-791.39-449.02)	0.589
Eosinophils	74.43	(-516.18-665.03)	0.805	-255.11	(-833.73-383.56)	0.469
Lymphocytes	-235.01	(-893.74-423.73)	0.484	-73.23	(-765.80-619.35)	0.836
Platelets	-590.85	(-1225.03-43.34)	0.068	171.06	(-482.63-824.75)	0.608
Ratio neutrophils / Lymphocytes	441.44	(-164.02-1046.89)	0.153	-48.55	(-684.75-587.65)	0.881
IgG	-422.46	(-1066.66-221.74)	0.199	45.96	(-612.38-704.29)	0.891
IgA	-228.88	(-885.91-428.15)	0.495	108.80	(-655.54-873.13)	0.780
IgM	-341.32	(-957.38-274.75)	0.278	143.95	(-489.80-777.71)	0.656
IgE	188.46	(-406.93-783.85)	0.535	-316.99	(-937.24-303.26)	0.316

Table 2.5 - The longitudinal effect of inflammatory biomarkers in LMM models on PD20, multivariate

Table 3. The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes

Table 3.1 The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes							
	LCT	Asthma				FEV1	
Blood samples		OR	CI	p-value	Estimates	CI	p-value
Neutrophils	LCT1	1.32	(0.68-2.51)	0.395	0.01	(-0.11-0.14)	0.821
	LCT3	0.59	(0.25-1.29)	0.215	0.04	(-0.09-0.17)	0.556
Eosinophils	LCT1	0.67	(0.31-1.37)	0.284	0.09	(-0.04-0.21)	0.163
	LCT3	1.29	(0.75-2.20)	0.353	0.03	(-0.08-0.13)	0.604
Lymphocytes	LCT2	1.02	(0.62-1.68)	0.929	-0.03	(-0.12-0.06)	0.547
Platelets	LCT2	0.93	(0.56-1.54)	0.778	-0.12	(-0.21--0.03)	0.013
Ratio Neutrophils / Lymphocytes	LCT2	1.36	(0.78-2.34)	0.271	0.05	(-0.06-0.15)	0.400
IgG	LCT2	0.86	(0.52-1.43)	0.566	-0.03	(-0.12-0.07)	0.559
IgA	LCT1	-	-	-	-0.05	(-0.66-0.55)	0.858
	LCT2	2.13	(0.28-11.71)	0.400	-0.05	(-0.41-0.30)	0.764
	LCT4	5.33	(0.85-41.98)	0.073	-0.22	(-0.65-0.20)	0.299

IgM	LCT1	0.16	(0.02-0.57)	0.016	0.00	(-0.17-0.17)	0.978
	LCT2	1.10	(0.33-3.18)	0.870	0.10	(-0.12-0.33)	0.375
	LCT4	0.88	(0.52-1.49)	0.639	-0.05	(-0.15-0.05)	0.350
IgE	LCT1	0.63	(0.26-1.39)	0.282	-0.03	(-0.17-0.10)	0.642
	LCT3	1.52	(0.86-2.65)	0.142	-0.04	(-0.15-0.07)	0.447

Table 3.1 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, multivariate

Table 3.2

**The longitudinal effect
of inflammatory
biomarkers in LCT
models on atopic
outcomes**

	LCT	FeNO			sRaw		
Blood samples		Estimates	CI	p-value	Estimates	CI	p-value
Neutrophils	LCT1	-1.76	(-8.22-4.70)	0.593	18.60	(-6.32-43.53)	0.144
	LCT3	0.43	(-6.29-7.16)	0.899	6.59	(-20.06-33.23)	0.28
Eosinophils	LCT1	-1.47	(-7.49-4.54)	0.631	0.03	(-0.06-0.12)	0.512
	LCT3	12.79	(7.82-17.75)	<0.001	-0.01	(-0.08-0.07)	0.814
Lymphocytes	LCT2	1.93	(-2.75-6.62)	0.418	0.01	(-0.05-0.08)	0.694
Platelets	LCT2	1.17	(-3.54-5.88)	0.626	-0.04	(-0.11-0.03)	0.229
Ratio Neutrophils / Lymphocytes	LCT2	0.03	(-5.33-5.40)	0.900	0.01	(-0.07-0.09)	0.795
IgG	LCT2	-3.85	(-8.65-0.95)	0.116	-0.00	(-0.07-0.07)	0.975
IgA	LCT1	-11.70	(-42.57-9.18)	0.458	-0.23	(-0.76-0.30)	0.401
	LCT2	-3.29	(-21.39-14.81)	0.722	0.16	(-0.07-0.38)	0.167
	LCT4	-2.70	(-24.51-19.10)	0.808	0.05	(-0.25-0.36)	0.735
IgM	LCT1	2.97	(-5.88-11.82)	0.510	-0.08	(-0.21-0.04)	0.191
	LCT2	5.21	(-6.32-16.73)	0.376	-0.06	(-0.22-0.10)	0.469
	LCT4	1.51	(-3.54-6.56)	0.558	0.01	(-0.06-0.09)	0.688

IgE	LCT1	-4.70	(-11.45-2.06)	0.173	-1.00	(-28.90-26.91)	0.944
	LCT3	9.67	(4.24-15.10)	<0.001	12.75	(-8.87-34.38)	0.248

Table 3.2 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, multivariate

Table 3.3 The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes				
Blood samples		LCT	PD20	
		Estimates	CI	p-value
Neutrophils	LCT1	-1153.81	(-2798.02-490.40)	0.169
	LCT3	239.28	(1515.68-1994.24)	0.789
Eosinophils	LCT1	-882.06	(-2589.17-825.05)	0.311
	LCT3	-973.07	(-2408.27-462.12)	0.184
Lymphocytes	LCT2	1159.96	(-122.38-2442.30)	0.076
Platelets	LCT2	-1343.84	(-2622.46-65.23)	0.039
Ratio Neutrophils / Lymphocytes	LCT2	-1336.34	(-2759.52-86.84)	0.066
IgG	LCT2	890.24	(-404.31-2184.80)	0.178
IgA	LCT1	-546.43	(-9850.25-8757.39)	0.908
	LCT2	-1017.73	(-5269.01-3233.56)	0.639
	LCT4	-2117.29	(-8611.35-4376.78)	0.523
IgM	LCT1	-546.43	(-9850.25-8757.39)	0.908
	LCT2	-1017.73	(-5269.01-3233.56)	0.639
	LCT4	-2117.29	(-8611.35-4376.78)	0.523
IgE	LCT1	680.75	(-1326.40-2687.89)	0.506
	LCT3	282.13	(-1171.50-1735.77)	0.704

Table 3.3 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, multivariate

Figure 1: Latent class trajectories of inflammatory biomarkers

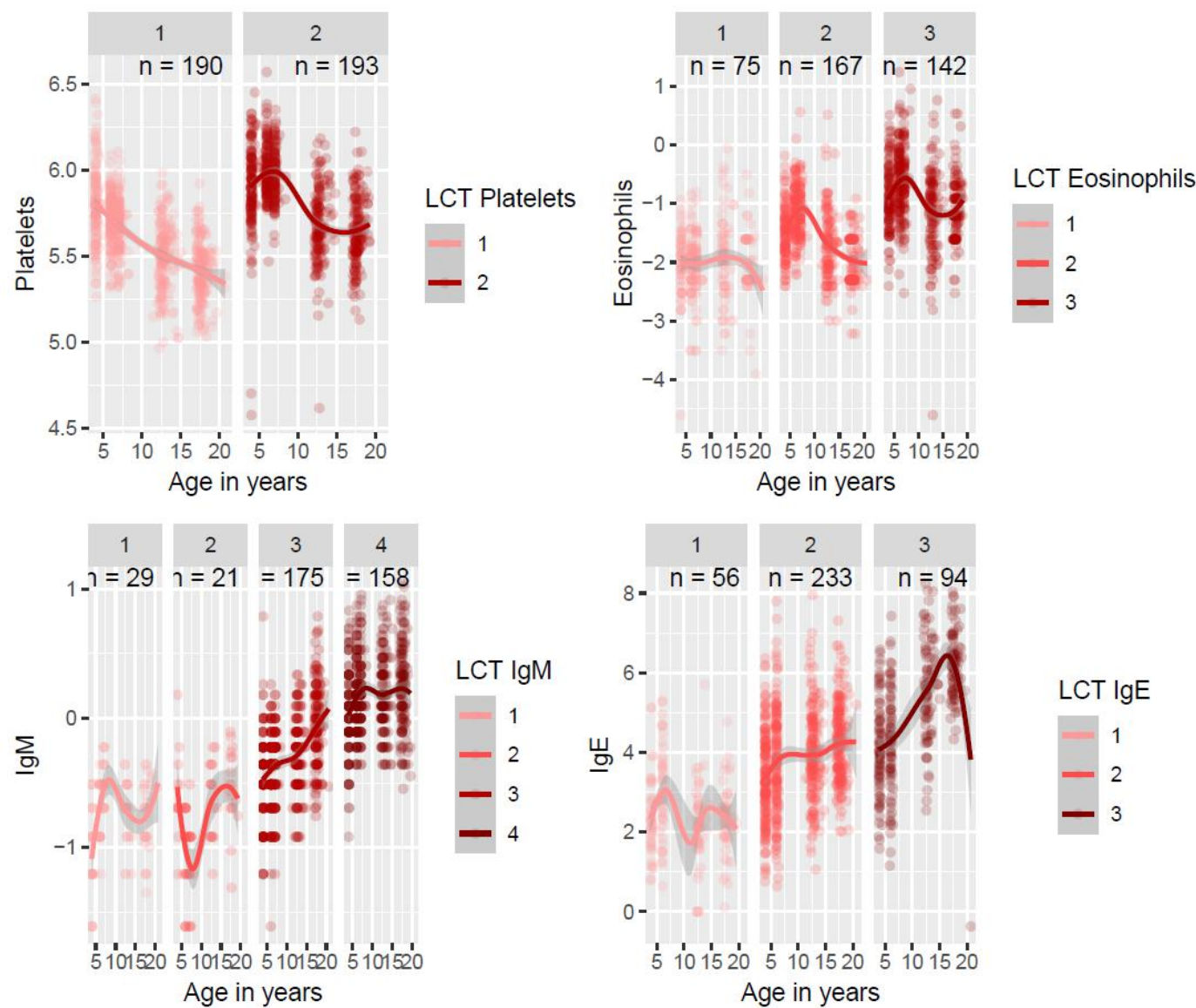
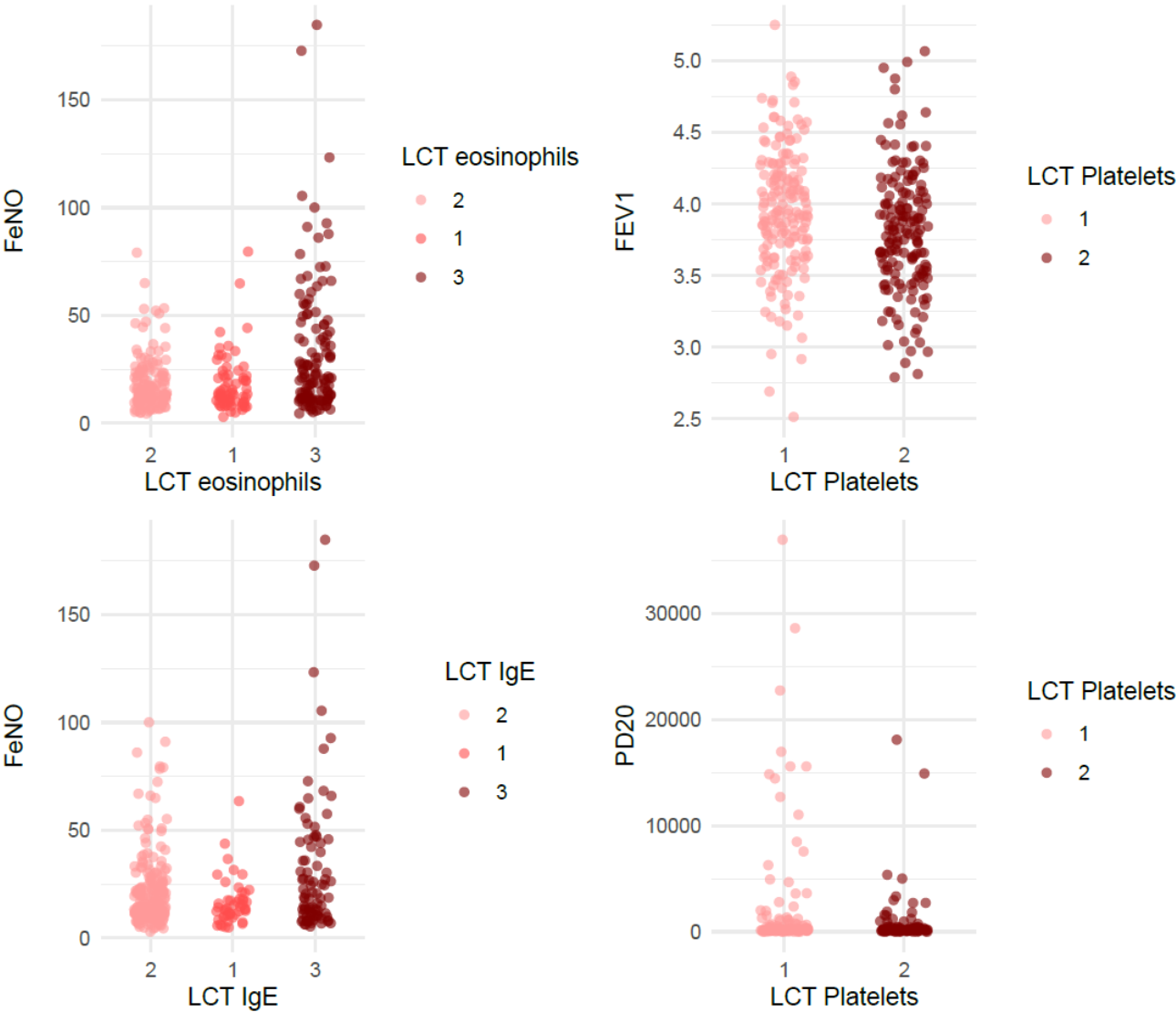


Figure 2. Scatterplot of significant associations between latent class trajectories of inflammatory biomarkers and outcomes



SUPPLEMENTARY APPENDIX

METHODS

Patient and public involvement:

The COPSAC₂₀₀₀ cohort study is conducted in collaboration with the 411 participating families. The families are queried through regular surveys about their motivations and attitudes towards participation in the close clinical follow-up at our research clinic. Our ongoing activities are adjusted according to the priorities and preferences expressed in the answers. In biannual newsletters, the families are updated on upcoming research activities and recently published results from the cohort. There was no direct patient- and public involvement concerning the development of outcomes, interpretation, or dissemination of the results of this specific study.

Ethics

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Local Ethics Committee (KF 01-289/96-, 18- & 24-year follow-up: H-16039498), and the Danish Data Protection Agency (2015-41-3696). Both parents gave written informed consent before enrolment. At the COPSAC₂₀₀₀ 18-year visit, the study participants gave written informed consent themselves.

Table S1. Baseline characteristics for the COPSAC2000 cohort

Baseline characteristics	Participated	Not participated	p-value
N = 411 (%)	370 (90.1)	41 (9.9)	-
Age (mean, SD)	17.7 (0.56)	-	-
Sex, female (%)	188 (50.8)	20 (48.8)	0.93
Race, caucasian (%)	358 (96.8)	39 (95.1)	0.93
Income, type (%)			< 0.001
Low	137 (37.0)	18 (42.9)	-
Medium	74 (20.0)	3 (7.1)	-
High	145 (39.2)	9 (21.4)	-
Maternal education, type (%)			<0.001
Low	205 (55.4)	21 (50.0)	-
Medium	97 (26.2)	3 (7.1)	-
High	49 (13.2)	3 (7.1)	-
Maternal age at birth (mean, SD)	30.1 (4.58)	29.4 (4.19)	0.29
Gestational age in days (mean, SD)	282.1 (10.7)	282.0 (10.7)	0.80
Maternal smoking in pregnancy (%)	83 (22.4)	16 (38.1)	0.03
Solely breastfeeding at 3 months (%)	264 (71.4)	19 (45.2)	< 0.001
Older siblings, yes (%)	141 (27.0)	14 (33.3)	0.28
Younger siblings, yes (%)	9 (2.4)	1 (2.4)	1.00
Delivery (cesarean section)	77 (20.8)	9 (21.4)	0.80

Season (%)			0.17
Winter	87 (23.5)	8 (19.0)	-
Spring	82 (22.2)	4 (1.1)	-
Summer	98 (26.5)	13 (31.0)	-
Autum	103 (27.8)	16 (38.1)	-

Table S1. Baseline characteristics of the cohort

Table S2. Unadjusted analyses of asthma outcomes and biochemistry measures at 18 years

Blood samples	Asthma			FEV1			FENO			sRaw			PD20		
	OR	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value	Estimates	CI	p-value
Neutrophiles	0.94	(0.77-1.14)	0.553	0.00	(-0.03-0.04)	0.883	-0.42	(-2.30-1.46)	0.662	-0.02	(-0.05-0.01)	0.123	154	(-363-671)	0.560
Eosinophiles	0.83	(0.15-3.43)	0.816	0.00	(-0.23-0.24)	0.971	31.12	(19.51-42.73)	<0.001	-0.06	(-0.22-0.11)	0.497	-1502	(-4543-1539)	0.333
Lymphocytes	0.85	(0.51-1.39)	0.516	0.02	(-0.07-0.12)	0.651	-0.39	(-5.22-4.45)	0.876	-0.03	(-0.10-0.04)	0.425	-1224	(-2579-132)	0.077
Platelets	1.00	(0.99-1.00)	0.740	-0.00	(-0.00--0.00)	0.006	-0.04	(-0.08-0.00)	0.068	0.00	(-0.00-0.00)	0.813	-8.9	(-20.2-2.4)	0.124
Ratio Neutrophiles / Lymphocytes	0.97	(0.73-1.25)	0.809	0.00	(-0.05-0.05)	0.890	-0.47	(-3.04-2.09)	0.718	-0.02	(-0.07-0.02)	0.282	521	(-259-1300)	0.191
IgG	1.02	(0.90-1.15)	0.800	0.00	(-0.02-0.03)	0.737	0.76	(-0.44-1.96)	0.212	-0.00	(-0.02-0.02)	0.923	-190	(-556-176)	0.308
IgA	0.86	(0.60-1.23)	0.420	0.02	(-0.05-0.09)	0.565	-0.71	(-4.15-2.74)	0.687	-0.02	(-0.07-0.03)	0.348	-65	(-1028-899)	0.895
IgM	1.24	(0.73-2.08)	0.424	-0.06	(-0.16-0.04)	0.226	-1.96	(-7.11-3.20)	0.457	0.05	(-0.02-0.13)	0.182	-801	(-2264-662)	0.283
IgE	1.00	(1.00-1.00)	0.637	-0.00	(-0.00-0.00)	0.195	0.01	(0.01-0.02)	<0.001	-0.00	(-0.00-0.00)	0.994	0.2	(-1.4-1.8)	0.778

Table S2. Cross-sectional 18 years - univariate

Table S3. The longitudinal effect of inflammatory biomarkers in univariate LMM models on atopic outcomes

Table S3.1 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on asthma				Asthma		
Blood samples	OR	Intercept CI	p-value	OR	Slope CI	p-value
Neutrophiles	0.78	(0.61-0.98)	0.038	1.26	(0.99-1.61)	0.062
Eosinophiles	1.50	(1.18-1.92)	0.001	0.80	(0.63-1.02)	0.069
Lymphocytes	0.99	(0.78-1.25)	0.910	0.95	0.75-1.20	0.691
Platelets	1.13	(0.90-1.43)	0.300	0.88	(0.69-1.11)	0.291
Ratio Neutrophiles / Lymphocytes	0.81	(0.64-1.02)	0.073	1.18	(0.93-1.48)	0.172
IgG	1.03	(0.81-1.30)	0.835	0.98	(0.77-1.23)	0.835
IgA	0.96	(0.76-1.22)	0.764	1.05	(0.83-1.34)	0.694
IgM	1.12	(0.89-1.43)	0.338	0.94	(0.74-1.19)	0.613
IgE	1.26	(0.99-1.60)	0.058	1.35	(1.07-1.72)	0.014

Table S3.1 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on asthma

Table S3.2 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on FEV1

Blood samples	FEV1					
	Estimate	Intercept CI	p-value	Estimate	Slope CI	p-value
Neutrophiles	-0.02	(-0.06-0.03)	0.494	0.02	(-0.03-0.06)	0.449
Eosinophiles	-0.02	(-0.07-0.03)	0.381	0.00	(-0.05-0.05)	0.999
Lymphocytes	-0.03	(-0.08-0.02)	0.202	0.03	(0.02-0.08)	0.187
Platelets	-0.08	(-0.12-0.03)	0.001	0.03	(-0.02-0.08)	0.203
Ratio Neutrophiles / Lymphocytes	0.01	(-0.03-0.06)	0.621	-0.02	(-0.06-0.03)	0.509
IgG	-0.00	(-0.05-0.04)	0.935	0.01	(-0.04-0.06)	0.674
IgA	0.03	(-0.02-0.08)	0.190	-0.02	(-0.07-0.03)	0.381
IgM	-0.02	(-0.06-0.03)	0.517	-0.01	(-0.05-0.04)	0.728
IgE	-0.00	(-0.05-0.04)	0.917	-0.02	(-0.06-0.03)	0.417

Table S3.2 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on FEV1

Table S3.3						
The longitudinal effect of inflammatory biomarkers in univariate LMM models on FeNO				FeNO		
Blood samples	Estimate	Intercept	p-value	Estimate	Slope	p-value
		CI			CI	
Neutrophiles	-0.84	(-3.08-1.40)	0.464	0.43	(-1.80-2.67)	0.706
Eosinophiles	3.54	(1.34-5.74)	0.002	1.96	(-0.27-4.20)	0.085
Lymphocytes	-1.78	(-4.14-0.58)	0.140	1.21	(-1.15-3.56)	0.316
Platelets	0.61	(-1.68-2.90)	0.600	-2.22	(-4.50-0.07)	0.057
Ratio Neutrophiles / Lymphocytes	0.25	(-2.02-2.52)	0.826	-0.35	(-2.59-1.89)	0.759
IgG	1.47	(-0.76-2.69)	0.196	-1.00	(-3.23-1.23)	0.381
IgA	1.12	(-1.20-3.44)	0.343	-1.56	(-4.12-1.00)	0.233
IgM	-0.26	(-2.88-1.67)	0.601	-0.48	(-2.74-1.79)	0.679
IgE	3.39	(1.18-5.60)	0.003	5.24	(3.08-7.39)	<0.001

Table S3.3 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on FeNO

Table S3.4 The longitudinal effect of inflammatory biomarkers in univariate LMM models on sRaw						
Blood samples	sRaw					
	Estimate	Intercept CI	p-value	Estimate	Slope CI	p-value
Neutrophiles	-4.33	(-13.09-4.44)	0.333	2.79	(-6.32-11.46)	0.572
Eosinophiles	0.00	(-0.03-0.03)	0.949	0.00	(-0.03-0.04)	0.810
Lymphocytes	-4.43	(-13.65-4.80)	0.347	1.42	(-8.28-11.13)	0.774
Platelets	-2.34	(-11.65-6.97))	0.622	1.31	(-8.08-10.71)	0.784
Ratio Neutrophiles / Lymphocytes	-0.50	(-9.27-8.26)	0.910	0.37	(-8.63-9.38)	0.936
IgG	-1.45	(-10.52-7.62)	0.754	1.61	(-7.56-10.77)	0.731
IgA	0.72	(-8.60-10.05)	0.879	-1.79	(-12.54-8.96)	0.744
IgM	6.87	(-2.10-15.84)	0.134	-2.04	(-11.17-7.08)	0.661
IgE	2.49	(-6.31-11.30)	0.579	2.33	(-6.71-11.36)	0.614

Table S3.4 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on sRaw

Table S3.5**The longitudinal effect of
inflammatory biomarkers in
univariate LMM models on PD20**

Blood samples	PD20					
	Estimate	Intercept CI	p-value	Estimate	Slope CI	p-value
Neutrophiles	387.55	(-190.21-965.31)	0.189	-255.88	(-864.00-352.25)	0.410
Eosinophiles	-9.46	(-583.94-565.02)	0.571	-173.38	(-773.39-426.64)	0.571
Lymphocytes	-235.71	(-870.55-399.13)	0.467	-49.50	(-724.76-625.77)	0.886
Platelets	-586.93	(-1209.73-35.87)	0.065	146.49	(-490.64-783.62)	0.652
Ratio Neutrophiles / Lymphocytes	532.77	(-53.59-1119.13)	0.075	73.32	(-698.33-551.70)	0.818
IgG	-422.29	(-1048.25-203.67)	0.186	30.63	(-599.70-660.95)	0.924
IgA	-142.00	(-780.32-496.32)	0.663	32.75	(-705.65-771.15)	0.931
IgM	-299.46	(-904.03-305.10)	0.332	26.84	(-595.22-648.90)	0.933
IgE	127.68	(-453.57-708.93)	0.667	-238.17	(-842.85-366.51)	0.440

Table S3.5 - The longitudinal effect of inflammatory biomarkers in univariate LMM models on PD20

Table S4. LCT models for neutrophils

Table S4 - Neutrophils	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	1142.97	1139.64	1157.37
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	1158.82	1160.94	1183.72
Entropy	0.32	0.34	0.30
Class 1	34.30	31.13	46.17
Class2	65.70	68.87	53.83
Model 3			
BIC	1173.3	1179.95	1204.86
Entropy	0.48	0.50	0.52
Class 1	16.89	16.89	15.57
Class 2	69.13	68.34	69.39
Class 3	13.98	14.78	15.04
Model 4			
BIC	1191.11	1197.81	1227.09
Entropy	0.296	0.59	0.61
Class 1	24.01	17.15	14.25
Class 2	0.00	66.23	67.02
Class 3	52.51	15.57	1.32
Class 4	23.48	1.06	17.41
Best model	3	3	3

Table S4. LCT models for neutrophils

Table S5. LCT models for eosinophils

Table S5 - Eosinophils	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	2819.61	2807.23	2759.67
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	2827.20	2826.66	2783.80
Entropy	0.94	0.41	0.44
Class 1	98.70	75.52	54.43
Class2	1.30	24.48	45.57
Model 3			
BIC	2842.82	2842.04	2803.35
Entropy	0.54	0.65	0.45
Class 1	65.36	0.52	19.53
Class 2	33.33	55.47	43.49
Class 3	1.3	44.01	36.98
Model 4			
BIC	2860.59	2854.00	2820.48
Entropy	0.56	0.70	0.57
Class 1	54.17	0.52	19.76
Class 2	0.26	5.,69	43.23
Class 3	44.01	43.49	0.26
Class 4	1.56	1.30	36.72
Best model	2	2	3

Table S5. LCT models for eosinophils

Table S6. LCT models for lymphocytes

Table S6 - Lymphocytes	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	-58.23	-34.50	-16.36
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	-40.12	-10.75	13.32
Entropy	0.0013	0.0013	0.0006
Class 1	49.34	49.87	31.13
Class2	50.66	50.13	68.87
Model 3			
BIC	-29.73	0.46	29.67
Entropy	0.86	0.81	0.79
Class 1	97.10	96.04	2.90
Class 2	1.85	1.85	94.20
Class 3	1.06	2.11	2.90
Model 4			
BIC	-14.59	21.54	34.22
Entropy	0.83	0.70	0.81
Class 1	0.26	87.86	3.69
Class 2	1.85	1.32	1.06
Class 3	95.78	6.86	92.35
Class 4	2.11	3.96	2.90
Best model	2	2	2

Table S6. LCT models for lymphocytes

Table S7. LCT models for platelets

Table S7 - Platelets	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	-747.38	-739.70	-817.67
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	-729.54	-715.91	-793.76
Entropy	0.0025	0.0023	0.354
Class 1	51.96	49.61	49.61
Class2	48.042	50.39	50.39
Model 3			
BIC	-723.15	-704.17	-766.91
Entropy	0.69	0.68	0.26
Class 1	7.31	6.53	37.60
Class 2	73.63	74.67	35.25
Class 3	19.06	18.80	27.15
Model 4			
BIC	-706,0305	-721,852	-812,4982
Entropy	0.41	0.75	0.70
Class 1	8.62	0.52	0.78
Class 2	24.28	6.27	80.68
Class 3	43.86	74.41	18.02
Class 4	23.38	18.80	0.52
Best model	3	3	2

Table S7. LCT models for platelets

Table S8. LCT models for the ratio of neutrophils/lymphocytes (NLR)

Table S8 - Ratio neutrophils/lymphocytes	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	1427.06	1428.27	1454.39
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	1443.81	1451.30	1482.77
Entropy	0.32	0.30	0.31
Class 1	26.12	25.07	27.97
Class2	73.88	74.93	72.03
Model 3			
BIC	1455.49	1462.01	1490.66
Entropy	0.33	0.79	0.83
Class 1	0.00	0.79	0.79
Class 2	96.31	94.99	95.25
Class 3	3.69	4.22	3.96
Model 4			
BIC	1473.30	1478.49	1529.65
Entropy	0.16	0.60	0.51
Class 1	0.00	0.00	23.48
Class 2	0.00	94.99	8.71
Class 3	94.72	3.956	62.01
Class 4	5.28	1.06	5.80
Best model	2	2	2

Table S8. LCT models for the ratio of neutrophils/lymphocytes

Table S9. LCT models for total-IgG

Table S9- total-IgG	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	-1134.69	-1429.34	-1420.81
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	-1116.85	-1405.55	-1442.17
Entropy	0.0005	0.007	0.99
Class 1	48.83	49.09	99.48
Class2	51.17	50.91	0.52
Model 3			
BIC	-1103.49	-1442.93	1420.48
Entropy	0.56	0.89	0.89
Class 1	3.92	3.11	3.39
Class 2	78.33	0.26	0.52
Class 3	17.75	96.61	96.08
Model 4			
BIC	-1085.64	-1420.35	1397.18
Entropy	0.33	0.88	0.78
Class 1	4.70	1.83	4.18
Class 2	0.78	0.26	0.52
Class 3	73.90	96.34	90.60
Class 4	20.63	1.57	4.70
Best model	2	2	2

Table S9. LCT models for total-IgG

Table S10. LCT models for total blood IgA

Table S10 - total IgA	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	45.28	1500.46	1511.41
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	1104.25	1472.43	1526.21
Entropy	0.0009	0.80	0.40
Class 1	47.00	11.22	43.27
Class2	53.00	88.78	56.73
Model 3			
BIC	1004.45	1475.68	1486.91
Entropy	0.98	0.68	0.62
Class 1	1.31	9.62	44.87
Class 2	97.39	25.32	44.55
Class 3	1.31	65.06	10.58
Model 4			
BIC	1022.30	1491.89	1489.66
Entropy	0.78	0.59	0.61
Class 1	1.31	9.94	24.04
Class 2	0.00	25.32	20.51
Class 3	97.39	13.14	44.87
Class 4	1.31	51.60	10.58
Best model	2	3	4
Table S10. LCT models for total blood IgA			

Table S11. LCT models for total-IgM

Table S11 - total-IgM	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	475.67	481.39	452.86
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	489.06	487.77	451.48
Entropy	0.70	0.90	0.86
Class 1	3.66	3.13	4.96
Class2	96.34	96.87	95.04
Model 3			
BIC	502.74	514.68	462.76
Entropy	0.58	0.60	0.85
Class 1	0.26	6.27	1.57
Class 2	67.10	67.62	4.96
Class 3	32.64	26.11	93.47
Model 4			
BIC	516.39	513.34	489.88
Entropy	0.62	0.75	0.57
Class 1	0.52	3.92	7.57
Class 2	3.66	0.52	5.48
Class 3	46.74	88.25	45.69
Class 4	49.09	7.31	41.25
Best model	2	3	4

Table S11. LCT models for total-IgM

Table S12. LCT models for total-IgE

Table S12 - total-IgE	LCT Linear	LCT Cubic	LCT Quadratic
Model 1			
BIC	3304.78	3119.25	2431.46
Entropy	1.00	1.00	1.00
Class 1	100.00	100.00	100.00
Model 2			
BIC	3320.03	3137.49	2459.67
Entropy	0.37	0.86	0.28
Class 1	73.70	98.63	39.18
Class2	26.30	1.37	60.82
Model 3			
BIC	3335.29	3159.32	2475.76
Entropy	0.48	0.50	0.61
Class 1	70.96	58.08	11.23
Class 2	26.58	40.27	65.21
Class 3	2.47	1.64	23.56
Model 4			
BIC	3352.00	3182.18	2461.00
Entropy	0.46	0.52	0.52
Class 1	40.27	52.60	0.82
Class 2	51.23	2.19	50.14
Class 3	4.93	41.92	46.85
Class 4	3.56	3.29	2.19
Best model	2	3	3

Table S12. LCT models for total-IgE

Table S13. The longitudinal effect of inflammatory biomarkers in univariate LCT models on atopic

Table S13.1							
The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, univariate		LCT	Asthma			FEV1	
Blood samples		OR	CI	p-value	Estimates	CI	p-value
Neutrophiles	LCT1	1.41	(0.76-2.54)	0.27	0.02	(-0.10-0.14)	0.75
	LCT3	0.63	(0.28-1.31)	0.24	0.06	(0.08-0.19)	0.40
Eosinophiles	LCT1	0.60	(0.29-1.20)	0.17	0.12	(-0.01-0.24)	0.06
	LCT3	1.29	(0.78-2.15)	0.33	0.01	(-0.09-0.11)	0.81
Lymphocytes	LCT2	1.07	(0.67-1.70)	0.79	-0.05	(-0.14-0.04)	0.30
Platelets	LCT2	1.07	(0.67-1.70)	0.79	-0.14	(-0.23--0.05)	0.002
Ratio Neutrophiles / Lymphocytes	LCT2	1.40	(0.83-2.33)	0.20	0.05	(-0.06-0.15)	0.38
IgG	LCT2	0.91	(0.57-1.46)	0.70	-0.02	(-0.11-0.07)	0.63
IgA	LCT1	1.41	(0.76-2.54)	0.27	-0.10	(-0.72-0.51)	0.74
	LCT2	0.63	(0.28-1.31)	0.24	-0.06	(-0.41-0.30)	0.76
	LCT4	-	-	-	-0.23	(-0.67-0.20)	0.30

IgM	LCT1	0.20	(0.03-0.71)	0.033	-0.03	(-0.20-0.15)	0.75
	LCT2	1.09	(0.37-2.85)	0.87	0.09	(-0.12-0.31)	0.39
	LCT4	0.86	(0.52-1.41)	0.56	-0.04	(-0.14-0.06)	0.43
IgE	LCT1	0.62	(0.27-1.29)	0.226	-0.01	(-0.14-0.12)	0.898
	LCT3	1.37	(0.80-2.33)	0.246	-0.03	(-0.14-0.08)	0.616

Table S13.1 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, univariate

Table S13.2

**The longitudinal effect
of inflammatory
biomarkers in LCT
models on atopic
outcomes, univariate**

	LCT	FeNO			sRaw		
Blood samples		Estimates	CI	p-value	Estimates	CI	p-value
Neutrophiles	LCT1	-0.85	(-6.95-5.26)	0.79	17.25	(-6.20-40.71)	0.15
	LCT3	-2.00	(-8.56-4.55)	0.55	2.94	(-23.38-29.27)	0.83
Eosinophiles	LCT1	-0.85	(-6.69-4.98)	0.774	0.00	(-0.09-0.09)	0.98
	LCT3	12.98	(8.16-17.80)	<0.001	-0.02	(-0.10-0.05)	0.59
Lymphocytes	LCT2	1.49	(-3.04-6.03)	0.52	0.01	(-0.06-0.08)	0.76
Platelets	LCT2	1.19	(-3.32-5.70)	0.60	-0.03	(-0.10-0.04)	0.38
Ratio Neutrophiles / Lymphocytes	LCT2	0.41	(-4.76-5.58)	0.88	0.01	(-0.06-0.09)	0.71
IgG	LCT2	-2.90	(-7.40-1.61)	0.207	0.02	(-0.05-0.08)	0.62
IgA	LCT1	-8.36	(-38.77-22.06)	0.59	-0.17	(-0.71-0.36)	0.53
	LCT2	-3.68	(-21.34-13.98)	0.68	0.16	(-0.06-0.38)	0.16
	LCT4	-2.08	(-23.65-19.48)	0.85	0.05	(-0.26-0.36)	0.75
IgM	LCT1	4.00	(-4.63-12.62)	0.36	-0.07	(-0.20-0.05)	0.26
	LCT2	4.43	(-6.20-15.06)	0.41	-0.10	(-0.26-0.06)	0.24

	LCT4	1.52	(-3.33-6.37)	0.54	0.00	(-0.08-0.07)	0.90
IgE	LCT1	-5.42	(-11.81-0.96)	0.096	1.22	(-25.92-28.36)	0.930
	LCT3	9.75	(4.53-14.98)	<0.001	10.53	(-10.69-31.75)	0.331

Table S13.2 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, univariate

Table S13.3				
The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, univariate				
	LCT	PD20		
Blood samples		Estimates	CI	p-value
Neutrophiles	LCT1	-1531.49	(-3079.09-16.11)	0.052
	LCT3	-0.39	(-1719.54-1718.75)	1.00
Eosinophiles	LCT1	-935.94	(-2573.43-701.54)	0.26
	LCT3	-1222.67	(-2606.37-161.03)	0.08
Lymphocytes	LCT2	1002.76	(-234.90-2240.41)	0.11
Platelets	LCT2	-1378.18	(-2605.28-151.08)	0.028
Ratio Neutrophiles / Lymphocytes	LCT2	-1569.31	(-2932.74-205.89)	0.024
IgG	LCT2	1073.54	(-161.77-2308.84)	0.09
IgA	LCT1	-1506.27	(-10654.37-7641.83)	0.75
	LCT2	-1374.66	(-5504.78-2755.46)	0.51
	LCT4	-1632.82	(-8116.96-4851.32)	0.62
IgM	LCT1	-1366.58	(-3977.30-1244.13)	0.31
	LCT2	-30.51	(-3061.81-3000.79)	0.98
	LCT4	-511.50	(-1827.18-804.17)	0.45
IgE	LCT1	705.08	(-1248.65-2658.81)	0.479
	LCT3	472.40	(-962.05-1906.85)	0.519

Table S13.3 - The longitudinal effect of inflammatory biomarkers in LCT models on atopic outcomes, univariate

Figure S1: Latent class trajectories for neutrophils during childhood and adolescence

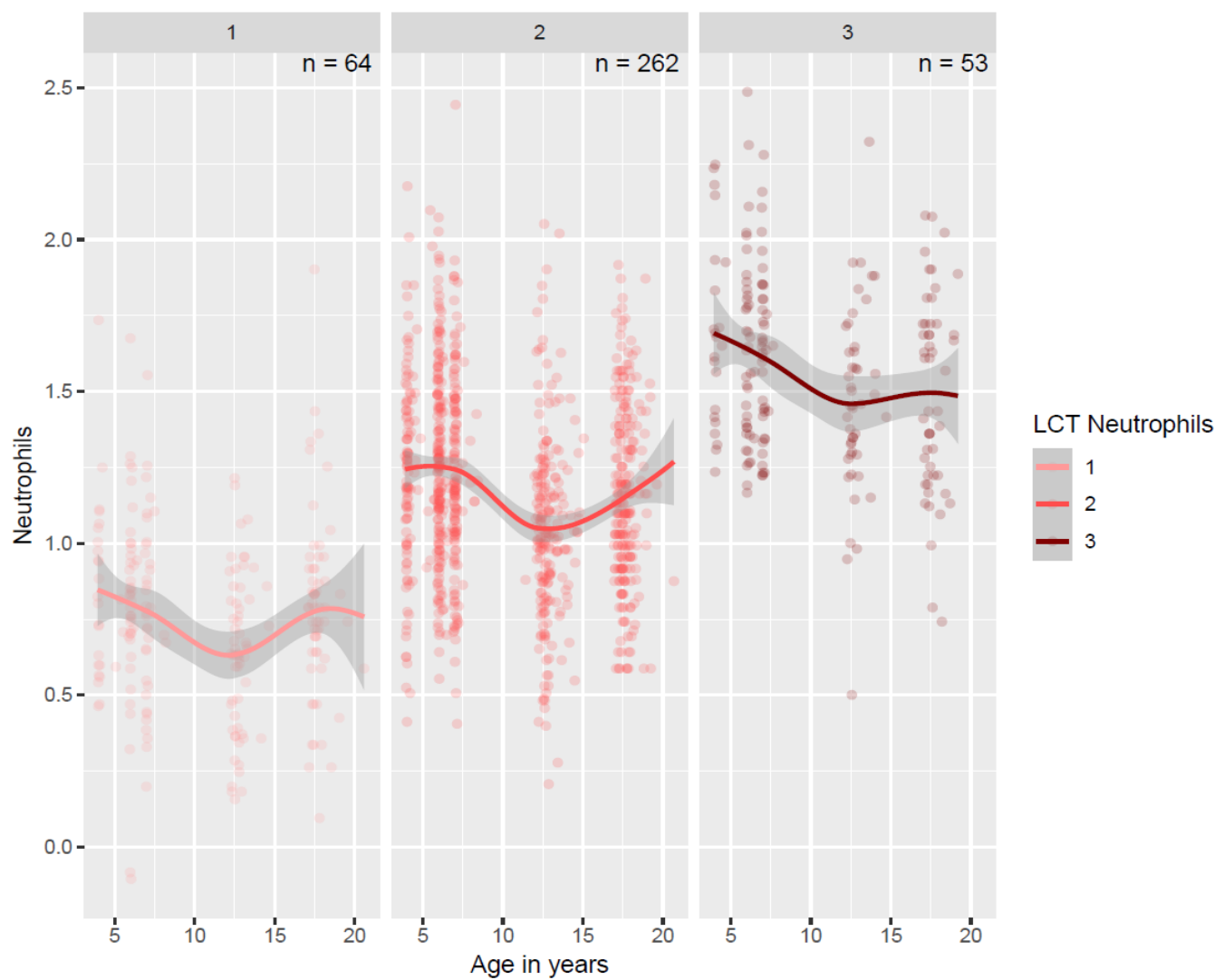


Figure S1 Latent class trajectories for neutrophils during childhood and adolescence.

Figure S2: Latent class trajectories for eosinophils during childhood and adolescence

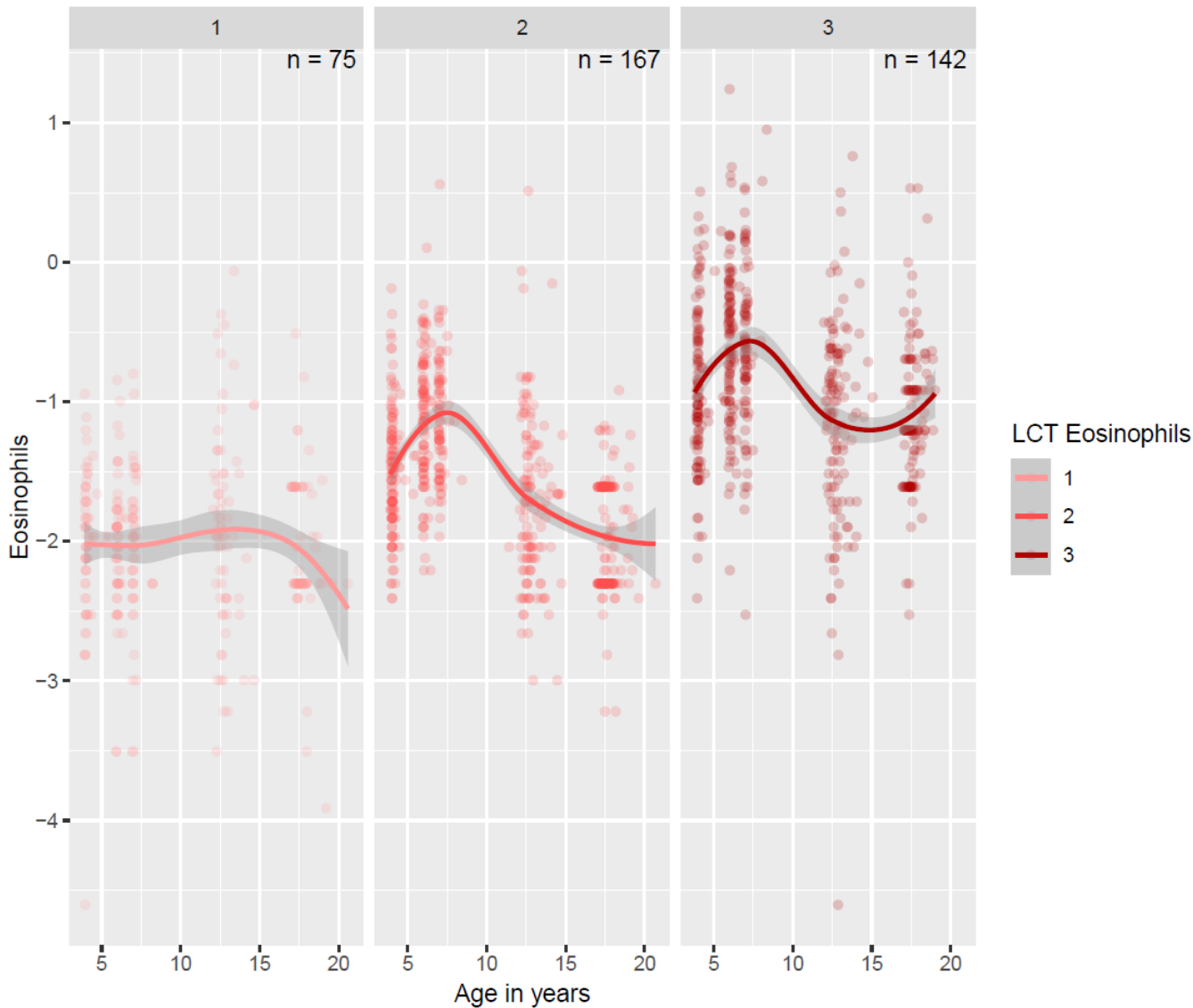


Figure S2. Latent class trajectories for eosinophils during childhood and adolescence

Figure S3: Latent class trajectories for lymphocytes during childhood and adolescence

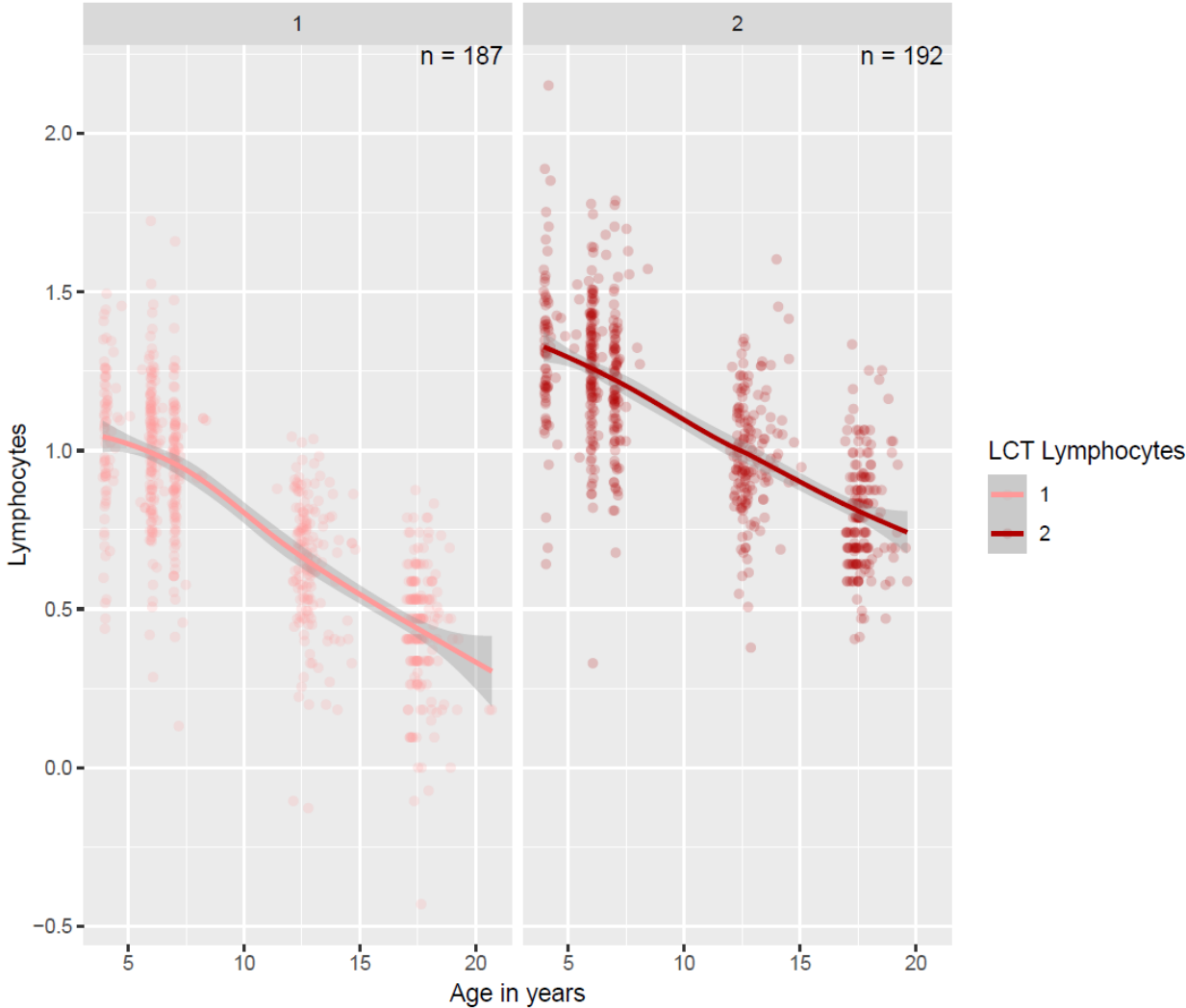


Figure S3. Latent class trajectories for lymphocytes during childhood and adolescence.

Figure S4: Latent class trajectories for platelets during childhood and adolescence

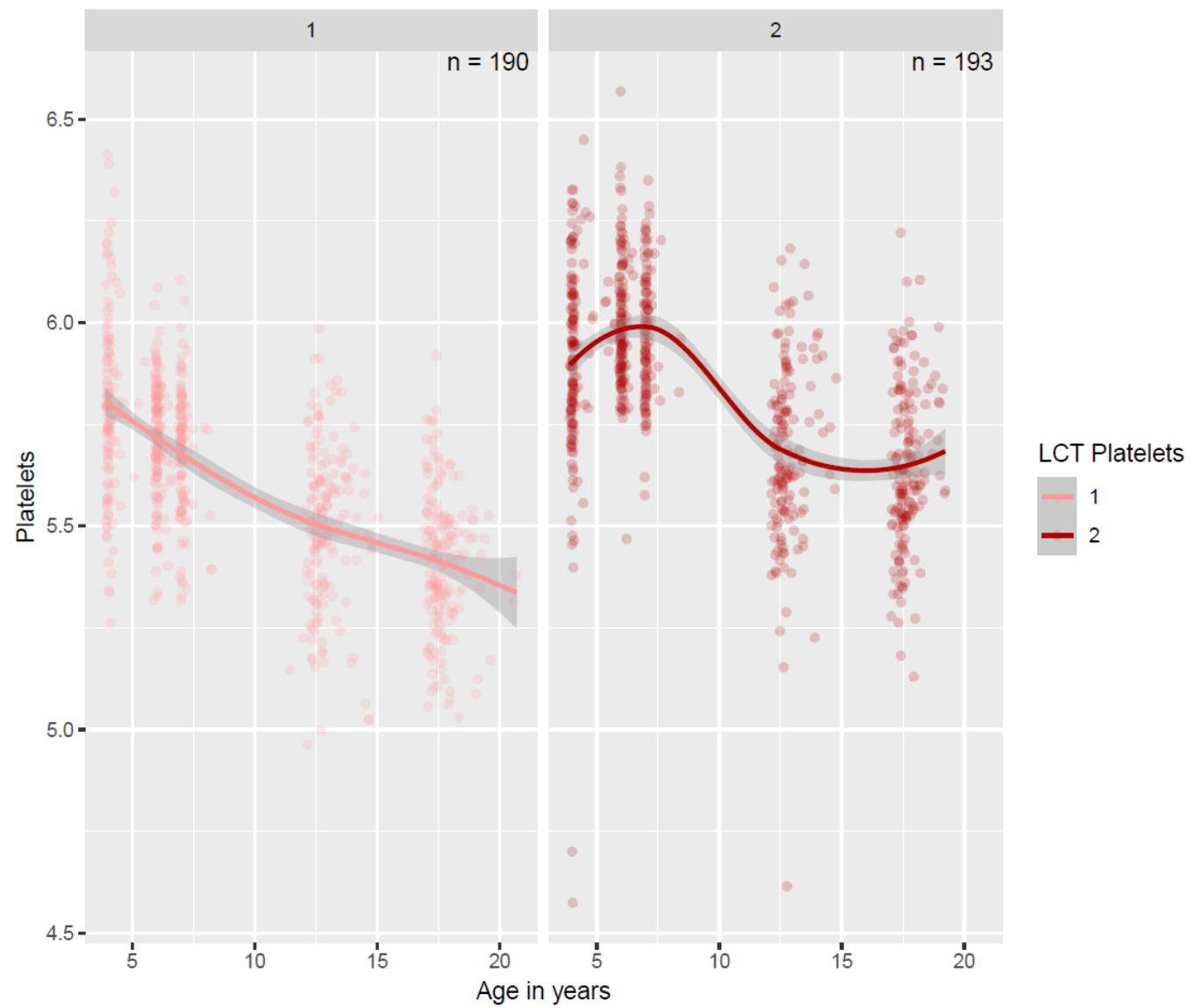


Figure S4. Latent class trajectories of platelets during childhood and adolescence.

Figure S5: Latent class trajectories for ratio neutrophils/lymphocytes during childhood and adolescence

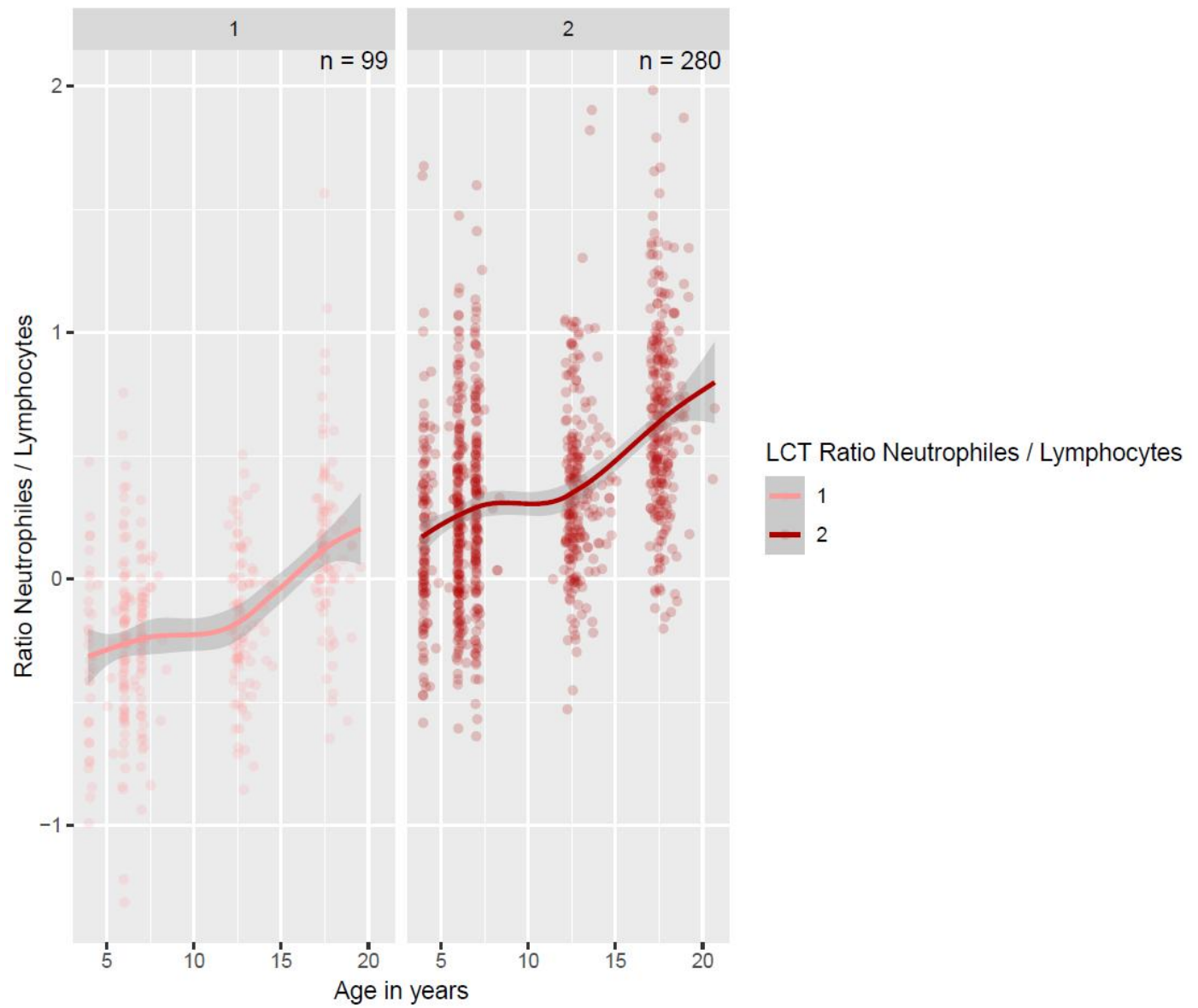


Figure S5. Latent class trajectories for ratio neutrophils/lymphocytes during childhood and adolescence.

Figure S6: Latent class trajectories for total-IgG during childhood and adolescence

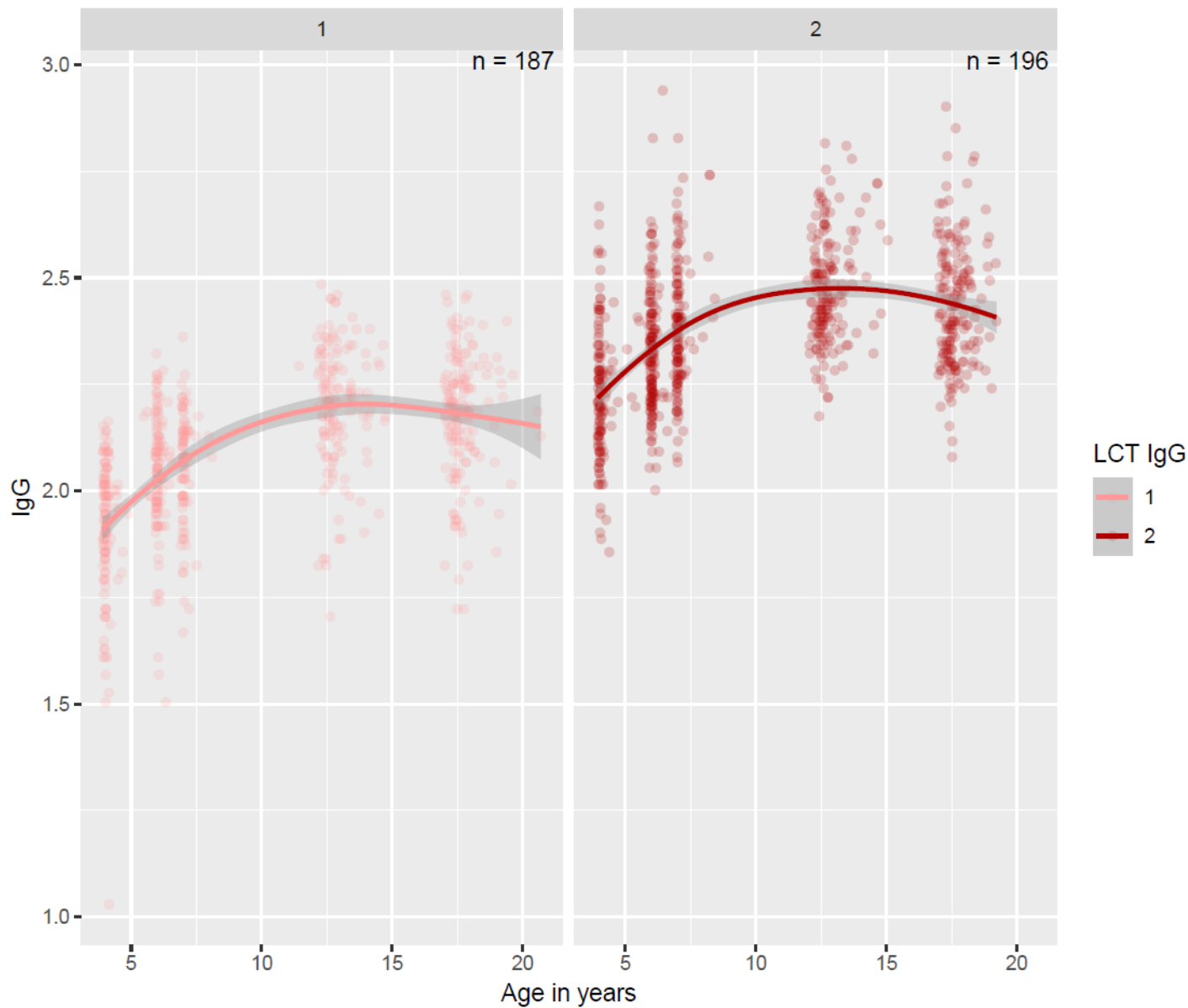


Figure S6. Latent class trajectories for total-IgG during childhood and adolescence.

Figure S7: Latent class trajectories for total-IgA during childhood and adolescence

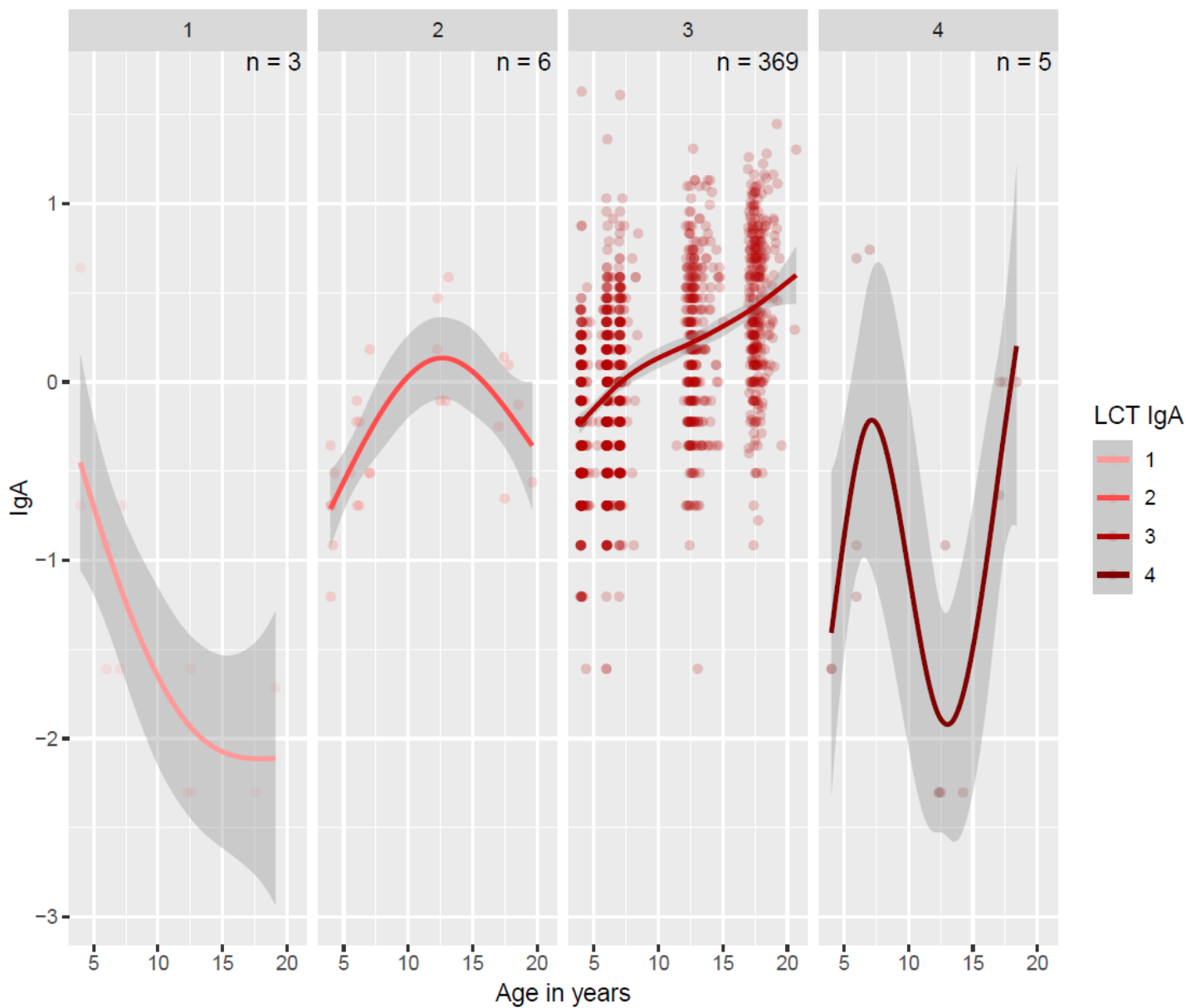


Figure S7. Latent class trajectories for total-IgA during childhood and adolescence.

Figure S8: Latent class trajectories for total-IgM during childhood and adolescence

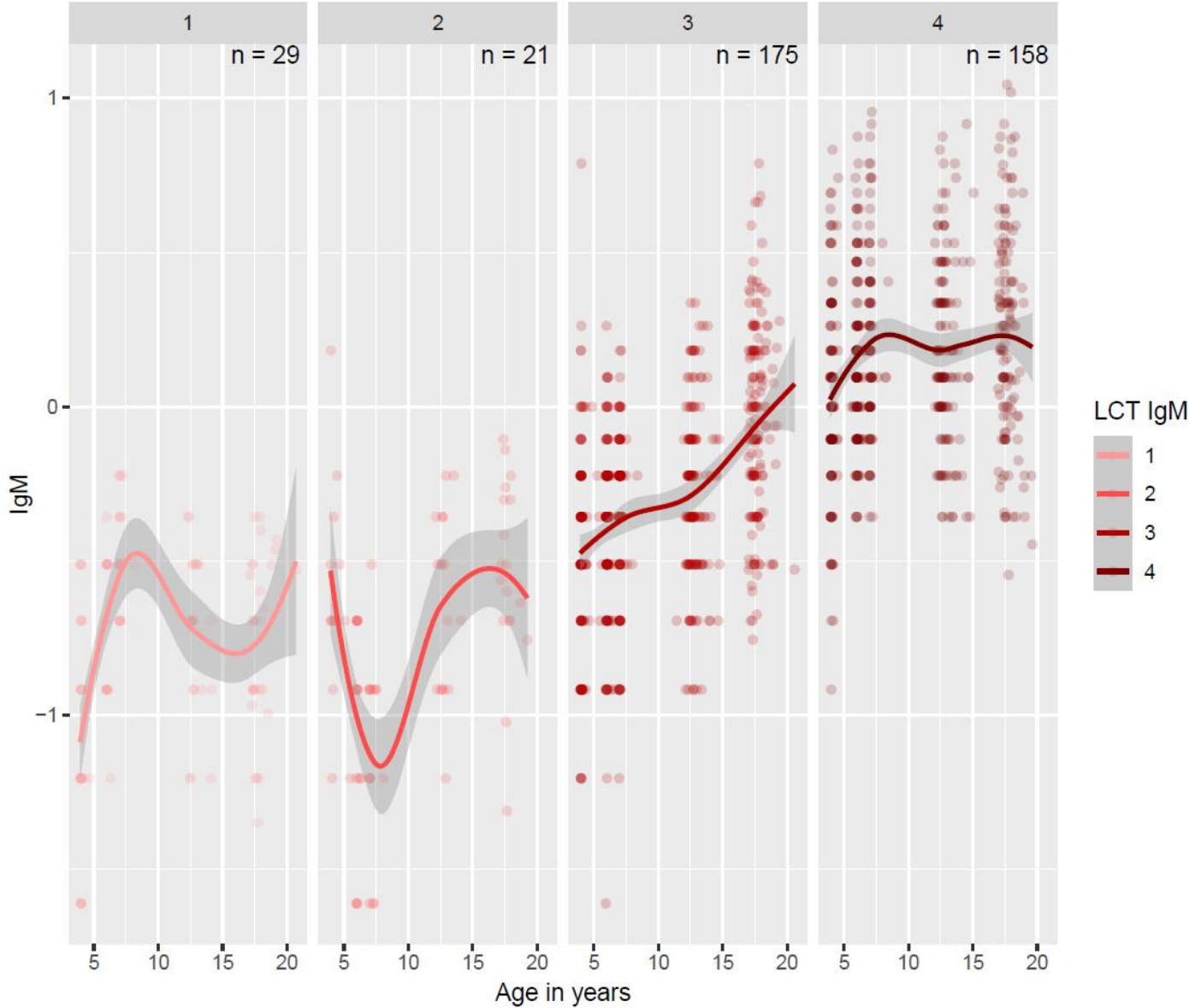


Figure S8. Latent class trajectories for total-IgM during childhood and adolescence.

Figure S9: Latent class trajectories for total-IgE during childhood and adolescence

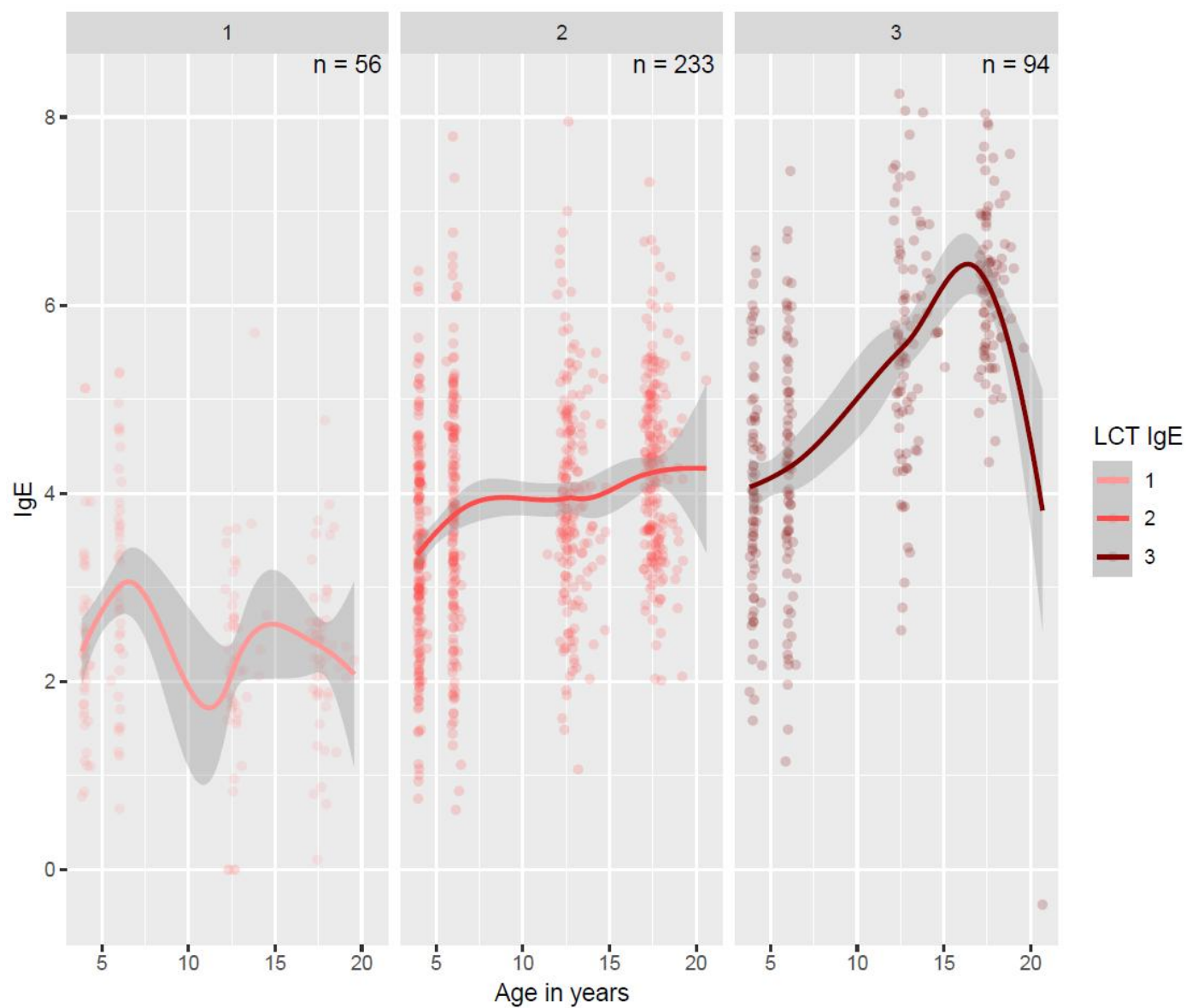


Figure S9. Latent class trajectories for total-IgE during childhood and adolescence.

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