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Kurt Taylor

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Bristol Medical School

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Abstract

Congenital heart disease (CHD) refers to the collection of diseases involving a problem with the structure of the heart present at birth. CHDs are a significant cause of infant morbidity and mortality. The causes of CHD are largely unknown. Observational epidemiological studies have reported links between a wide range of maternal lifestyle factors during pregnancy and CHD in the offspring. However, the causal relevance of these is unclear. This PhD thesis explores relationships between maternal pregnancy exposures and offspring CHD by employing a range of epidemiological techniques using multiple independent data sources.

Chapter 1 introduces CHDs, summarises the literature in relation to maternal pregnancy exposures and offspring CHD, puts forward the case for using pregnancy metabolomics and outlines epidemiological methods that could help improve causal inference. **Chapter 2** describes cases of congenital anomalies and CHD in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. In **Chapter 3**, I used parental negative exposure control analyses to explore the effects of maternal pre-pregnancy body mass index (BMI), and pregnancy smoking and alcohol on offspring CHD. I found that maternal pregnancy smoking may increase offspring CHD risk via intrauterine mechanisms but did not find evidence to suggest maternal overweight or obesity increase risk. Results for alcohol were inconclusive. In **Chapter 4**, I used Mendelian randomisation (MR) to explore the effects of the same exposures studied in **Chapter 3**. I found no robust evidence of an effect for maternal BMI or smoking on offspring CHD using MR. Using a genetic risk score of drinks per week, there was some evidence of a potential causal effect for maternal alcohol intake on offspring CHD. In **Chapter 5**, I examined the relationship of maternal gestational mass spectrometry-derived metabolites with offspring CHD using multivariable regression and MR analyses. I found evidence that amino acid metabolism during pregnancy, androgenic steroid metabolites, and levels of succinylcarnitine could be important contributing factors to CHD. In **Chapter 6**, I explored the relationship of maternal gestational nuclear magnetic resonance-derived metabolic traits with offspring CHD. I replicated the findings for amino acids seen in **Chapter 5** and found evidence of potential effects for some fatty acid and very low-density lipoprotein traits, albumin, and citrate. **Chapter 7** provides an overview of the primary findings for analyses included in each chapter along with strengths and limitations. It then considers the public health and clinical implications of my findings and provides recommendations for future research.

Preface

Research outputs related to this thesis

This PhD dissertation integrates publications as individual chapters. It has been formatted following guidance on the integration of publications as chapters within a dissertation as stipulated by the Academic Quality and Policy Office. All researched materials included in these publications derive from original research undertaken during my PhD study period. Chapters 2-6 are "publication chapters". Chapters 2 and 3 have been published in peer-reviewed journals and Chapters 4 and 5 are available as pre-prints and have been submitted for publication in a peer-reviewed journal. Chapter 6 has been formatted as a publication and will be available as a pre-print and submitted to a peer-reviewed journal at a later date. My contributions to these publication chapters are listed below. For simplicity, throughout this thesis, I refer to the work as "mine" instead of changing between using "I" and "we" when referring to the work and what was done. I acknowledge that this work would not have been possible without all my co-authors (listed below) and collaborators.

Chapter 2

Taylor K, Thomas R, Mumme M, Golding J, Boyd A, Northstone K, Caputo M & Lawlor DA. Ascertaining and classifying cases of congenital anomalies in the ALSPAC birth cohort. *Wellcome Open Research* (2020). https://doi.org/10.12688/wellcomeopenres.16339.2.

Responsibilities: I co-conceived this study, performed all analyses and data management, and prepared all manuscript versions. Co-authors contributed writing in sections where they described their data contributions in detail (Thomas R, Mumme M, Boyd A, Golding J).

Chapter 3

Taylor K, Elhakeem A, Thorbjørnsrud Nader JL, Yang TC, Isaevska E, Richiardi L, Vrijkotte T, Pinot de Moira A, Murray DM, Finn D, Mason D, Wright J, Oddie S, Roeleveld N, Harris JR, Nybo Andersen A, Caputo M & Lawlor DA. Effect of Maternal Prepregnancy/Early-Pregnancy Body Mass Index and Pregnancy Smoking and Alcohol on Congenital Heart Diseases: A Parental Negative Control Study. *The Journal of the American Heart Association* (2021). https://doi.org/10.1161/JAHA.120.020051.

Responsibilities: I co-conceived this study, performed all analyses (or prepared analysis code for collaborators to perform analyses), and prepared all manuscript versions.

Chapter 4

Taylor K, Wootton R, Yang Q, Oddie S, Wright J, Yang TC, Magnus M, Andreassen OA, Borges MC, Caputo M & Lawlor DA. The effect of maternal BMI, smoking and alcohol on congenital heart diseases: a Mendelian randomization study. *medRxiv* (2022). https://doi.org/10.1101/2022.01.27.22269962.
Responsibilities: I co-conceived this study, performed all analyses, and prepared all manuscript versions.

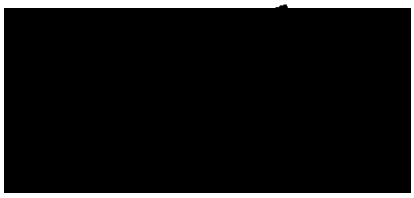
Chapter 5

Taylor K, McBride N, Zhao J, Oddie S, Azad R, Wright J, Andreassen OA, Stewart ID, Langenberg C, Magnus M, Borges MC, Caputo M & Lawlor DA. The relationship of maternal gestational mass spectrometry-derived metabolites with offspring congenital heart disease: results from multivariable and Mendelian randomization analyses. *medRxiv* (2022). https://doi.org/10.1101/2022.02.04.22270425.
Responsibilities: I co-conceived this study, performed all analyses, and prepared all manuscript versions.

Chapter 6

To be submitted for publication at a later date.

We declare that the above articles/chapters were written by the author of this thesis, Kurt Taylor, with support from supervisors.



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Professor Deborah Lawlor

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Professor Massimo Caputo

Research outputs and activities completed during my studentship that are not included in this thesis

1. **Taylor K,** Davey Smith G, Relton CL, Gaunt TR, Richardson TG. Prioritizing putative influential genes in cardiovascular disease susceptibility by applying tissue-specific Mendelian randomisation. *Genome Medicine* (2019). https://doi.org/10.1186/s13073-019-0613-2.
2. **Taylor, K**, L Santos Ferreira D, West J, Yang TC, Caputo M & Lawlor DA. Differences in Pregnancy Metabolic Profiles and Their Determinants between White European and South Asian Women: Findings from the Born in Bradford Cohort. *Metabolites* (2019). https://doi.org/10.3390/metabo9090190.
3. Richardson TG, Mykkänen J, Pahkala K, Ala-Korpela M, Bell JA, **Taylor K**, Viikari J, Lehtimaki T, Raitakari O & Davey Smith G. Evaluating the direct effects of childhood adiposity on adult systemic metabolism: a multivariable Mendelian randomisation analysis. *International Journal of Epidemiology* (2021). https://doi.org/10.1093/ije/dyab051.
4. McBride N, Yousefi P, Sovio U, **Taylor K**, Vafai Y, Yang TC, Hou B, Suderman M, Relton C, Smith GCS & Lawlor DA. Do Mass Spectrometry-Derived Metabolomics Improve the Prediction of Pregnancy-Related Disorders? Findings from a UK Birth Cohort with Independent Validation. *Metabolites* (2021). https://doi.org/10.3390/metabo11080530.
5. Corbin LJ, White SJ, Taylor A, Williams CM, **Taylor K**, van den Bosch MT, et al. Epigenetic Regulation of F2RL3 Associates with Myocardial Infarction and Platelet Function. *Circulation Research.* 2022. https://doi.org/10.1161/CIRCRESAHA.121.318836.
6. Hughes DA, **Taylor K**, McBride N, Lee MA, Mason D, Lawlor DA, Timpson NJ & Corbin LC. metaboprep: an R package for pre-analysis data description and processing. *Bioinformatics* (2022). https://doi.org/10.1093/bioinformatics/btac059.
7. Supervision: Qui-Yi Lim – final year dental student at the University of Bristol. My role involved co-supervising Qui-Yi with Dr Thomas Dudding in a final year research project. Qui-Yi has now submitted the work for publication in the journal: *Community Dentistry and Oral Epidemiology.* Preprint also available: https://doi.org/10.1101/2021.10.27.21265567.
8. Grant: British Heart Foundation Accelerator Award Pump Priming Grant (£50,000), March 2020 - Cardiovascular risk factors other than the heart defect in children with congenital heart disease. Cocomello L, **Taylor K**, Cornish R, Skeffington K, Lawlor DA & Caputo M.

9. Prize: University of Bristol Open Research Prize for Increasing Quality (£100 prize), March 2021. https://www.ukrn.org/2021/04/16/university-of-bristol-open-research-prize-winners-announced/.

Acknowledgements

Firstly, I'd like to thank my supervisors – Professor Deborah Lawlor and Professor Massimo Caputo for their guidance and overwhelming support throughout my studentship. Deborah, your advice and constructive feedback has helped me grow as a researcher and I look forward to continuing working with and learning from you in the future. Massimo, your clinical insight has been invaluable - you are a true hero with what you do on a daily basis, so thank you for giving up some of your time for me.

I could not have completed this PhD without colleagues within the MRC Integrative Epidemiology Unit and various external collaborators. There are too many to name here, but special mentions of course go to Dr Nancy McBride and Miss Charlie Hatcher – you've been there since day one and we've enjoyed our journeys together; thank you, my OF28 friends! Dr Tom Richardson, you were awesome during my first-year mini project, and you've always been there for a chat when I've needed help and advice or to just talk about tennis and football – thank you, sir. I would also like to acknowledge Professor Abi Fraser and Dr Neil Davies for our annual discussions about my work and for the advice they have given me.

I would like to thank my fiancé, Melissa for supporting me in whatever I choose to do. You packed your bags and moved to Bristol with me, and I will forever be grateful for that. Thank you, mum and dad - you've taught me so much and always shown interest in what I do, whether it was painting pipes on a building site or pursuing a PhD!

Finally, I would like to acknowledge the British Heart Foundation for generously funding my PhD studentship.

Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed:  Date: 07/02/2022

COVID-19 Statement

There were some delays arising from the pandemic such as getting used to homeworking and performing some analyses remotely, but these were largely mitigated by being granted a 6-month extension by my funders, The British Heart Foundation, for which I am grateful. The key disruption in relation to the work presented in this thesis was due to delays in data generation. I have not been able to access the pregnancy metabolomics data in the Norwegian Mother Father and Child Cohort in time, which would have been used for external replication analyses in Chapter 6. As a result of this, without replication in an independent large sample, the results in Chapter 6 are not as robust as they could have been. I still intend on performing these analyses at a later date and including them in the eventual publication.

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List of Tables

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List of Figures

N.B. any figure label beginning with "S" (e.g., Figure S1.1) can be found in Appendices.

List of abbreviations

- ABCD: The Amsterdam Born Children and Their Development Study
- ACHD: adult congenital heart disease
- ALL IN: allergy and infection study
- ALSPAC: Avon Longitudinal Study of Parents and Children
- ApoB: apolipoprotein B
- AVSD: atrioventricular septal defect
- BASELINE: Cork Scope Baseline Study
- BiB: Born in Bradford
- BMI: body mass index
- BNSSG: Bristol, North Somerset and South Gloucestershire
- BRI: Bradford royal infirmary
- CA: congenital anomaly
- CCG: clinical commissioning group
- CHD: congenital heart disease
- CI: confidence interval
- COMETS: consortium of metabolomics studies
- CRA: community research administrators
- CT: clinical term
- DAG: directed acyclic graph
- DNBC: Danish National Birth Cohort
- DNPR: Danish national patient register
- EDTA: ethylenediaminetetraacetic acid
- EUROCAT: European surveillance of congenital anomalies.
- FASP: fetal Anomaly Screening Programme
- GlycA: glycoprotein acetyls
- GP: general practice
- GRS: genetic risk score
- GWAS: genome-wide association study
- HDL: high-density lipoprotein
- HDP: hypertensive disorders of pregnancy
- HES: hospital episode statistics
- HLH: hypoplastic left heart
- HMDB: human metabolome database
- HRA: health research authority
- HRH: hypoplastic right heart
- HWE: Hardy-Weinberg equilibrium
- ICD-10: international classification of disease codes – Version 10

- IMD: index of multiple deprivation
- IV: instrumental variable
- LD: linkage disequilibrium
- LDL: low-density lipoprotein
- MAF: minor allele frequency
- MBRN: medical birth registry of Norway
- MoBa: Norwegian Mother, Father and Child Cohort Study
- MR: Mendelian randomisation
- MS: mass spectrometry
- NCARDRS: national congenital anomaly and rare diseases registration service
- NHS: national Health Service
- NINFEA: Nascita e Infanzia: gli Effetti dell'Ambiente (Birth and Childhood: Effects of the Environment)
- NIPE: new-born and infant physical examination
- NMR: nuclear magnetic resonance
- OGTT: oral glucose tolerance test
- ONS: office for national statistics
- OR: odds ratio
- PC: principal component
- PCM: partially characterised molecule
- PDA: patent ductus arteriosus
- PDS: patient demographic system
- POPs: pregnancy outcome prediction study
- QC: quality control
- SD: standard deviation
- SEP: socioeconomic position
- SeRP: secure eResearch platform
- SNP: single nucleotide polymorphism
- SSRI: selective serotonin reuptake inhibitors
- STROBE-MR: strengthening the reporting of observational studies in epidemiology using Mendelian randomisation
- SWCAR: southwest congenital anomaly register
- TGA: transposition of the great arteries
- ToF: Tetralogy of Fallot
- UHBT: University hospital's Bristol Trust
- UKBB: UKBiobank
- UPBEAT: UK pregnancies better eating and activity trial
- VLDL: very low-density lipoprotein
- VSD: ventricular septal defect
- WHO: world health organisation

Chapter 1. Introduction

This chapter introduces the topic, provides an overview of the current literature, and places my aims and objectives in the context of current gaps in the literature. It provides a justification for the need of novel causal epidemiological methods to address my aims and a description of each of the methods I have used, including their assumptions. The use of metabolomics in the context of epidemiological studies is introduced as a potential tool to uncover biological pathways that may relate to congenital heart disease (CHD). In the final section, I summarise the chapter, present my thesis aims and objectives and provide a description of the work undertaken and how it is set out within the remaining chapters.

1.1. Congenital anomalies

Congenital anomalies (CAs) occur in utero and can be identified prenatally, at birth or during later life. CAs can be defined as structural (e.g., missing limb) or functional (e.g., metabolic disorders). The exact cause of most CAs is unknown; however, causes can include single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens and micronutrient deficiencies during pregnancy [1]. Consequences vary depending on the type and severity of the anomaly, but many children and their families are burdened with lifelong complications. Worldwide, at least 3.3 million children under the age of 5 die from CAs each year [2]. In Europe and the UK, CAs affect approximately 2-3% of births [3]. CAs are a major cause of fetal death, infant morbidity, and long-term disability. Undoubtedly, CAs represent a significant public health issue.

CAs can occur in isolation or in combination with other anomalies. Around 76% of cases with a CA occur in isolation. The remaining cases have multiple structural anomalies, with around 70% of those cases with multiple anomalies being attributed to a chromosomal syndrome or genetic defect [4]. Generally, CAs are mostly classified by a combination of the organ system affected and whether they occur in isolation, affect multiple systems and/or have chromosomal/genetic cause. However, it is important to note that this can vary depending on the nature and location of the research. **Table 1.1** exhibits the sub-classes of CAs by organ type along with their incidence obtained from the European surveillance of congenital anomalies (EUROCAT).

Table 1.1. Incidence of different types of congenital anomalies in Europe between 1980-2016.

Congenital anomaly	Incidence per 10,000 births (95% CI) [a]	Live-birth incidence per 10,000 births (95% CI) [b]
Nervous system	25.5 (25.2 – 25.7)	12.7 (12.6 – 12.9)
Eye	4.9 (4.8 – 5.0)	4.5 (4.4 – 4.6)
Ear, face, and neck	3.9 (3.8 – 4.0)	3.3 (3.2 – 3.4)
Congenital heart defects	74.6 (74.1 – 75.0)	67.0 (66.6 – 67.4)
Respiratory	3.5 (3.4 – 3.6)	2.7 (2.7 – 2.8)
Oro-facial clefts	15.2 (15.0 – 15.4)	13.7 (13.5 – 13.9)
Digestive system	19.8 (19.6 – 20.0)	17.0 (16.8 – 17.2)
Abdominal wall defects	6.0 (5.8 – 6.1)	3.5 (3.4 – 3.6)
Urinary	33.6 (33.3 – 33.9)	28.4 (28.2 – 28.7)
Genital	20.4 (20.2 – 20.6)	19.5 (19.3 – 19.8)
Limb	47.6 (47.3 – 48.0)	42.8 (42.5 – 43.1)

[a] Calculated as the number with each congenital anomaly divided by those without the anomaly in the European population during the specified years including all known pregnancies irrespective of whether there was a live birth or not.
[b] Live birth incident rates are calculated in the same way as above but only include livebirths.
Data obtained from: http://www.eurocat-network.eu/AccessPrevalenceData/PrevalenceTables [5].

1.2. Congenital heart disease

1.2.1. Background: Prevalence, definition and public health

CHDs are the most prevalent type of CA affecting approximately 6-8 per 1000 live births and 10% of still births and remain the leading cause of death from CAs [6]. According to Ottaviani and Buja's definition, CHD, "*consists of a wide variety of anomalies and malformations involving the heart and great vessels that develop in utero, are present at birth, and come to clinical attention in infancy, adolescence, or adulthood*" [7]. Many CHD patients present with sequela from surgical intervention resulting in health problems persisting throughout the life course into adulthood. Around 20% of CHDs can be attributed to known chromosomal anomalies, gene disorders or teratogens. The cause of the remaining 80% are unknown, however, it is suggested that these CHDs are multifactorial in nature caused by a combination of genetic and non-genetic modifiable risk factors [8].

Advancements in paediatric cardiology and surgical techniques have improved short- and long-term outcomes in CHD patients, which is evident in the 35% decrease in annual deaths attributed to CHDs from 1990 to 2017. Despite this, the incidence rates of CHDs have changed little over the same period [9] suggesting that more work is needed on identifying causes and implementing preventive interventions to

reduce disease burden. Due to the complex nature of CHDs, research from multiple scientific disciplines including embryology, basic science, population health sciences and others is warranted.

Babies born with CHD require highly specialised health care, often including multiple surgical procedures. Financial analyses from 2013/2014 estimate the cost of CHD services to National Health Service (NHS) England as £175m per annum with this estimated to rise to between £186m-£207m by 2025 [10]. The substantial improvements in short- and long-term outcomes in CHD patients has meant that the adult congenital heart disease (ACHD) population is growing, with more than 95% of CHD patients reaching adult life [11]. Studies have shown that CHD patients could be at greater risk of later life health outcomes such as cardiovascular disease [12] and cancer [13] in comparison with the general population. CHD patients are also more likely to have neurodevelopmental deficits and are less likely to complete higher education [14]. It is also important to consider the implications that a CHD diagnosis could have on close family members. For example, many parents of a child with CHD report significant stress and negative impact of the medical condition on the family [15]. In summary, the major burden of CHDs to patients, their family, health care provision and hence society in the UK and globally, highlight the need to identify causes in order to prevent them.

1.2.2. Cardiac development

Understanding cardiac morphogenesis and exploring important molecular pathways is a key area of research in the paediatric cardiology field. The heart is the first organ to form, as it is required to support the rapidly developing embryo. The heart begins to beat from 2 weeks gestation and by day 50 (~8 weeks) the mature heart has formed [16]. There are several prominent steps that occur throughout heart formation. These steps are briefly described below, however, for a detailed account of human heart development the interested reader is directed to embryology textbooks or reviews on this topic [17].

Between weeks 3 and 4, the heart develops into a primitive tube (**Figure 1.1A**). The tubular heart continues to elongate, eventually forming the first sign of the heart's chambers – the truncus arteriosus, the bulbus cordis, the primitive ventricle and the primitive atrium (**Figure 1.1B**) [18]. By day 28, the heart tube has formed a U-shaped loop resulting in the placement of the atria above and behind the truncus arteriosus, bulbus cordis and ventricle (**Figure 1.1C & 1.1D**). Septation, the separation of the heart into the four recognisable chambers occurs between weeks 4 and 6 gestation. Atrial septation begins when a ridge of tissue, called the septum primum grows from the base towards the apex. The foramen ovale is formed, a valve (which usually closes at birth) that allows blood to pass from the right to the left atrium.

Simultaneously, formation of the interventricular septum ensues septation of the ventricles takes place resulting in a fetal heart with 4 distinct chambers (**Figure 1.1E**). At week 5, the aorta and pulmonary artery form as a result of the division of the bulbus cordis and truncus arteriosus. In utero, a small vessel called the ductus arteriosus connects the pulmonary artery to the aorta to ensure blood is directed to the placenta for oxygenation. The ductus arteriosus usually closes soon after birth, triggered by breathing whereby blood is then directed to the lungs for oxygenation. One-way valves are formed to connect the hearts chambers. The tricuspid and mitral (atrioventricular) valves form to drive blood from the atria to the ventricles. Then, the pulmonic and aortic (semilunar) valves form, driving blood out of the heart and preventing backflow. More recently, seminal papers published in the early 21st century described the phenomena (known as the second heart field) that the heart tube elongates by addition of cells to the arterial pole. The heart tube elongates by addition of myocardium from progenitor cells lying outside the heart itself [19–21].

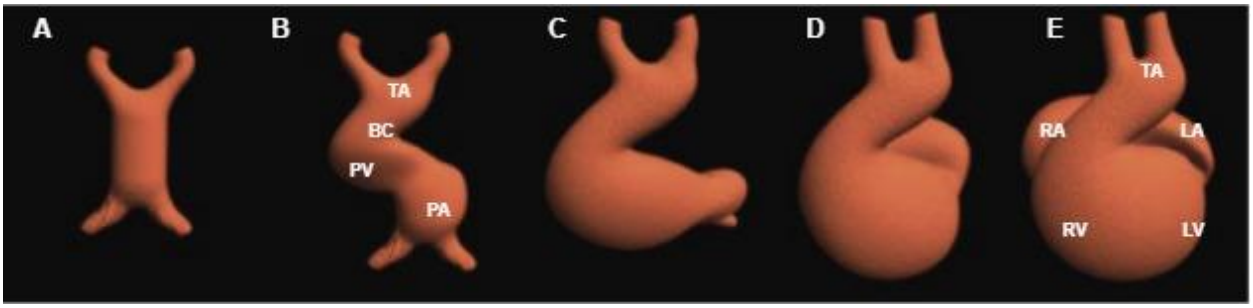


Figure 1.1. An illustration of normal cardiac looping.
A. The straight heart tube at approximately 22 days. **B.** at 23-24 days, the tube loops to the right folding into an S-shape. **C,D,E.** Looping eventually places the atria above and behind the primitive ventricles. Abbreviations: TA: Truncus Arteriosus, BC: Bulbus cordis, PV: Primitive ventricle, PA: Primitive atrium, RV: Right ventricle, LV: Left ventricle, RA: Right atrium, LA: Left atrium. Figure obtained and adapted with permission from the author [16].

1.2.3. Classification

CHDs can occur in isolation, with other structural anomalies or with chromosomal/genetic syndromes. CHD can be further categorised into subtypes (**Table 1.2**). There are multiple classification systems that can be used to define CHD subtypes. Arguably the most commonly used and one that is preferred by epidemiologists is the World Health Organisation's (WHO) International Classification of Disease (ICD) codes [22]. Congenital malformations of the circulatory system correspond to ICD version 10 (ICD-10) codes Q20-Q28. The ICD-10 subcategories of CHD correspond to anomalies of: the cardiac

chambers and connections (Q20), the cardiac septa (Q21), the pulmonary and tricuspid valves (Q22), the aortic and mitral valves (Q23), the heart (Q24), the great arteries (Q25) and the great veins (Q26), the peripheral vascular system (Q27) and the circulatory system (Q28). In line with the aforementioned definition of CHD described by Buja, CAs of the peripheral vascular and circulatory system (ICD-10: Q27-28) would generally not be classed as CHD. Furthermore, minor CHDs which have little clinical impact (e.g., isolated patent ductus arteriosus (PDA) or peripheral pulmonary artery stenosis in pre-term infants) are not generally classed as CHD. Indeed, EUROCAT define CHDs using ICD-10 codes Q20-Q26.

Other coding systems include The International Society for Nomenclature of Paediatric and Congenital Heart Disease and the European paediatric cardiac coding. These also adopt a similar method in which CHDs are broken down into subtypes. However, they tend to be more geared towards clinicians with subtypes being further categorised according to the surgeries used to treat the CHD subtype, the exact location of the CHD subtype and the severity of the CHD subtype.

Classification systems have been proposed for use in epidemiological studies investigating CHD aetiology [23]. Botto et al acknowledged that CHDs are anatomically, clinically, epidemiologically, and developmentally heterogeneous. However, they go on to say that basing classification and analysis on phenotype alone can lead to too many groups with too few cases for meaningful risk factor estimation. They describe an approach which classifies CHDs into cardiac phenotypes based on a hierarchical system. This approach has since been used in many large-scale epidemiological studies that aimed to explore CHD aetiology [24,25].

Table 1.2. Descriptions of CHD subtypes.

CHD subtype	Description	ICD 10 code[a]
Common arterial trunk	The two large arteries that leave the heart, the aorta and the pulmonary artery, are combined in one large vessel. Usually occurs with VSD [26].	Q20.0
Double outlet right ventricle (Taussig-bing syndrome)	"Double outlet" refers to the transposition of the aorta to the right ventricle. Patients also have subpulmonic VSD.	Q20.1
Transposition of the great arteries (TGA)	Pulmonary artery and aorta are switched in position.	Q20.3
Double inlet ventricle	Multiple abnormalities including left and right atrium drain into one ventricle, the other ventricle is abnormally small. Patients also have VSD.	Q20.4
Ventricular septal defect (VSD)	A hole in the ventricular septum, which separates the hearts lower chambers.	Q21.0

Atrial septal defect	A hole in the wall separating the two upper chambers of the heart.	Q21.1
Atrioventricular septal defect (AVSD)	Holes between the heart's chambers and an impairment of valve function.	Q21.2
Tetralogy of Fallot	Involves four malformations which present together: VSD, pulmonary stenosis, overriding aorta and right ventricular hypertrophy.	Q21.3
Pulmonary valve atresia	Pulmonary valve does not form properly.	Q22.0
Pulmonary valve stenosis	An obstruction of blood flow through the pulmonary valve resulting in a restriction of blood to the lungs.	Q22.1
Tricuspid atresia	Tricuspid valve does not form or does not form properly resulting in an underdeveloped ventricle and lack of oxygen to the body.	Q22.4
Ebstein's anomaly	Insufficiency of the tricuspid valve causing backflow and a less efficient heart.	Q22.5
Hypoplastic right heart (HRH)	HRH syndrome refers to the underdevelopment of the right-side structures of the heart, notably a small or non-existent right ventricle.	Q22.6
Aortic stenosis	Valve disorder that narrows or obstructs the aortic valve opening.	Q23.0
Mitral stenosis	Thickened leaflets of the mitral valve.	Q23.2
Hypoplastic left heart (HLH)	HLH syndrome refers to the underdevelopment of the left-side structures of the heart, notably a small or non-existent left ventricle.	Q23.4
Patent ductus arteriosus (PDA)	An unclosed hole in the aorta allowing a portion of oxygenated blood flow back to the lungs.	Q25.0
Coarctation of the aorta	Narrowing of the aorta.	Q25.1

[a] International Classification of Disease codes (version 10).
Abbreviations: CHD, congenital heart disease; VSD, ventricular septal defect; TGA, transposition of the great arteries; AVSD, atrioventricular septal defect; HRH, hypoplastic right heart; HLH, hypoplastic left heart; PDA, patent ductus arteriosus.

1.2.3.1. Classification of CHDs in this thesis

In this thesis, I have used any CHD as the main outcome. I defined any CHD using ICD-10 codes where available according to EUROCAT guidelines. However, I have also included CHD cases in cohorts that did not have ICD-coded data available and have used their own definition. Although it is discussed above that CHD subtypes are heterogenous in several ways, I believe there is value for prospective parents, clinicians and policy makers in knowing effects on any CHD. Where possible, I have also categorised cases into severe CHD (heterotaxia, conotruncal defect, atrioventricular septal defect, anomalous pulmonary venous return, left ventricle outflow tract obstruction, right ventricle outflow tract obstruction, other complex defects) and the remainder as non-severe CHD (PDA [in full term infants], valvular pulmonary stenosis, ventricular septal defect [VSD], atrial septum defects [ASD], unspecified

septal defects, isolated valve defects, other specified heart defects, unspecified heart defects). These definitions are based on the classification system described above (which has previously been adopted in recent large-scale epidemiological studies [24,25]) combined with clinical expertise and judgement from a clinician. The classification of CHDs used in this thesis where ICD-10 codes are available is summarised below in **Table 1.3**.

Table 1.3. Classification system used to define CHD in this thesis.

Category	CHDs included	ICD-10 codes
All	Any CHD as defined by EUROCAT[a] PDA with gestational age (GA) < 37 weeks not considered a CHD case. Peripheral pulmonary artery stenosis with GA < 37weeks not considered as a CHD case.	Q20-Q25, Q260, Q262-Q269[b]
Severe	Heterotaxia, Conotruncal defect, Atrioventricular septal defect, Anomalous pulmonary venous return, Left ventricle outflow tract obstruction, Right ventricle outflow tract obstruction, Other complex defects	Q240, Q241, Q206, Q200, Q251, Q252, Q253, Q254, Q203, Q213, Q201, Q214, Q212, Q26, Q262, Q264, Q268, Q269, Q234, Q251, Q230, Q231, Q221, Q224, Q225, Q255, Q204
Non-severe	PDA (in full term infants), valvular pulmonary stenosis, VSD, ASD, unspecified septal defects, isolated valve defects, other specified heart defects, unspecified heart defects	Non-severe cases that are All=1 and Severe=0.

[a] Definitions taken from here: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/EUROCAT-Guide-1.4-Section-3.3.pdf ; [b] Q250 and Q256 not a case if isolated and GA<37weeks.
Abbreviations: CHD, congenital heart disease; ICD-10, international classification of disease codes (version 10); EUROCAT, European surveillance of congenital anomalies; GA, gestational age; PDA, patent ductus arteriosus; VSD, ventricular septal defect; ASD, atrial septal defect.

1.2.4. Diagnosis and treatment

1.2.4.1. Prenatal diagnosis

The Fetal Anomaly Screening Programme (FASP) was introduced in 2001. A fetal anomaly ultrasound scan between 18 to 20 completed weeks of gestation (hereafter 'weeks') is offered to all pregnant women in the UK. The screening offered will depend on where the pregnant woman lives in the UK. The fetal anomaly scan screens for 11 conditions which includes serious cardiac anomalies: transposition of the great arteries (TGA), atrioventricular septal defects (AVSD), Tetralogy of Fallot (ToF), hypoplastic left heart syndrome (HLHS). Suspected cases of CHD are referred to a fetal cardiology service

where fetal echocardiography is performed to confirm the diagnosis. The pathway for fetal diagnosis of CHD is illustrated in **Figure 1.2**. Pregnancies deemed "high-risk" will be referred to fetal cardiology services regardless of the result of the fetal anomaly scan. Pregnancies at increased risk for fetal CHD include a range of maternal indications (e.g., maternal CHD or maternal disorders such as diabetes), familial indications (e.g., paternal CHD or previous child with CHD) and fetal indications (e.g., fetal arrhythmias). The full criteria for pregnancies that are deemed high-risk is described in the British Congenital Cardiac Association fetal cardiology standards [27]. Obtaining a current and accurate estimate of prenatal diagnosis numbers is difficult due to the ever-improving rates as a result of technological and medical advance [28]. These rates can substantially differ depending on geographic region, technology available, the sonographer and the type of CHD. According to the National Institute for Cardiovascular Outcomes Research and (then) Public Health England, almost half of CHDs receive a prenatal diagnosis in the UK using data from 2016 [29,30]. The importance of having a prenatal diagnosis of CHD is underlined in a meta-analysis conducted by Holland et al which showed that prenatal diagnosis of critical CHD significantly improved preoperative neonatal survival when comparing cases with similar forms of CHD [31].

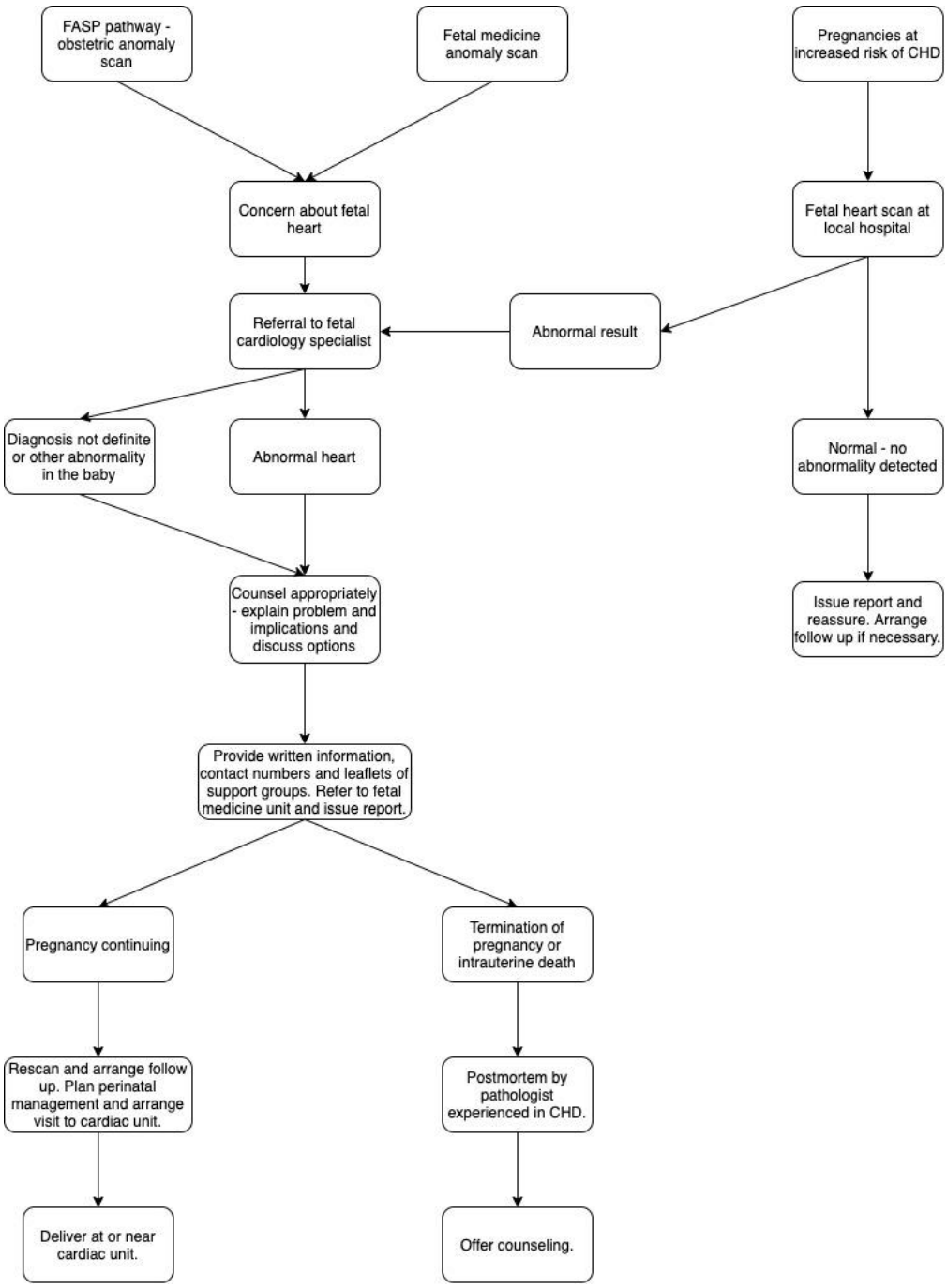


Figure 1.2. Pathway for fetal diagnosis of congenital heart disease.
Adapted from the British Congenital Cardiac Association's fetal cardiology standards: http://www.bcs.com/documents/Fetal_Cardiology_Standards_2012_final_version.pdf [27]. Abbreviations: FASP, fetal anomaly screening programme; CHD, congenital heart disease.

1.2.4.2. Postnatal diagnosis

A new-born and infant physical examination (NIPE) should be conducted within 72 hours of birth and then again at 6 to 8 weeks of age [32]. Within the NIPE, the examination of the heart is broken down into 3 broad categories to give the physician the best chance of identifying presenting abnormalities: observation, palpation, and auscultation. Observation covers identifying any obvious signs that indicate cardiac stress such as cyanosis (bluish discoloration of the skin), palpation covers femoral and brachial pulses as well as other key areas and auscultation involves listening to the heart for quality of sound and presence of murmurs. Although most postnatally diagnosed CHD cases are diagnosed immediately or soon after birth, some cases can remain undiagnosed all the way through to adulthood as shown by others and research I conducted for this thesis (Chapter 2) [33–35]. Common subtypes where a delayed diagnosis might occur are bicuspid aortic valve, septal defects and coarctation of the aorta [36].

1.2.4.3. Treatment including surgical intervention

Treatment and interventions vary considerably depending on CHD subtype. Those with mild heart defects may not require any treatment at all. Although, regular check-ups and careful monitoring will likely occur throughout life. More severe CHDs often require surgical intervention or catheterisation of the heart. Medications are often prescribed pre- or post-surgery to stabilise the condition to get the best possible outcome. Common medications include diuretics, anti-platelet therapies such as warfarin and angio-tensin-converting-enzyme inhibitors [37]. The following CHD subtypes typically require open heart surgery to survive (N.B. this is not an exhaustive list): single ventricle, tricuspid atresia/stenosis, HLHS, ToF, transposition of the great vessels, common arterial truncus, atrioventricular septal defect, pulmonary valve atresia with ventricular septal defect (VSD) and total anomalous pulmonary venous connections. Individuals with aortic valve atresia/stenosis and coarctation of aorta generally require either surgical or catheter intervention, with the timing dependent on severity.

1.3. CHDs: What role does the maternal pregnancy environment play? A literature review.

CHDs have been introduced and described in the preceding sections and now, a critical review of the scientific literature relating to the aims of this thesis is presented. An ever-expanding body of epidemiological literature has explored associations of potential maternal (intrauterine) risk factors during pregnancy with CHDs in the offspring [38]. Whether these risk factors are causal remains unknown. Identifying modifiable causal risk factors for CHDs is necessary to improve aetiological understanding and to develop preventive interventions that target the most promising causal and modifiable risk factors. The purpose of this review is to summarise the evidence on non-inherited intrauterine risk factors for offspring CHDs in the form of an umbrella review (i.e., a review of systematic reviews). This section aims to provide the reader with an overview of the current literature base including limitations and potential sources of bias.

1.3.1. Methods

To identify all previous systematic reviews of association of any intrauterine exposure with CHDs I conducted a systematic search of PubMed using the following terms: *((Systematic review OR meta-analysis OR meta analysis)) AND (congenital heart OR congenital heart disease OR congenital heart defect OR congenital cardiac OR tetralogy of Fallot OR pulmonary stenosis OR pulmonary valvar stenosis OR fontan circulation OR cavo-pulmonary connection OR univentricular heart OR conotruncal OR hypoplastic left heart OR single ventricle OR septal defect).* I specified that these words had to be contained in the title or abstract of the articles. I also searched the reference list of articles that were eventually included. The search was conducted on 13th April 2021 and identified 585 articles (**Figure 1.3**). I read all titles to decide whether they should be included. After excluding articles that were not a systematic review/meta-analysis or those not related to pregnancy characteristics and congenital outcomes, 101 abstracts were screened. Of these, 49 were not eligible for inclusion (see reasons in **Figure 1.3**) and 52 full text articles were screened for inclusion. I identified 39 systematic review/meta-analysis articles summarizing maternal pregnancy characteristics on CHDs that were eligible. There were multiple systematic reviews for several exposures (e.g., 4 meta-analyses published between 2014 and 2018 on maternal body mass index [BMI] and offspring CHDs). In these instances, I included the most recent article. However, I also checked each individual review for which main studies were included and the concordance of results. There were no

instances where the most recent systematic review was deemed to be not the most appropriate to include. After removing duplicates, there were 18 articles included. As the focus is specifically on CHDs, I restricted the search to systematic reviews of CHDs. However, when screening abstracts, any papers where the main focus was on all CAs but where it was likely that associations with CHDs were presented separately were kept for review of the full paper and included if CHD results were presented. I extracted data from included systematic reviews, including the inclusion criteria, the number of studies, total participants, and number of CHD cases, risk of bias assessment, whether results were pooled, the number of studies included in the pooled analyses, any sensitivity analyses and the numerical results. I did not formally assess the quality of included studies; however, I include comments and potential sources of bias to assist in the interpretation of the quality of evidence.

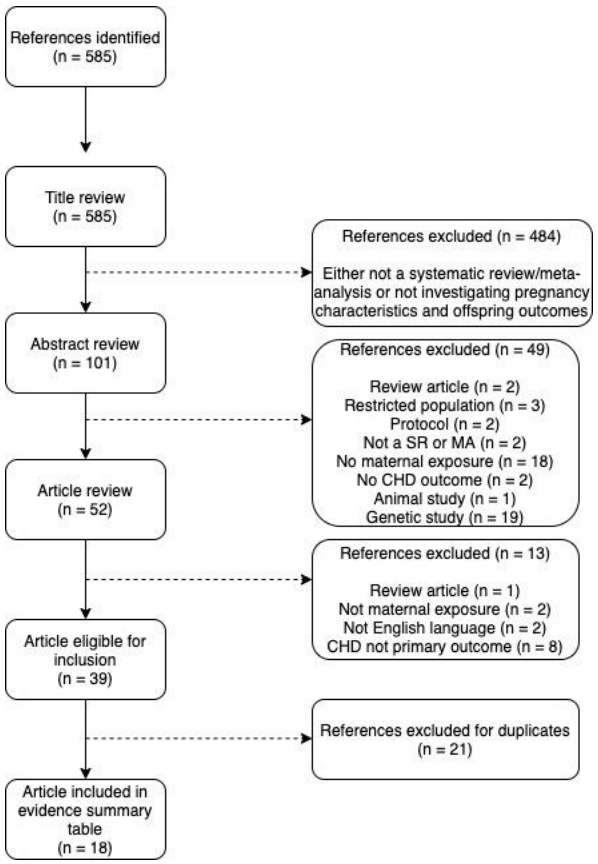


Figure 1.3. Flow chart of articles included in the synthesis of evidence.

1.3.2. Results

Table 1.4 summarises the evidence from 18 systematic reviews corresponding to different maternal exposures and CHDs.

Table 1.4. Summary of findings of associations between maternal risk factors and CHD in the offspring.

Exposure (reference)	N of studies and participants	Main findings	Author's risk of bias/study quality assessment	Comments and potential sources of bias
Air pollution (Hu et al 2020 [39])	26 studies included (10 cohort, 16 case-control). Up to 6,000 CHD cases depending on exposure or outcome subtype.	No results reported for any CHD. 37 separate meta-analyses presented for different types of air pollution and CHD subtypes. High carbon monoxide (CO) exposure associated with ToF (OR = 1.21 (1.04-1.41, I^2 = 0%), 8 studies, 688 CHD cases). A small increased risk was found for ASDs for each increment increase in particulate matter (OR = 1.04 (1.00, 1.09, I^2 = 43%).	Critical Appraisal Skills Programme checklist and Newcastle-Ottawa scale (NOS). Authors deemed the included studies to be of sufficient quality. Some of the limitations identified relate to potential risk of misclassification and non- representative samples.	Authors focused on 'statistically significant' findings. There were many analyses performed for different exposures and different CHD outcomes with no discussion on multiple testing. Confounders varied by study (high risk of residual confounding). 8 out of 26 studies adjusted for smoking. Exposure assessment also varied by study. Air pollution is notoriously difficult to measure accurately, therefore measurement error could possibly be influencing results [40].
Alcohol (Zhang et al 2020 [41])	45 studies included (3 cohort, 42 case-control). 34,638 CHD cases and 290,425 controls. The study also included paternal alcohol as an exposure. 55 studies were included in total, but 45 reported associations for maternal alcohol and CHDs.	Any alcohol between 3 months before the pregnancy and during the first trimester with any CHD: OR = 1.16 (1.05, 1.27, I^2 = 74%, 45 studies). Binge drinking: OR = 1.16 (1.02, 1.32, I^2 = 12%, 10 studies).	NOS – NOS results displayed in Supplementary Material. 44 out of 55 included studies had a score of at least 7 which is the cut-off used to deem a study of high methodologic quality. Authors do not comment on results of NOS.	31 out of the 45 studies did not adjust for confounders. Effect estimates were similar for any alcohol and binge drinking. The authors also report results for paternal alcohol (OR = 1.44 (1.19, 1.74, I^2 = 90%). The magnitude of the effect was stronger than that of maternal alcohol suggesting that results could be confounded. Carefully conducted parental negative control analyses could elaborate on this. The timing of the exposure (i.e., 3 months before pregnancy) does not solely reflect the critical period for fetal heart development.
Antidepressants (De Vries et al 2020 [42])	20 studies included (15 cohort, 5 case-control). 5,507,872 pregnancies.	Any anti-depressant during the first trimester of pregnancy and offspring CHD: OR = 1.28 (1.17, 1.41, I^2 = 49%). Serotonin norepinephrine reuptake inhibitors OR: 1.69 (95% CI 1.37–2.10, I^2 = 25%). Selective serotonin reuptake inhibitors 1.25 (95% CI 1.15–1.37, I^2 = 33%) tricyclic antidepressants OR: 1.02 (95% CI 0.82–1.25, I^2 = 0%) Analyses of individual SSRIs also produced positive associations.	Modified version of the NOS. Strict inclusion criteria: Cohort studies were excluded if any of the high- or medium- impact criteria were poor. If four of the high-impact criteria were ideal and at least two of the low-impact criteria were ideal for case-control studies, the study was included.	15 out of 20 studies had no data on confounder adjustment. Large majority of studies used linked electronic health records. Authors performed a rigorous bias assessment and excluded studies that were high risk of bias. Possibility of residual confounding and/or confounding by indication. Majority of studies contained live births only.

Body mass index (Liu et al 2019 [43])	19 studies included (6 cohort, 13 case-control) 2,416,546 participants including 57,172 CHD cases.	Maternal overweight versus normal weight with any CHD: RR = 1.08 (1.03, 1.13, I^2 = 54.5%, 18 studies). Maternal obesity versus normal weight: RR = 1.23 (1.17, 1.29, I^2 = 48.3%, 23 studies (23 studies due to duplicates from obesity severity categories)).	All included studies considered as high quality (≥ 7) based on the NOS.	No results reported for maternal underweight (two previous meta analyses published in 2018 reported null associations for maternal underweight [44,45]). 10 of the 19 studies did not provide information on confounder adjustment. There was inter-study heterogeneity, with smaller studies having weaker associations.
Diabetes (Chen et al 2019 [46])	52 studies included (16 cohort, 32 case-control). 16,929,835 participants including 259,917 CHD cases.	Any maternal diabetes and any CHD: OR = 2.71 (2.28, 3.23, I^2 = 98%, 52 studies). Pre-gestational diabetes and any CHD: OR = 3.18 (2.77, 3.65, I^2 = 79%, 31 studies). Gestational diabetes and any CHD: OR = 1.98 (1.66, 2.36, I^2 = 90%, 27 studies). Positive associations for most subtypes.	NOS – 46 out of 52 included studies (including 99.98% of the participants) had a score of at least 7.	Large number of studies and participants included. Associations for pre-gestational diabetes stronger than gestational diabetes which is biologically plausible because fetal heart development takes place during early pregnancy at which point gestational diabetes is not fully manifested. 27 of the 52 studies did not control for any confounders. Cannot rule out residual confounding. There was significant heterogeneity in the results from meta-analyses.
Fever (Yang et al 2021 [47])	16 studies included (1 cohort, 15 case-control). 31,922 CHDs cases among 183,563 participants.	Maternal fever experience during preconception and conception periods and any offspring CHD, OR: 1.45 (1.21, 1.73, I^2 = 80%). There were also positive associations for specific CHD phenotypes including CTDs, ASDs, TGA and RVOTO.	NOS – 11 out 16 studies considered of higher methodological quality.	10 out of the 16 studies were not adjusted for confounders. Many studies used different definitions to define maternal fever. All but 1 of the studies had a case-control design.
Folic acid supplementation (Xu et al 2016 [48])	20 studies included (20 case-control). 33,270 participants.	Folic acid supplementation associated with a decreased risk of CHDs: OR = 0.60 (0.49, 0.71, I^2 = 88.8%, 20 studies).	NOS – 15 out of 20 included studies had a score of at least 7.	Significant heterogeneity reported which was partly driven by geographical location. There was evidence of publication bias. The authors do not discuss confounder adjustment in the included studies. Brief manuscript (< 3 pages) with all figures and tables in the supplementary material.
Hypertension (Ramakrishnan et al 2015 [49])	16 studies included (6 cohort, 9 case-control, 1 cross-sectional). 41,172 CHD cases included in study population.	Untreated maternal hypertension and any CHD, RR = 1.38 (1.15, 1.67, $P_{heterogeneity}$ = <0.001, 7 studies). The effect estimate was also positive for the association between untreated maternal hypertension and each of the seven CHD subtypes. Treated maternal hypertension in the first trimester of pregnancy and any CHD, RR = 2.03 (1.54, 2.68, $P_{heterogeneity}$ = 0.001, 8 studies). The effect was positive for the association between maternal hypertension treated with each of three specific types of hypertension medications (angiotensin converting enzyme inhibitors, beta-blockers, and calcium channel blockers).	NOS – Authors do not report NOS score for each study but report the range (range 5–9). Authors repeated analyses among studies with a total score of >6, and results were similar to the main results.	Large sample size with a good number of cohort studies included. 4 out of 16 studies did not adjust for any confounders. Confounder adjustment in the remaining studies varied. 4 out of the 12 studies that adjusted for at least one confounder adjusted for BMI. 3 studies adjusted for smoking and alcohol use. No evidence of publication bias. Insufficient data to assess the effects of pregestational versus chronic hypertension.

IVF (Giorgione et al 2018 [50])	8 cohort studies included. 313,851 participants including 1,952 cases.	OR of CHDs in IVF pregnancies = 1.45 (1.20, 1.76, I^2 = 44%, 8 studies). Subgroup associations reported (major and minor CHD) but results imprecise with CIs spanning the null.	NOS – 13 out of 41 included studies had a score of at least 7.	Meta-analysis results based on relatively small numbers of exposed and outcome (N with IVF = 25,856 (of which 337 had CHD), N without IVF = 287,995 (of which 1,952 had CHD)). 5 out of 8 studies did not adjust for confounders. Large numbers difficult to obtain given that IVF is rare (~1-2% of births in developed countries) and CHD is a rare outcome.
Nitrofurantoin (Goldberg et al 2015 [51])	6 studies included (4 cohort, 2 case-control). 1,404,619 participants including 23,620 CHD cases.	Exposure of Nitrofurantoin (an antibiotic commonly used to treat urine infections in pregnancy) and any CHD, OR = 0.94 (0.69, 1.28, I2 = 68%, 6 studies).	No formal assessment of risk of bias or study quality.	Only 165 events (CHD cases) from 6 studies in the exposed group in meta-analyses, therefore it is difficult to draw any strong conclusions. Confounder adjustment not reported by the authors. Associations were also null for any major malformation (from cohort studies).
Parity (Feng et al 2014 [52])	17 studies included (3 cohort, 14 case-control). 39,757 CHD cases.	RR of CHD for parous vs nulliparous = 1.01 (0.97, 1.06, I^2 = 54%, 16 studies). RR of CHD for the highest versus lowest parity categories = 1.20 (1.10, 1.31, I^2 = 83%, 14 studies).	NOS – 11 high quality studies (scores $\geq$ 7) and 7 low quality (scores $\leq$ 7).	15 out of the 17 studies did not adjust for any confounders. Authors performed subgroup analyses stratified by maternal age and found consistent results. Significant heterogeneity between studies in the highest versus lowest parity analyses.
Reproductive history (Feng et al 2015 [53])	18 studies included (17 case-control, 1 nested case-control). 10,132 CHD cases.	Ever pregnant versus nulligravidity and any CHD, OR = 1.18 (1.03, 134, I^2 = 62%, 10 studies). Dose-response analysis: Each increment in number of pregnancies compared with no prior pregnancy, OR = 1.13 (1.08, 1.18, I^2 = 43%, 7 studies). Prior abortion and any CHD, OR = 1.24 (1.11, 1.38, I^2 = 46%, 11 studies).	NOS – 4 out of 18 included studies had a score of at least 7.	Confounder adjustment in included studies not reported by the authors. All but one of the studies included were of the same design (case-control) and therefore susceptible to common biases such as selection bias.
RTI/influenza (Xia et al 2019 [54])	17 studies included (17 case-control). 11,911 cases and 74,358 controls.	Random effects meta-analysis for maternal RTI/influenza and any CHD: OR = 1.43 (1.24, 1.63, I^2 = 37%, 17 studies). Similar associations for simple and complex CHD.	No formal assessment of risk of bias or study quality.	Authors do not comment on confounder adjustment in the included studies. Some evidence of publication bias when any CHD was used as the outcome. Some studies exposure data relied on retrospectively self-reporting which can be susceptible to information bias due to differential recall between cases and controls.
Smoking (Zhao et al 2019 [55])	125 studies included (108 case-control, 17 cohort). 8,770,837 participants including 137,574 cases.	Maternal active smoking and CHD: RR = 1.25 (1.16, 1.34, I^2 = 89%). Maternal passive smoking and CHD: RR = 2.24 (1.81, 2.77, I^2 = 92%). Sub-type associations also reported.	NOS – 76 out of 125 studies (60.8%) were considered of higher methodologic quality (scores $\geq$ 7).	Large sample size and number of studies included. 84 out of 125 studies did not control for any confounders. Significant heterogeneity reported. Associations for maternal passive smoking and paternal active smoking were stronger than maternal active smoking suggesting results could be confounded. There was some evidence of publication bias.

Socioeconomic status (Yu et al 2014 [56])	33 studies included (2 cohort, 31 case-control). 53,358 CHD cases.	Maternal educational attainment (highest vs lowest) and any CHD, RR = 1.11 (1.03, 1.21, I^2 = 61%, 29 studies). Income level (highest vs lowest) and any CHD, RR = 1.05 (1.01, 1.09, I^2 = 0%, 6 studies). The study also reported positive associations for different levels of occupational prestige in comparison to the highest level of occupation.	No formal assessment of risk of bias or study quality.	Results were inconsistent between developed and developing countries (29 out of the 33 studies included were from high-income countries). Classification and definition of the exposures vary between studies. All but 2 of the studies included were case-control studies. Confounder adjustment in included studies not reported by the authors.
SSRIs (Gao et al 2018 [57])	Up to 18 studies included depending on analysis (all studies included in the meta-analysis were cohort studies). Participant numbers not stated for CHD analyses.	Exposure to any SSRI in the general population and any CHD, RR = 1.24 (1.11, 1.37, I^2 = 59%, 18 studies) and in women with a psychiatric diagnosis, RR = 1.06 (0.90, 1.26, I^2 = 33%, 6 studies). Citalopram and any CHD, RR = 1.24 (1.02, 1.51, I^2 = 53%, 11 studies). Fluoxetine and any CHD, RR = 1.30 (1.12, 1.53, I^2 = 29%, 14 studies). Paroxetine and any CHD, RR = 1.18 (1.05, 1.32, I^2 = 0%, 16 studies). Sertraline and any CHD, RR = 1.42 (1.12, 1.80, I^2 = 64%, 13 studies).	NOS – Analysis of the included studies indicated that 23 studies were low risk and 6 were high risk for bias.	Attempted to account for confounding by indication but authors acknowledge that this could still be an important potential source of bias. Authors found that RRs for the association between use of SSRIs and outcomes were lower in the restricted cohorts (it is plausible that smaller cohorts might have more complete confounder data available). One study with a discordant sibling design found no association [58], suggesting results from meta-analyses could be confounded.
Valproic acid (VPA) (Tanoshima et al 2015 [59])	20 studies for any VPA exposure (all included studies were of a prospective or retrospective cohort design). 19,284 participants including 194 CHD cases.	VPA exposure and CHDs, RR = 2.08 (1.55, 2.79, I^2 = 0%, 20 studies).	NOS – 24 out of 59 studies were considered of higher methodologic quality (scores ≥ 7). Results similar when including those studies of higher quality.	Low participant numbers (N = 67 exposed cases in meta-analyses from 20 studies) meaning it is difficult to draw any robust conclusions. Authors did not examine confounder adjustment. Although fetal valproate syndrome generally considered as an established risk factor for CHD.
Viral infection (Ye et al 2019 [60])	17 studies included (17 case-control). 67,233 participants.	History of viral infection in early pregnancy and any CHD: OR = 1.83 (1.58, 2.12, I^2 = 78%, 17 studies). Associations for specific virus's also reported.	NOS – 15 out of 17 studies were considered of higher methodologic quality (scores ≥ 7).	16 out of the 17 studies conducted in China, therefore results might not generalise to other populations. There was significant heterogeneity, although results were consistent in both fixed and random effects meta-analyses. Only 6 out of 17 studies controlled for confounders.
Confidence intervals are 95% unless stated. Abbreviations: CHD, congenital heart disease; OR, odds ratio; CI, confidence interval; RR, relative risk; NOS, Newcastle-Ottawa scale; CTDs, conotruncal defects; ASDs, atrial septal defects; TGA, transposition of the great arteries; RVOTO, right ventricular outflow tract obstruction; FA, folic acid; BMI, body mass index; IVF, in vitro fertilisation; SSRI, selective serotonin reuptake inhibitors; VPA, valproic acid.				

Studies included were predominantly of a case-control design (N = 493 total studies from 18 systematic reviews, of which 358 (73%) were case-control). The systematic reviews covered a diverse range of exposures from BMI, smoking and alcohol through maternal conditions (e.g., hypertension and fever) to maternal treatments during or before pregnancy (e.g., IVF, antidepressants, and epilepsy treatments). All but 1 of the 18 systematic reviews reported an association between the maternal exposure and risk of CHD. The exception was for Nitrofurantoin (an antibiotic commonly used to treat urine infections in pregnancy) which included 6 studies (1,404,619 participants and 23,620 CHD cases [of which, 165 were exposed to Nitrofurantoin]) with the authors concluding that they found no increased risk for CHDs. The results suggested a weak protective effect with wide confidence intervals that included potentially clinically meaning full protective or detrimental effects (OR: 0.94 (95%CI: 0.69, 1.28). Of the 17 other systematic reviews that reported an association, 16 reported positive associations (i.e., increased exposure was associated with higher risk of CHD), whereas folic acid supplementation was found to be associated with lower CHD risk.

3 out of 18 systematic reviews did not formally assess the risk of bias or study quality of the included studies. The remaining studies all used some form of the Newcastle Ottawa Scale. 7 out of the 18 systematic reviews did not comment on confounding variables in the included studies. Of the remaining 11, confounder adjustment significantly varied. 67%, 53%, 69% and 52% of studies did not report confounder adjustment in the alcohol, BMI, diabetes and smoking systematic reviews, respectively (4 of the most studied exposures). Heterogeneity between studies was common, with I^2 (a measure of the proportion of variation across studies that is due to between study heterogeneity rather than chance) provided in 17 studies and ranging from 0% to 98% (with 12/17 of the studies having a value >=50%). This was particularly the case for some of the larger studies mentioned above (I^2 > 70% for alcohol, diabetes and smoking meta-analyses), although it is worth noting that larger studies have more power to detect heterogeneity. Potential sources of heterogeneity could arise from study samples reflecting different populations and associations being different between these populations, or differences in outcome definitions (i.e., some studies only including specific CHD subtypes or defining CHD cases differently to others), or by the way the exposure is assessed or the quality and differences of the study design. 17 of the 18 systematic reviews assessed for evidence of publication bias with 6 of the studies uncovering evidence of publication bias.

1.3.3. Exposures not identified in the Umbrella Review

The advantage of performing a review of systematic reviews in the form of an umbrella review is that it focuses on where there has been a body of work that has been summarised, meta-analysed (where possible) and critiqued. The limitation is that it focuses on exposures that have strong hypotheses and available data to be explored in several studies, which may miss important risk factors that are not in the 'cognitive and political bias set'. Seminal studies that were not a systematic review would not have been included. Some exposures that might be pertinent but were not identified by the review above could include diet, illegal drug use and a range of biomarkers (e.g. glucose). There were no studies of metabolomics, which would reflect a more hypothesis free approach to exploring how the pregnancy environment relates to offspring CHDs and I discuss this further in section 1.4. Since writing this section, a large umbrella review exploring environmental risk factors and CHDs has been published. The interested reader is directed towards this publication for a more in-depth review [61].

1.3.4. Risk of bias for causal inference

73% of studies included in the systematic reviews identified for this umbrella review used a case-control design. CHDs are relatively rare (~1% incidence) and case-control studies are an efficient study design for studying rare outcomes. Furthermore, case-control studies are cost-effective and less time-consuming than other study designs. However, case-control studies and other observational study designs are prone to biases that could influence findings and therefore cast doubt on causality. I discuss these biases below and consider the extent to which the biases might have influenced findings from the review.

1.3.4.1. Selection bias

Selection bias can occur when groups of participants differ in ways other than the exposure of interest. For example, case-control studies are prone to selection bias as response rates in controls are commonly low and some controls are selected from hospitals or clinics which may not be representative of the target population. Prospective cohort studies can go some way to addressing this, although, selection into and dropout from these studies may bias associations, as previously discussed in the context of UK Biobank [62,63], the largest prospective cohort study in the UK. The magnitude and direction of effect that selection bias is having on a study is often difficult to determine [64]. More recently, studies have made

use of large electronic linked datasets often including large proportions of a country's population which provide opportunities to obtain truly representative samples and reduce the chance of selection bias influencing findings [65,66]. Preventive steps for observational studies include exploring replication across different study populations, dealing with missing data appropriately and selection of populations should be as broad as possible and openly reported in the recruitment/inclusion criteria.

1.3.4.2. Misclassification/measurement error of exposure or outcome

Case-control studies are susceptible to information bias due to differential recall and reporting of the exposure between cases and controls [67]. For example, the systematic review exploring respiratory tract infections (RTIs) and influenza on CHDs included some studies that retrospectively ascertained self-reported exposure status once an offspring CHD was already diagnosed [54]. It is plausible that women might recall the exposure differently after an offspring CHD diagnosis. If pregnancy data is collected prospectively for all women in a population before offspring CHD diagnoses are made, this would reduce the possibility of differential recall influencing the results.

The timing of the assessment of putative risk factors is also important to consider. The embryonic heart is developed between week 3 to week 8. It is important that exposure measurement closely resembles this crucial period of heart development. Retrospective data covering a specific period such as this could be difficult to reliably obtain. In the air pollution systematic review, air pollution exposures were largely assessed on air pollutant measurements carried out at fixed monitoring stations close to the residential address of the mother. This assumes that the measures at a fixed station then reflect air pollution exposure in the mother during early pregnancy which could be a difficult assumption to reliably assess.

The classification of CHDs can be somewhat complicated and is not a clear-cut exercise. Some studies may only include live births, although, it is well known that CHDs are major cause of still births (e.g. the largest study included in the BMI systematic review including >1.2 million singletons and accounting for >15% weighting in meta-analyses only included live births [66]). Despite this, recent findings suggest that livebirth bias is unlikely to affect studies of risk factors for most CAs [68]. Outcome misclassification (offspring who would be diagnosed with a CHD later in life are treated as not having CHD) can also be a problem in CHD research if studies only recruit cases in early life. It is well known that most CHDs are identified in utero or at birth, however, many are still diagnosed throughout childhood and even

in adulthood [35,69,70]. This misclassification (wrongly assigning a participant as a "control" instead of a "case") would bias results towards the null.

1.3.4.3. Confounding

As mentioned above, many of the studies included in the literature review did not adjust for confounders (67%, 53%, 69% and 52% of studies did not report confounder adjustment in the alcohol, BMI, diabetes and smoking systematic reviews). Studies that do not adjust for any confounders have no way of discerning whether an association is being caused by their exposure of interest or other common causes (i.e., confounders: illustrated below in **Figure 1.4**). Results from studies using conventional multivariable approaches that have adjusted for confounders may also be explained by residual confounding because of incomplete identification or adjustment for confounders or poorly measured confounders. The authors of the systematic review looking at selective serotonin reuptake inhibitors (SSRI) attempted to account for confounding by indication (underlying psychiatric diagnosis) by comparing women using SSRIs vs. those with unmedicated psychiatric illness [57]. No other studies attempted to explore the impact of possible residual confounding meaning that there is a high risk of confounding influencing results from the systematic reviews discussed above. There is a pressing need for research involving maternal exposures and offspring CHDs to make use of study designs that can address the issue of confounding. I discuss some of these methods in section 1.5 which I subsequently use throughout this thesis.

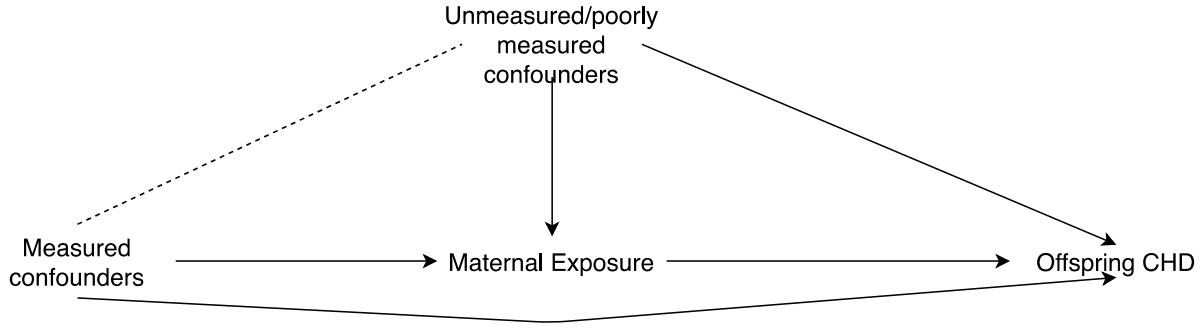


Figure 1.4. Causal diagram for the effect of a maternal exposure on offspring CHDs, with both measured (controlled) and uncontrolled confounders that cause both the exposure and outcome. The dashed line between measured and unmeasured confounders indicates that either may cause the other, and they may share common causes.

1.3.4.4. Publication bias

A major limitation of reviewing the epidemiological literature for CHD risk factors is that studies uncovering null results may be less likely to be published [71]. Of the 18 systematic reviews reported in **Table 1.4**, 6 found some evidence of publication bias, whereas 1 study did not explore the potential impact of publication bias. This can skew the evidence base for a particular topic, affect meta-analyses and compromise the credibility of the scientific literature. This issue is not specific to CHD research but is a problem for science in general [72]. More and more scientists and journals are beginning to adopt 'pre-registered' studies as part of a bid to prevent publication bias and put an emphasis on robust methods and scientific analysis as oppose to 'significant' results [73]. Pre-registering a study requires the authors to openly report their background, rationale, and study methods before undertaking any analyses and should be adopted more widely in the field of aetiological CHD research. Questionable research practices such as only reporting favourable results, 'P-hacking' and post hoc theorising can be identified by comparing published articles to their pre-registered protocols. Confirmatory studies seeking to replicate previous work and studies reporting null results are crucial for improving our scientific understanding of CHD aetiology.

1.3.5. Summary

I have provided a summary of evidence on maternal risk factors for offspring CHDs in the form of an umbrella review. It highlights a focus on 'expected' candidate exposures, including treatments such as IVF, antidepressants, sodium valproate, and behaviours such as smoking and alcohol, that have been hypothesised to influence fetal development and CAs in general for decades. In conclusion, there are many exposures associated with CHD, but studies that use causal methods are lacking. I have discussed sources of bias for causal inference which I am interested in and that I aim to address in this thesis.

1.4. Metabolomics in aetiological CHD research

In this section, I will introduce and discuss the emerging role of metabolomics technologies and how they could improve our understanding of how the pregnancy environment may relate to offspring CHDs. In this context, metabolite(s) are deemed exposures; they might mediate some of the behaviours mentioned above or they may be independent risk factors. To date, there are no systematic reviews that

have looked at maternal metabolites/metabolomics and offspring CHDs and therefore this topic was not discussed in the previous section. The rationale for exploring this is that detailed information on the metabolome can provide information on a range of medications, diet and lifestyle factors that are being used/adopted by the mother which can be difficult to reliably obtain via self-report.

Metabolomics falls under the 'omics' umbrella term, which includes genomics, transcriptomics, proteomics, and epigenomics. It involves the measurement of small molecule compounds (metabolites). The metabolic state of an organisms depends on a complex and continuous interaction between its genome, transcriptome, proteome, epigenome, microbiome and the external environment [74]. Changes in metabolite levels are a result of enzymatic reactions and physical, pathological and environmental influences at the molecular level [75]. Therefore, metabolites are thought to be the 'omics' that most closely reflect profiles of phenotypes in health **(Figure 1.5)**. Metabolomics can provide us with a detailed exploration of an organism's current physiological state. The metabolome represents the complete set of metabolites in an organism. Advances in high-throughput technologies have introduced the use of metabolomics into epidemiological studies, providing opportunities to improve our understanding of molecular mechanisms that underpin health and disease [76]. For example, metabolomics studies have uncovered potential new biomarkers for cardiovascular disease and contributed to the metabolic changes that underpin the disease [77]. The two most common platforms currently being used for metabolomic profiling are mass spectrometry (MS) and nuclear magnetic resonance (NMR). MS offers an untargeted approach with comprehensive coverage of the metabolome (>1000 metabolites) due to its high sensitivity. However, MS only provides relative quantification based on peak area without comparison to a metabolite reference standard. NMR offers less coverage of the metabolome, but with absolute quantification possible in clinically relevant units (i.e. mmol/l). In this section, I focus on the use of metabolomics (during pregnancy) in CHD research.

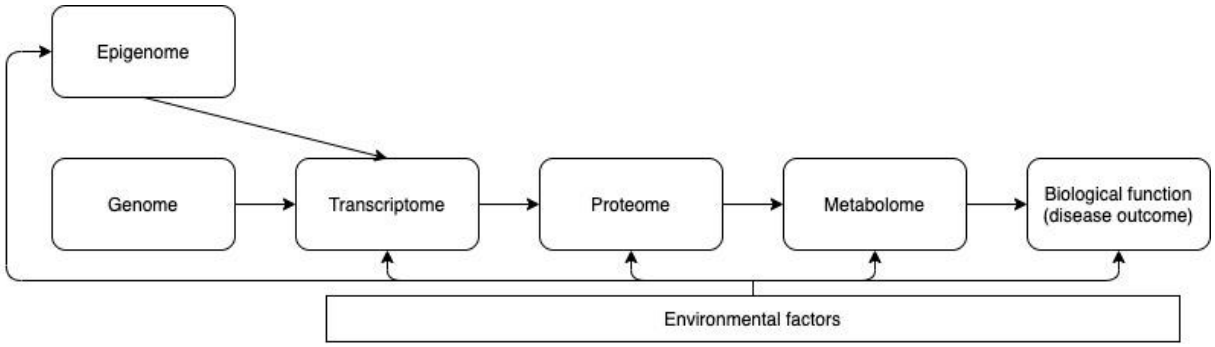


Figure 1.5. Hierarchy of biological information leading to biological function or disease outcome.

The development of metabolomics has provided opportunities to improve our understanding of the physiological changes that occur during pregnancy [78]. For example, differences in pregnancy metabolomic profiles have been reported in relation to changes in pregnancy characteristics [79–81]. Metabolomics provides scope to assess whether these molecular changes that affect the mother during pregnancy can lead to complications in the offspring through possible intrauterine mechanisms by identifying clinically relevant biomarkers. Currently, there are no widely adopted maternal pregnancy biomarkers available for the detection of CHDs. As depicted in **Table 1.4** above, there is an extensive body of research exploring maternal risk factors and CHDs, however, the mechanisms by which putative risk factors influence offspring cardiac development are not clearly understood. Laboratory studies are vital for mechanistic research of this nature, however, in a real-world population setting, metabolomics could provide opportunities to enhance our molecular understanding of CHDs.

To date, there have been several studies that have used pregnancy metabolomics data to study offspring CHDs which I discuss below. Some of the studies have aimed to use metabolomics for early diagnosis rather than attempting to establish causal links. For example, one study including 27 CHD cases and 59 controls aimed to identify metabolomic markers in maternal serum during pregnancy for the detection of CHDs [82]. They found more than 100 metabolites that differed between CHD cases and non-cases concluding that abnormal lipid metabolism was a significant feature of CHD pregnancies. However, the sample size was relatively small, and their results have not been externally validated. Other work has explored potential biomarkers of maternal urine metabolomics with offspring CHDs (N = 70 CHD cases and 70 controls) [83]. Their results indicated that short chain fatty acids and aromatic amino acid metabolism in a Chinese population may be relevant to CHDs. Recent work using an untargeted metabolomics approach using maternal amniotic fluid samples discovered that the metabolites uric acid and proline, were significantly elevated in CHD cases [84]. Replication of these studies are warranted.

Some studies have explored more traditional molecular markers (as opposed to a metabolomic platform) and found that women with a compromised vitamin D status (defined as 25-hydroxyvitamin D < 50 nmol/l in comparison to adequate defined as > 75 nmol/l) [85] and lipid profile [86,87] could be important maternal risk factors for CHDs. However, some of these studies measured the biomarkers after pregnancy and used the measurements as a proxy for pregnancy levels. Other work explored one-carbon metabolite levels during pregnancy (including a variety of analytes: homocysteine, methylmalonic acid, folate, vitamin B12, pyridoxal phosphate, pyridoxal, pyridoxic acid, riboflavin, total choline, betaine, methionine, cysteine, cystathionine, arginine, asymmetric and symmetric dimethylarginine). The authors concluded

that they observed no statistical differences, although there were several associations that had potentially clinically meaningful point estimates with the confidence intervals spanning the null [88].

In summary, there have been some promising studies uncovering potentially important molecular pathways associated with offspring CHDs. However, pregnancy metabolomic studies are still relatively novel and the evidence presented above is preliminary. Going forward, larger studies that possess metabolomic measurements during pregnancy coupled with offspring outcomes will be required to further explore associations between maternal metabolomic profiles and CHDs and to replicate previous findings. Metabolomics studies are still susceptible to biases discussed above (i.e., selection bias and confounding). Future studies should explore associations using a range of metabolomic platforms and employ causal methods (as discussed in the next section) where possible to improve confidence in findings.

1.5. Causal methods

Over the last 15-20 years novel approaches to improve causal inference in observational epidemiology have been proposed and increasingly used. These include the use of genetic variants as instrumental variables (IVs) (known as Mendelian Randomisation (MR)), within sibship analyses, negative control studies, and cross-context comparisons. These are increasingly developed and applied specifically to explore intrauterine effects, for example of maternal pre-/early pregnancy BMI, glucose, lipids, blood pressure, vitamin D and smoking on offspring fetal growth, birthweight, later life BMI and cardiometabolic risk [89–95]. However, their use in identifying modifiable intrauterine effects on risk of CHDs appears limited, potentially because of the very large numbers of participants that would be needed for such studies.

In the field of aetiological CHD research, there is a need for evidence to be integrated from multiple epidemiological approaches (such as those mentioned above) with differing and unrelated key sources of bias to improve our causal understanding of maternal risk factors and CHDs. This approach is referred to as triangulation [96]. The use of multiple different study designs to improve our causal understanding of maternal risk factors for offspring CHDs is likely to require considerable data sharing and multidisciplinary collaboration. The adoption of data sharing and collaboration is particularly pertinent for the topic I discuss here due to the rarity of CHDs within the general population. In contrast, for an outcome such as birthweight which is ubiquitous in the general population, larger sample sizes are easier to obtain. I have tried to improve causal inference in this thesis by triangulating evidence from either two or all three

of the following methods: multivariable regression, parental negative control analyses and MR analyses. I describe each method below by including a directed acyclic graph (DAG) for each, their assumptions, and the key sources of bias in relation to the research presented in this thesis (including the likely direction of that bias).

1.5.1. Multivariable regression

Multivariable regression is a statistical model with one outcome (here CHD) and multiple exposures that potentially influence the outcome **(Figure 1.6).** Key assumptions of this method are: (i) all confounders are accounted and controlled for (no residual confounding), (ii) participants are not selected on in a specific way (no selection bias), (iii) misclassification of exposure or confounders is not related to the outcome and vice versa. Sources of bias include: (i) unmeasured or poorly measured confounding (residual confounding) distorting the results. Unmeasured confounders would bias estimates away from the null, however, measurement error (i.e. poorly measured confounders) leading to residual confounding could bias estimates in either direction [97]. (ii) Misclassification of exposure, outcome, and confounders. The direction of bias as a result of misclassification will depend on what is being misclassified (i.e., exposure, confounder or outcome) and the direction of misclassification; I provide further details and discussion in relevant subsequent chapters reflecting the specific data available. (iii) Differential missing data between exposure or outcome levels. (iv) Selection bias (discussed above).

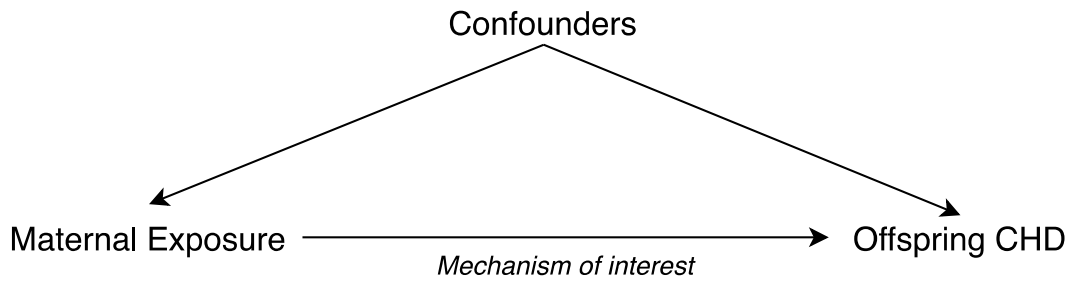


Figure 1.6. Directed Acyclic Graph of use of multivariable regression to assess the effect of maternal exposures on offspring congenital heart disease.

1.5.2. Parental Negative Exposure Controls

The idea behind negative control studies is that either the exposure or the outcome in the real experiment is substituted for a negative control exposure (or outcome) that is not a plausible risk factor but would have similar sources of bias or confounding as in the main experiment. Parental negative exposure control analyses involve comparing the observed confounder adjusted associations of the maternal exposure with CHDs to the same associations of paternal exposures with CHDs **(Figure 1.7)** as a way of exploring potential residual confounding [98,99]. The assumptions of this approach are that: (1) measured and unmeasured confounders influence the exposures in the same direction and with a similar magnitude in mothers and fathers and (2) there is no plausible reason why the exposure in the father would affect the offspring outcome (or at a minimum the paternal association would be much weaker than in the mother). Under these assumptions, if there is a causal intrauterine effect of a maternal pregnancy exposure, one would expect to see a maternal-specific association, with no (or a much weaker) association with the equivalent paternal exposure. Similar associations in mothers and fathers would suggest that results are largely driven by residual confounding. Sources of bias include: (i) confounders and/or biases differing between the real (mothers' exposure) and the negative control exposures (paternal exposure). (ii) The negative control exposure having a causal effect on the outcome. (iii) The real and negative control exposure not being similarly scaled and/or assessed during a similar period. I discuss these sources of bias further during Chapter 3 of this thesis.

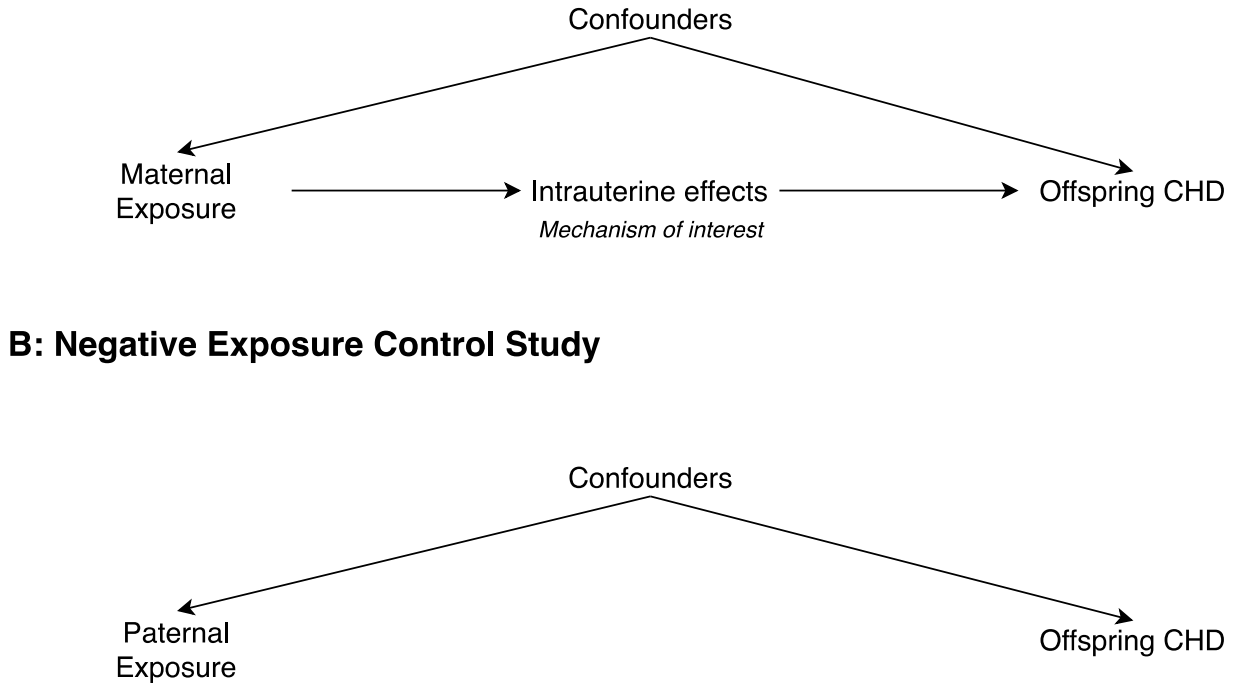


Figure 1.7. Directed Acyclic Graph illustrating the use of negative controls in assessing the effects of maternal intrauterine exposures on offspring congenital heart disease.
The negative paternal exposure has the same incoming arrows as the maternal exposure of interest but has no arrow to the outcome. Therefore, any association observed between the negative control and the outcome will be due to confounding in the model. **A** illustrates the "real" study whereby the maternal exposure could plausibly influence the offspring outcome (CHD) via our hypothesised mechanism of an intrauterine effect. **B** illustrates the negative exposure control whereby the exposure cannot plausibly influence the outcome via the hypothesised mechanism.

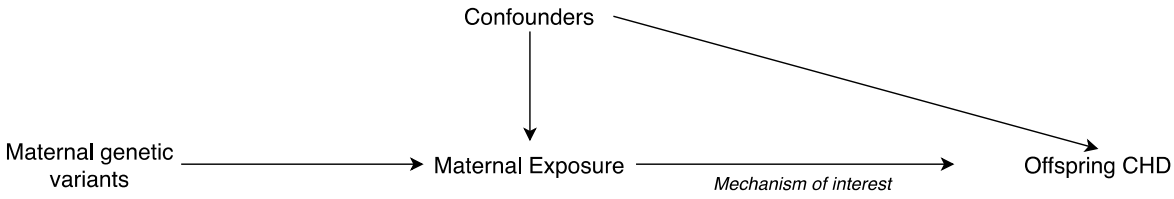
1.5.3. Mendelian Randomisation

MR involves using genetic variants as IVs for an exposure **(Figure 1.8)** [100,101]. The key assumptions for MR are: (i) relevance assumption – the genetic instruments are robustly associated with the exposure (often defined as below genome-wide p-value threshold and replicates across at least two independent studies). (ii) Independence assumption – there is no confounding of the IV-outcome association. (iii) Exclusion restriction criteria – The genetic variant is not related to the outcome other than via its association with the exposure (pleiotropy). As well as these three core assumptions, additional

assumptions are required that include: homogeneity assumptions, no effect modification and monotonicity [102].

Sources of bias for MR studies include: (i) The genetic instruments are not truly associated with the exposure in the population being studied. This is particularly pertinent for using MR to determine causal effects of maternal pregnancy exposures on offspring outcomes as done in this thesis. This is because, genome-wide association studies (GWAS) tend to be performed in non-pregnant populations. Therefore, it is important that a robust association of the maternal genetic IV with the exposure assessed during pregnancy is demonstrated [89]. (ii) Population stratification (population subgroups experiencing different disease rates and different allele frequencies) may confound the genetic instrument-outcome association which would violate the independence assumption [101]. To avoid the issue of population stratification, it is recommended to use ethnically homogeneous populations and/or controlling for principal components that reflect different population subgroups [100,101,103]. (iii) Violation of the exclusion restriction criteria (horizontal pleiotropy, where a genetic variant(s) influences multiple traits). The exclusion restriction criteria could also be violated by fetal genotype as well as horizontal pleiotropy when exploring maternal pregnancy exposures on offspring outcomes [89]. Offspring get 50% of their genetic variation from their mother (i.e., the mothers genetic instrument will associate with the same genotype in fetus) and if those fetal genetic variants relate to the outcome, the exclusion restriction criteria is violated. This is generally the case when there is overlap between offspring genetic variants that are related to their outcome and the maternal IV genetic variants (such as when the maternal exposure and offspring outcome are the same (e.g., exploring the effect of maternal BMI on offspring BMI)). Therefore, it is recommended to adjust for offspring genotype in these analyses.

A: Mendelian randomisation



B: MR to assess intrauterine causal effects on offspring outcomes, showing how the exclusion restriction criteria may be violated

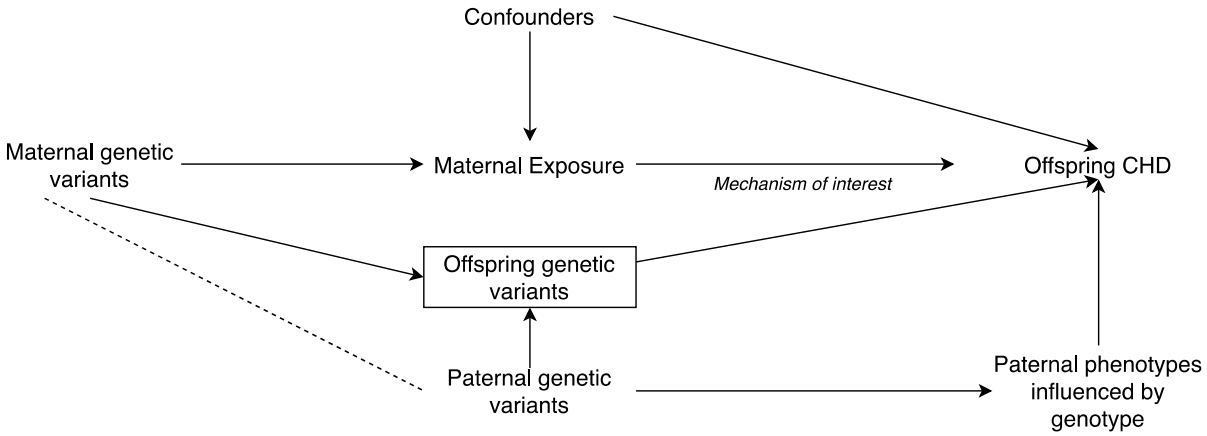


Figure 1.8. Directed Acyclic Graph of use of Mendelian randomisation to assess causal effects of maternal exposures on offspring congenital heart disease.
Dashed line reflects a spurious association that could arise from collider bias by conditioning on offspring genetic variants.

1.6. Summary and overview of thesis aims

CHDs are a significant burden to patients, their families and society. Currently, there are limited treatments or interventions to prevent them. Previous research has mainly focused on expected common exposures and is likely to be compromised in relation to causality by residual confounding. I aimed to address key aspects of gaps or limitations in current research in my PhD by (i) exploring potential effects of exposures that have previously been well studied (maternal BMI, smoking and alcohol) with an attempt to use methods that improve causal inference and (ii) exploring potential effects of multiple metabolites that in themselves have not been previously well studied and that could also proxy other exposures such as metabolic health, diet and a range of medications without relying on maternal report. As with the more established exposures mentioned above I will also triangulate evidence from different approaches to improve causal inference with the metabolite exposures.

1.6.1. Thesis outline

In **Chapter 2** I describe the CA and CHD data in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort in the form of a data note. I collated, cleaned and coded the data from multiple sources and also made these data available to other researchers that wish to carry out important work on CAs and/or CHDs. I use these data in subsequent chapters throughout this thesis. In **Chapter 3** I undertook a parental negative exposure control study to explore the effects of maternal pre-/early-pregnancy BMI and pregnancy smoking and alcohol on CHDs using data from seven European birth cohorts. In **Chapter 4** I conducted MR analyses to triangulate results and explore the effects of the same exposures studied in **Chapter 3** (BMI, smoking and alcohol). **Chapters 5 and 6** involve metabolomics data. In **Chapter 5,** I used metabolomics data from an untargeted MS platform (~1,000 metabolites) to explore associations between pregnancy metabolites and offspring CHDs. Where possible, I seeked validation of metabolite associations using other data sources and MR. In **Chapter 6,** I explored associations between pregnancy metabolic profiles assessed by an NMR platform and offspring CHDs. As in Chapter 6, I used MR analyses to seek validation. In **Chapter 7**, I summarise findings presented in this thesis including strengths and limitations in the context of previous research and consider their potential public health and clinical implications. I consider future directions in aetiological CHD research.

Chapter 2. Ascertaining and classifying cases of congenital anomalies in the ALSPAC birth cohort

2.1. Chapter summary

This chapter has been published:

Taylor K, Thomas R, Mumme M, Golding J, Boyd A, Northstone K, Caputo M & Lawlor DA. Ascertaining and classifying cases of congenital anomalies in the ALSPAC birth cohort. *Wellcome Open Research* (2020). https://doi.org/10.12688/wellcomeopenres.16339.2.

In **Chapter 1** I introduced congenital heart disease (CHD) and provided the case for research that uses causal methods to investigate maternal risk factors. I discovered that there was no comprehensive data source describing cases of congenital anomalies (CAs) in the Avon Longitudinal Study of Parents and Children (ALSPAC). ALSPAC is a truly unique cohort that contains data in mothers, fathers, and offspring across multiple generations. To make use of ALSPAC in this thesis, I set out to define CHD cases. In this Chapter (**Chapter 2**) I used multiple sources of data to retrospectively define all cases of CAs in ALSPAC with a specific focus on CHDs. I demonstrate that combining multiple sources of data including data from antenatal, delivery, primary and secondary health records, and parent-reported information can improve case ascertainment. The approach identified 590 participants (385 per 10,000 live births) with a CA according to the European Surveillance of Congenital Anomalies (EUROCAT) guidelines, 127 of whom had a CHD (80 per 10,000 live births). I use these data for analyses in subsequent chapters throughout this thesis. I published this work in the form of a data note so that the scientific community can find out more and make use of these data.

2.2. Introduction

Congenital anomalies (CAs) occur in utero and can be identified prenatally, at birth or during later life. CAs can be defined as structural (e.g. limb reduction defects) or functional (metabolic disorders). The exact cause of most CAs is unknown; however, causes can include single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens and micronutrient deficiencies during pregnancy [1]. Consequences vary depending on the type and severity of the anomaly, but many children and their families experience lifelong complications. Worldwide, at least 3.3 million children under the age of 5 die from CAs each year [2]. In European countries, including the UK, CAs affect approximately 2–3% of births [3]. CAs are a major cause of fetal death, infant morbidity and long-term disability. CAs represent a significant public health concern requiring further research around their causes, consequences and long-term implications.

Birth cohorts can be useful for studying the aetiology and longer-term consequences of CAs as they aim to include all births in a defined population over a defined period of time and often follow them into adulthood. Many have the added advantage of recruiting during pregnancy and recording all birth outcomes, whether live or stillborn. This reduces selection bias (in comparison to studies that focus solely on those with CAs or those at risk), provides a comparison group of those without CA from the same underlying population, and with postnatal follow-up allows for all CAs to be identified [70,104]. Follow-up supports research into the natural history and impacts of CAs on future health and wellbeing. The latter is important as modern treatments, including advancements in surgery, mean higher proportions of those with CAs now live through to adulthood [105]. On the other hand, as CAs are relatively rare disorders, statistical power in any single birth cohort is likely to be low, meaning effects will be imprecisely estimated in comparison to case-control studies. Some birth cohorts exclude infants with known CAs from being in the study population or collect information at birth but often then exclude those with known CAs from specific studies [106]. Other birth cohorts, such as the Born in Bradford (BiB) study, seek data on all CAs, and demonstrate the importance of continuing to identify cases postnatally, for example through linkage to primary and secondary care, in order to identify participants whose clinical diagnoses came later in life [70].

In this paper, I describe how I have attempted to identify all cases of major CAs in the UK-based Avon Longitudinal Study of Parents and Children (ALSPAC), a birth cohort which started following participants in the early 1990s. To date, there has been no systematic approach to doing this in ALSPAC. Consequently, it has contributed little to research about CAs [107]. This is likely because around the time the

original women were recruited in pregnancy the routine ultrasound scan screening of all pregnant women was not advanced enough to identify many fetal anomalies [108]. Additionally, linkage of cohort participants to clinical records was limited as centralised national sources were in their infancy [109] and many local datasets remained paper based or in the early stages of digitisation. Here, I demonstrate that combining multiple sources of data including data from antenatal, delivery and neonatal, primary and secondary care health records, as well as parental-reported information can improve case ascertainment. I show that this approach captures more cases than relying on any single data source.

2.3. Methods

2.3.1. Aims

To: (1) combine a range of data sources to ascertain cases of major CAs in the ALSPAC birth cohort, with a specific focus on congenital heart diseases (CHDs) and (2) code cases of CAs with International Classification of Diseases (ICD) codes (version 10) according to the European Surveillance of Congenital Anomalies (EUROCAT) guidelines [110].

2.3.2. Cohort

ALSPAC is a prospective birth cohort, which was devised to investigate the environmental and genetic factors of health and development. Detailed information about the methods and procedures of ALSPAC is available elsewhere [111–113]. In brief, pregnant women with an expected delivery date between April 1991 and December 1992, residing in and around the city of Bristol, UK were eligible to take part. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible participants who had failed to join the study originally (i.e., any child born during the same years and in the same geographical area that defined the original cohort). As a result, for all ALSPAC variables collected from the age of seven onwards there are data available for more than the 14,541 pregnancies mentioned above. The total sample size for analyses using any data collected after

the age of seven is 15,454 pregnancies, resulting in 15,589 fetuses. Of these, 14,901 were alive at 1 year of age [113].

In 2012 recruitment of the next generation (children of the original children born in the early 1990s began) and since then researchers have described the generations as ALSPAC-G0 (women recruited during pregnancy in the early 1990s and their partners), ALSPAC-G1 (the index children of those women who have been followed since birth) and ALSPAC-G2 (the children of ALSPAC-G1 and grandchildren of ALSPAC-G0) [114]. This data note is about ascertaining and coding CAs in the ALSPAC-G1 cohort. Data on CAs in G2 are being, and will continue to be, prospectively collected, but currently there will be very few cases amongst the ~1000 G2 participants that have been recruited. All three generations have continued to be followed via questionnaires, research clinics and record linkage. The study website contains details of all the data that Is available through a fully searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/access/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (http://www.bristol.ac.uk/alspac/researchers/research-ethics/). Consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time. All G0 and G1 participants have been informed about the study's intention to link to and use their routine health records in the study's research program. Participants are free to object to this use of their records, and the records of those objecting have not been used in this research. When it becomes practicable, explicit consent for linkage to health records is collected (e.g., at study assessment visits). The use of National Health Service (NHS) records in this way has approval from a Health Research Authority (HRA) Research Ethics Committee and the HRA Confidentiality Advisory Group.

2.3.3. Data sources and methods of obtaining CAs from them

Five data sources were used to identify children with CAs in ALSPAC (**Table 2.1**). Four of these were able to identify any CA, one (data source 2) was specific to CHDs. I included diagnoses made at any age. Restricting diagnoses to a specific age bracket could lead to incomplete case ascertainment [70].

Table 2.1. Data sources used to identify cases of congenital anomalies in ALSPAC.

#	Data source	Data collection method	Description and data coverage
1	NHS Primary Care records	Record linkage to Primary Care	Linkage of ALSPAC participants to primary care records. Last extract was October 2016. Capability to capture any CA diagnosed on an ALSPAC participant registered with a participating GP in England/Wales between 1990 and 2016. Further extracts will continue to be made.
2	Paediatric cardiology & cardiothoracic surgery records	Record linkage to paediatric cardiology & cardiothoracic surgery records	The *HeartSuite* patient management system is designed specifically for paediatric cardiology and cardiothoracic surgery. It covers data on diagnoses and procedures between 1992 to 1994 and 2002 to 2019 for a regional referral centre. It would include ALSPAC participants' residing in and around Bristol who had cardiac/ cardiothoracic surgery or procedures such as catheterisation at the UHBT during the periods covered. Data were provided by UHBT, in November 2019.
3	Data on fetal, infant and child deaths	Birth notification system, ONS, post-mortem reports.	Includes data on fetal deaths of gestation 20 weeks or more in England, Scotland or Wales, including spontaneous and therapeutic abortions for malformations or genetic defects, and deaths of livebirths up to ~104 weeks of age. Data were captured from multiple sources including: The birth notification system of deaths to livebirths in Avon, the Office for National Statistics (ONS), post-mortem reports and the regular clinical discussions of all such deaths in the two major maternity hospitals. This provides the ability to capture CAs that resulted in antenatal or early postnatal death, which might not be captured in other sources.
4	Diagnoses from Avon Child Health Services	Diagnoses from Avon Child Health Services	CAs from Child Health (formerly known as Avon Child Health Services). These data cover the Avon region from December 1990 to February 1993 and would identify children diagnosed at any postnatal age during that period.
5	ALSPAC	i. Antenatal, labour and neonatal records ii. Questionnaire completed by research nurses iii. Participant's mother self-report	i. *Abstractions from clinical records* – This database comprises detailed abstractions from the clinical records covering midwife, obstetrician, paediatric and additional (e.g., blood test results and ultrasound scans) entries from the antenatal, intrapartum and first two weeks of the postnatal period. Abstractions were conducted by ALSPAC employed research nurses on different subgroups. These included several clinical subgroups (e.g., preterm births and multiple pregnancies) as well as a random sample. In total, detailed data has been extracted from 8,369 ALSPAC-G0 pregnancies. In addition, extracted text data with descriptions of all abnormalities of the fetus and neonate were available for 6,343 ALSPAC-G1 fetuses and infants with known birth outcomes and used in this data note. ii. *Neonatal admissions questionnaire* – For each neonate (<28 days of age) admitted to hospital, a detailed questionnaire was completed by a neonatal

			nurse. In total, 994 questionnaires were completed. Of these, all but 5 were from the two main hospitals in Bristol at the time. iii. *Child-based questionnaires* – I undertook a search of the text answers from ALSPAC parent (mostly mothers) completed child-focused questionnaires between birth and ~14years. These data would only include G1 participants whose mothers (or another main carer) filled in and returned at least one child-based questionnaire. Questionnaires were searched for key words relating to CAs in response to general questions about the child experiencing diseases, being admitted to hospital, outpatient investigations or a free text space at the end of each questionnaire that carers were invited to use for any other information they thought would be valuable to the study.
Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; CA, congenital anomaly; GP, general practice; NHS, National Health Service; UHBT, University Hospital's Bristol Trust; ONS, office for national statistics.			

2.3.3.1. NHS Primary Care Records

ALSPAC have established a linkage between participants and their information on the NHS Patient Demographic System (PDS): the national patient register for England, Wales, and the Isle of Man. This linkage provides a participant's NHS ID number and can also be used to identify which General Practice (GP [primary care]) a participant is registered with. The NHS provides free primary and secondary health care to all UK residents. Access to secondary care is via referral from primary care and even when someone has care from a private provider a discharge note will be sent to their GP. Therefore, NHS record linkage will provide health data for the vast majority of the population. It is possible that some participants were or are not registered with a GP, although, I would expect this to be a small minority. To date, ALSPAC have extracted primary care information in two batches:

I. In 2013 a pilot exercise was conducted, which aimed to extract the records of 2,806 G1 participants registered in 523 primary care practices across England and Wales. ALSPAC gained approval from 290 of these practices to extract life-course GP coded records. These were extracted by EMIS Health Ltd or Apollo Ltd clinical software system providers. This resulted in the extract of 2,249 participants records from 180 practices (the high level of achieved participant coverage reflects that the 180 practices disproportionately included those with high numbers of ALSPAC participants, including those in and around the city of Bristol) [115].
II. In 2016 an additional extract was conducted to extract the records of 11,955 G1 participants from participating practices in the Bristol, North Somerset and South Gloucestershire (BNSSG) clinical commissioning group (CCG), which has the same geographical coverage as the ALSPAC catchment area. This resulted in the extract of 11,087 participants records [116] This second extract included most, but not all of the participants in the 2013 pilot, meaning that the final number of ALSPAC-G1 participants (i.e. the participants considered in this work) with primary care data is 11,810.

For the data described in this manuscript, the majority of primary care records which contributed to the case definition were those extracted from BNSSG GPs in 2016, when participants were aged ~26 years old. There were a small number of additional extracted records from across England and Wales taken in 2013 when participants were aged ~23. However, not all participants will have complete records up to the date of the extract (record loss can have occurred during any of the following: (i) transferring paper-based to electronic records; (ii) when participants move practice; (iii) if practices change record keeping software systems; or (iv) during any amendments made to electronic records made by health

professionals). It is also important to note that ALSPAC do not have the governance approvals to extract linked health records for participants who died before the age of 18. In total, there were data on 11,810 participants linked with at least one record, with approximately 3.5 million coded entries in total. I compiled a list of GP Read Codes (the health coding system used in primary care in the UK) used to code diagnoses (see Appendices, Table S2.1) to narrow down the dataset with the aim of identifying cases of CAs.

2.3.3.2. Paediatric cardiology and cardiothoracic surgery records (HeartSuite)

HeartSuite is a fully integrated patient management system designed specifically for paediatric cardiology and cardiothoracic surgery. It includes records of paediatric cardiology and cardiothoracic surgery undertaken at University Hospital's Bristol Trust (UHBT, previously only known as Bristol Royal Infirmary). The data was sought from the UHBT cardiac team through the UHBristol Congenital Cardiac Services Information Analyst and Clinical Data Team. NHS numbers were provided to ensure accurate capture of records after the widespread adoption of the modern NHS number in 1996 (i.e., 5 years after the birth of the oldest ALSPAC-G1 participant). However, some of the medical records pre-dated the advent of NHS numbers and so I used other probable identifiers to link to these. The probable identifiers used were: ALSPAC-G0 (parents) and -G1 names, dates of birth and addresses (at recruitment and subsequently when participants moved). Many individuals had multiple records in order to capture changes in address or even name. The identifiers included not only the child's details but also, where possible, the mother's details because the antenatal, perinatal and very early post-natal tests were performed before the child was given a name. A total of 48,326 records were provided for 12,338 individuals. Early electronic records from UHBT, the STORK maternal and delivery database, contained the individual hospital numbers for each mother and child from 1991–92. These were provided back to UHBT, however, the record system had changed at some stage between then and now and so these were unfortunately not of any benefit. It was unclear which address was held by the HeartSuite database and so this necessitated that all known addresses of each member of the ALSPAC cohort be provided so as to maximise the possibility of generating a match between the databases, although the risk of duplication needed to be accounted for. All transfers of data were performed using AES-256 (a 256 bit) encryption and password protected through a secure data portal.

The data was provided by UHBT in November 2019 and included all matches found up to that date. There were 377 events, relating to 303 individuals, the majority of which (93%) were a full match including NHS number and the remaining 7% were matched using the probable identifiers mentioned above (the IDs for the records matches using probable identifiers can be made available to researchers using the data if required). There were 11 events between 1st April 1992 and 31st March 1994 with the remaining 366 events identified after January 2002 (though it should be noted that no paediatric surgery was undertaken in Bristol between these two time periods). UHBT started using Heart Suite in May 2009 and the Bristol Royal Hospital for Children in December 2004 (some previous diagnoses and procedure data from a previous system called Cardiobase were obtained which went back a further ~6 years). Of these matched records, 68 had details in the diagnosis section (including conditions such as CHDs, benign murmur, chest pain and family history of heart condition). The remainder had no diagnosis provided and may have been tested for a suspected cardiac issue, but no problem found. Participants who had CHD but who did not have surgery/a procedure or those treated at a different hospital would not be included. UHBT is a regional referral centre for paediatric cardiovascular surgery with no other hospital in the South West region providing this over the period covered by HeartSuite. It is possible some CHDs may have been detected at a very young age but were unable to be successfully treated and therefore not survivable, this may have excluded some of the early and more severe CHDs from being matched via the HeartSuite database. However, it is plausible that these cases would be identified by the fetal and child deaths data source described below.

2.3.3.3. Fetal and child deaths

I wanted the data on CAs in ALSPAC-G1 to be as comprehensive as possible, and as CAs are a cause of fetal and early child deaths I obtained data on miscarriages, terminations, fetal deaths and deaths in the first years of life. Presence of malformations, chromosome abnormalities or genetic defects were recorded whether or not they were thought to be the cause of death or reason for termination. These data came from multiple sources: (i) ALSPAC were notified by the Birth Notification System of deaths (including still births) within Avon. Whenever a baby had died outside of the Avon Health authority area, this system was notified, therefore meaning ALSPAC would have obtained information about any baby who had died in the first year of life. (ii) All deaths occurring in England and Wales were notified to the study by the Office for National Statistics (ONS). Death certificates were provided with these notifications. ALSPAC also had an arrangement to obtain any deaths that might have occurred in Scotland. (iii)

Chromosome abnormalities in some of the fetal or early childhood deaths, as well as the survivors were identified via the Cytogenetics laboratory at Southmead Hospital, who analysed samples in the South West region where a chromosomal abnormality was suspected or in those with a family history. The way that these data were collected were by ALSPAC team members visiting the cytogenetics department periodically. The records were all classified according to date of birth and details were recorded. Linkage was then independently performed for any that may have been enrolled in ALSPAC.

Professor Jean Golding, who established the ALSPSAC study, was responsible for obtaining details from the clinical records, post-mortems and death certificates and summarising these in a single document. The deaths were classified according to the system involved (nervous system, chromosomal, renal, CHD, syndrome, other, genetic). Information used for the classifications has relied on post-mortem and clinical evidence. Classes of perinatal death were based on a scale adapted from the Wigglesworth classification [117]. The Wigglesworth classification is one that, in addition to major malformations, classifies the deaths according to when the death mainly occurred or was initiated (i.e., antenatal; intrapartum (including livebirths dying of asphyxia) and features associated with preterm delivery to a livebirth. There was a miscellaneous group into which deaths that did not fall into these categories was put. If a baby born at 29 weeks died after 6 months having been suffering from immature development throughout, he/she would still be classified as a death due to preterm delivery.

2.3.3.4. Diagnosis from Avon Child Health Services

Data from the congenital malformation records of the NHS Avon Child Health Services ('child health'), who provided early years community health care services, such as school-based vaccination programmes in the ALSPAC catchment area, were linked to existing ALSAPC-G1 participants data. Only the records of children with one or more recorded CA were linked. This data source includes cases diagnosed between December 1990 and February 1993 (the date of birth range for the eligible study sample) in those living in the original ALSPAC catchment area. Diagnoses were originally reported as categories depending on the bodily system affected as well as diagnoses as free text and were given ICD codes as part of the derivation of a comprehensive set of CA data for this report (see application of ICD codes below). The linked file contained 129 children.

2.3.3.5. Sources derived from the ALSPAC cohort

(i) Antenatal, labour and postnatal (first 2 weeks) records

The database comprises detailed extractions from the clinical records covering midwife, obstetrician, paediatric (almost every baby was examined by a paediatrician) and additional (e.g. blood test results and ultrasound scans) entries from during the antenatal, labour and first two weeks of the postnatal period [118]. The data source used in the present data note comprised 6,343 babies or fetuses from the overall original ALSPAC cohort with a known birth outcome. The source was derived from all the free text in section F: 'The Liveborn Baby – at Delivery', from the 'Delivery Questionnaire' which is available to view on the ALSPAC website (https://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/). Free text descriptions of CAs were initially abstracted by a clinical geneticist according to ICD classification.

(ii) Neonatal admissions questionnaire

For each neonate (<28 days of age) admitted to hospital, whether to a Special Care Baby Unit, the Children's Hospital or elsewhere, a detailed questionnaire was completed by a single neonatal research nurse working for ALSPAC. The questionnaire was first developed by the neonatal paediatrician Dr Heather White for use in Special Care Baby Units by the Jamaican Perinatal Morbidity and Mortality Survey of Jamaica [119,120]. In total, there were 994 completed questionnaires. Of these, 989 were from the two main hospitals in Bristol at the time (Bristol Maternity Hospital and Southmead). The locations for the remaining five were 'elsewhere' with the exact location not reported on the questionnaires that were examined. In total, 60% of admissions were male and 95% were alive at discharge. I searched through each questionnaire separately and retrieved all cases of reported CAs and assigned ICD-10 codes.

(iii) Child-based questionnaires

I systematically searched questionnaire data completed by the main caregiver of the ALSPAC-G1 (for most participants the mother) in relation to the children covering the period April 1991 to December 2006 (corresponding to ALSPAC-G1 ages 1 month to 166 months). This consisted of searching free text responses from questions, mostly in relation to the health of the child. All of the questions used are listed in Appendices (Table S2.2) and can be linked back to the ALSPAC questionnaires which are available on the website (http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/). In total, I used

questions from 21 questionnaires. Response rates varied for each questionnaire, ranging from 88% completion for the first one sent at 1 month to 47% completion for the final child-based questionnaire that I considered sent at 166 months. Response rates for all 21 questionnaires can be found in the Table S2.2 (Appendices). I developed a search strategy of key terms for CAs and applied this to the text fields. I then read a small subsample of these fields to see what proportion of cases might be missed by this search (e.g., because of incorrect spelling) and updated the search with the additional (misspelt) terms. This process was repeated until it was felt all cases had been identified. The search strategy can be found in the Appendices (Table S2.3).

2.3.4. Application of ICD-10 codes to identified CA cases

In this section I describe the methods used to assign ICD-10 codes to data from the 5 data sources described above. CAs were grouped by system affected. A child could contribute to more than one system group when they had been diagnosed with multiple CAs. The ICD-10 codes used to define cases can be found in the Appendices (Table S2.4).

The EMIS and Apollo primary care data assigns any diagnosis a clinical term version-2 (CTV2) medical 'Read Code' as well as a SNOMED clinical term (CT) code. I mapped SNOMED CT codes to ICD-10 codes using the NHS digital SNOMED CT browser (SNOMED International 2017 v1.36.4, https://termbrowser.nhs.uk/). The cross-mapping of SNOMED to ICD-10 is vulnerable to discrepancies due to multiple codes sometimes presenting as a possible match. To account for this, I used best judgement with the data I had by matching the text diagnosis to the ICD-10 code text as closely as possible. There were no instances where I could not find a probable match. HeartSuite data was partially provided with ICD-10 codes. In some instances, there was a text diagnosis without an ICD-10 code. In these cases, I assigned an ICD-10 code based on the text diagnosis. The data on fetal, infant and child deaths were provided with detailed text on the anomaly present in each death. From this text, I assigned ICD-10 codes to CA cases. Diagnoses from child health were originally categorised by subgroup with text of the specific diagnoses. I assigned ICD-10 codes based on the subcategories and text. The ALSPAC delivery questionnaire data was initially assessed by a clinical geneticist who assigned ICD-10 codes based on free text descriptions. For neonatal and child-based (self-report) questionnaires, assigning codes was initially done by myself. In the first instance I grouped text diagnoses by organ or system. Any uncertainty was checked with MC and DAL. Sub-types were then assigned where possible by myself in discussion with MC and DAL.

Once I had ICD codes assigned to all three ALSPAC data sources, I then explored the overlap. Some of the reports in the ALSPAC questionnaires may be less reliable than those from other sources, such as the primary care linked data. For example, either the caregiver or I may have misattributed an abdominal problem that is not a CA to CA status. Therefore I *a priori* decided that I would only include cases where the same case (at an organ or system level) appeared in at least two of the questionnaires (**Figure 2.1**). Of all the participants with at least one ICD-10 system/organ code at the end of the initial assignment (N = 672), 64 (9.5%) appeared in at least two of the questionnaires. These (including which questionnaires they were identified in and the remaining 608 (90.5%) that only appeared in one questionnaire are shown in the Appendices (Tables S2.5 & S2.6), including which organ/system they came under. To test the assumption that those only found in one questionnaire were more likely to be false positives, I checked how many were defined as a case in the primary care dataset. Of those that appeared in one questionnaire, 21% were a CA case in the primary care data. Of those that appeared in two questionnaires, 50% were a CA case in the primary care data. I labelled the 608 that appeared in one questionnaire as 'possible CAs'. This variable will be made available to researchers that use the data described in this data note. I have not included these 608 participants with possible CAs in the following sections presenting results (overlap and description of population).

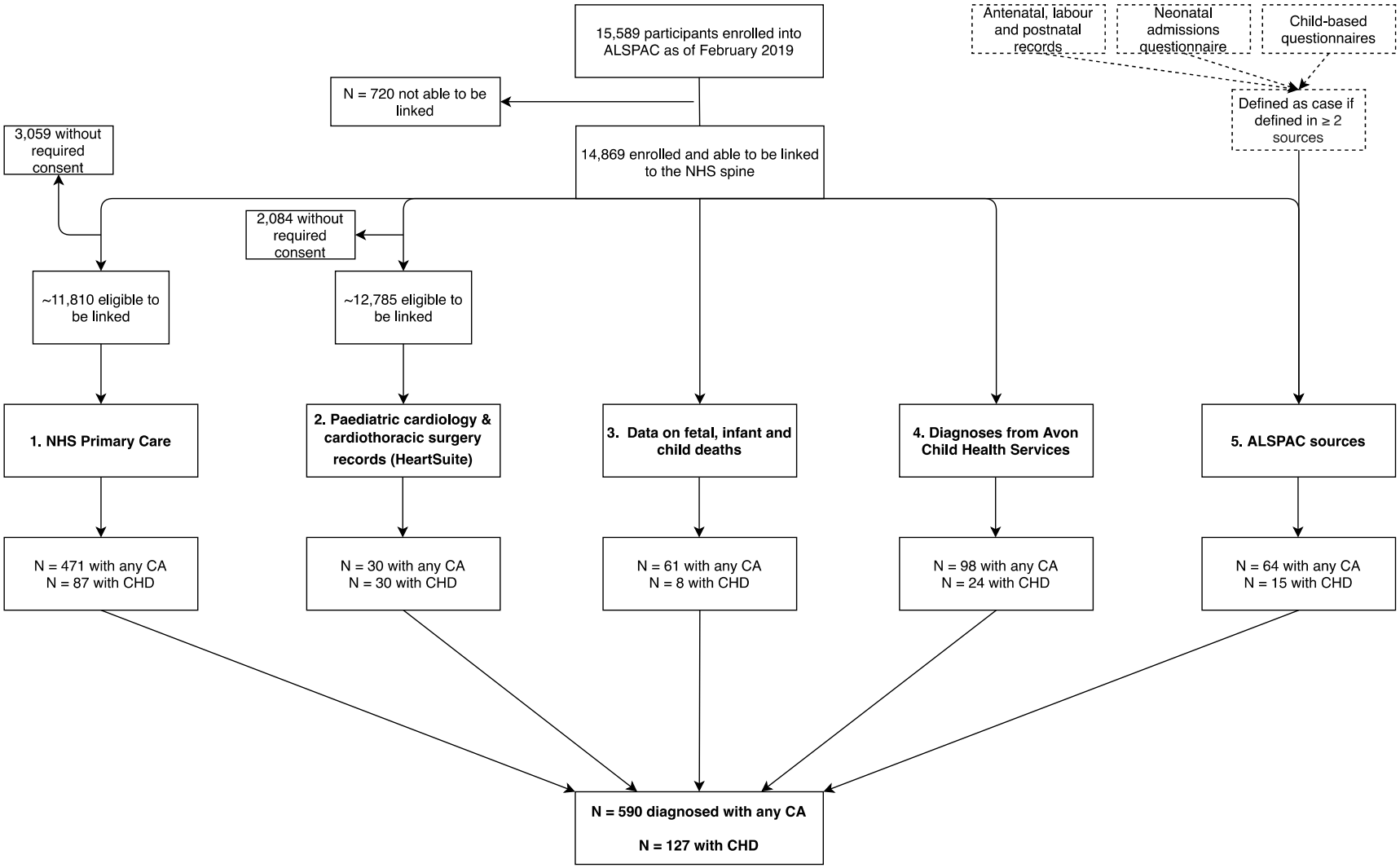


Figure 2.1. Flow diagram illustrating the multiple sources used to formulate the cases of major congenital anomalies in the ALSPAC cohort. All 30 CA cases within HeartSuite had a CHD diagnosis. Note that the potential capture population for each source may differ and cannot be definitively quantified. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; CA, congenital anomaly; CHD, congenital heart disease; NHS, National Health Service.

2.3.5. Overlap across cases and case definition in ALSPAC

I considered an ALSPAC-G1 participant to have a CA if they were identified in any of the 5 sources for the total of 'any CA' (**Figure 2.1**). For specific types of CA, these were also defined as occurring in a participant if there was evidence from any of the 5 sources. This is a liberal approach that I hope will minimise false negatives (i.e., missed cases). It might mean that I have included some false positives. I demonstrate overlap between the sources (using a Venn diagram) and future uses of the data will be able to select which sources they use (see Data access statement).

2.4. Description of population

In total, 590 ALSPAC participants were identified as having a CA with a prevalence of 385.5 per 10,000 live births (calculated using 14,791 as the total number of live births for ALSPAC). Of these 590 participants, 151 (25.6%) had a CA occurring in the presence of other anomalies. **Figure 2.2A** is a Venn diagram of the number of CA cases from each data source and how they overlap. Primary care data provided the largest number of cases with 471 of the 590 being identified via linkage to primary care. Of the 471 identified via primary care 82 were also identified in at least one other data source. The HeartSuite database contained 30 cases of any CA, all of which had CHD, whilst the mortality data included 61 cases. The child health services data source identified 98 cases and the ALSPAC data source (after limiting to the cases found in at least two of the sub-data sources) included 64 cases. **Figure 2.2B** provides the numbers for CHDs only. Of the 127 CHD cases, 87 were identified in the primary care data, with 24 of these also being identified in at least one other data source. Of the 30 cases of CHD identified by HeartSuite, 16 cases were also identified in at least one other data source. The list of deaths contained 8 cases of CHD, child health included 24 cases of CHD and the ALSPAC data source contained 15 cases of CHD. For those 8, 24 and 15, the number of cases found in at least one other data source were 7, 14 and 9, respectively.

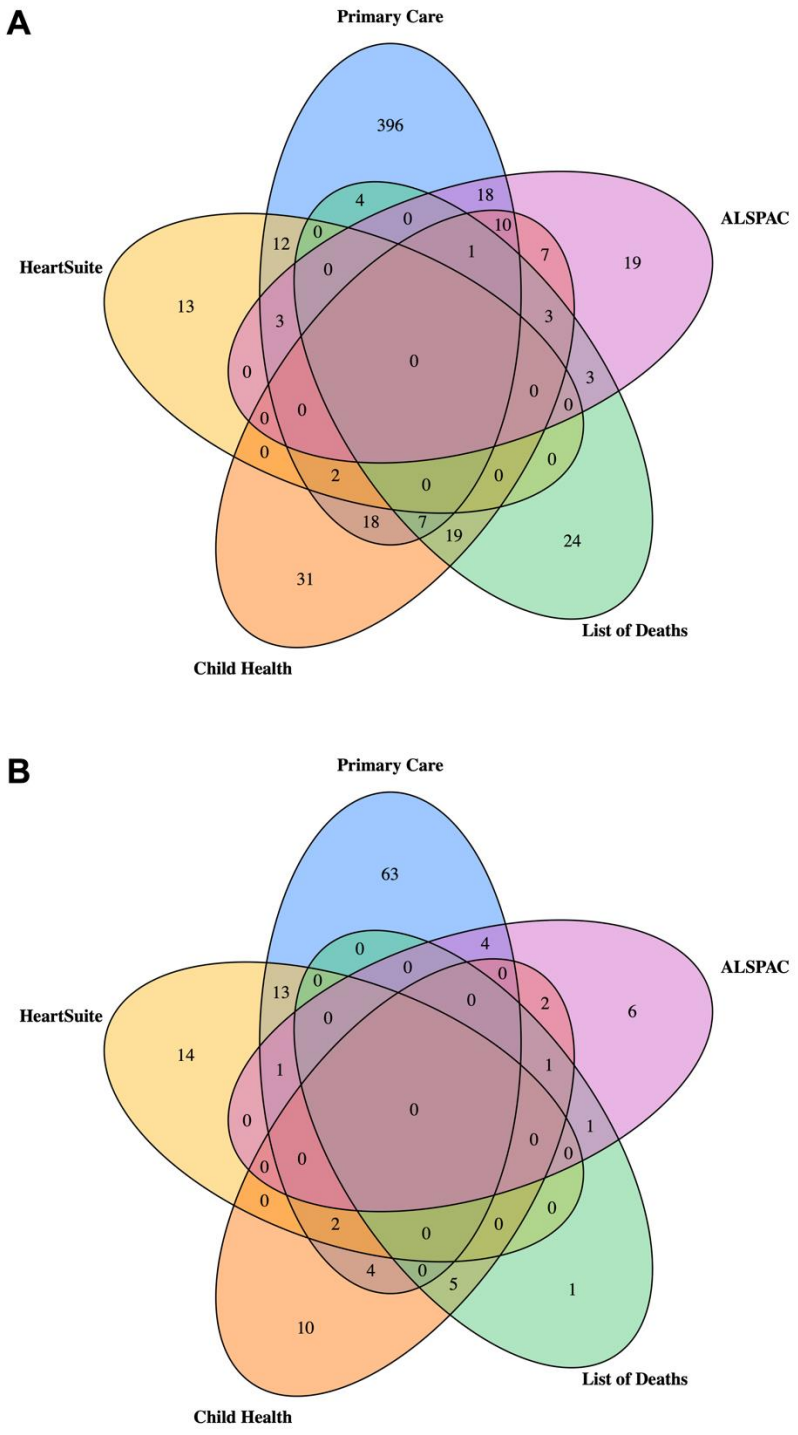


Figure 2.2. Venn diagrams illustrating the overlap between the 5 data sources for any major congenital anomaly (A; total n = 590) and any congenital heart disease (B; total n = 127) as defined by the European surveillance of congenital anomalies.

Table 2.2 reports the total number of anomalies in each subcategory and compares the prevalence in ALSPAC to the EUROCAT recorded prevalence for CAs from full European registries between the years 1990–1992 (https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence_en).

Table 2.2. Total numbers of congenital anomalies, numbers in those live born and prevalence per 10,000 live born in ALSPAC-G1 participants (total N live born = 14,791 of the 14,869 enrolled and linkable).

Anomaly subtype[a]	Total N (N born alive) [b]	Prevalence per 10,000 live births	EUROCAT prevalence per 10,000 live births [5]
Any CA	590 (570)	385.3	205.7
CHD	127 (119)	80.5	56.0
Nervous system	18 (15)	10.1	13.5
Respiratory	5 (5)	3.4	1.9
Orofacial clefts	16 (16)	10.8	14.2
Eye	29 (29)	19.6	5.6
Ear, face, neck	*	*	5.7
Digestive system	16 (14)	9.5	20.3
ABWD	*	*	2.7
Urinary	48 (44)	29.7	28.6
Genital	64 (64)	43.3	10.9
Limb	197 (196)	132.5	48.4
Other	60 (57)	38.5	-
Chromosomal	42 (39)	26.4	15.8
Teratogenic/genetic syndromes, microdeletions and chromosomal abnormalities	67 (63)	42.6	-

Abbreviations: CA, congenital anomaly; CHD, congenital heart disease; ABWD, abdominal wall defects; * used when there were fewer than 5 cases in a given category all of these would have prevalence per 10,000 <3.4.

[a] International classification of disease (ICD) codes used to define subtypes can be found in the *Appendices*.

[b] I have included all cases in ALSPAC including whether they resulted in a fetal death. I give the number live born in brackets and this is used to estimate live born prevalence for comparison with EUROCAT results.

Minor anomalies according to EUROCAT are not included. Numbers represent cases of congenital anomalies; if a child had multiple anomalies affecting different systems, they would contribute to more than one category. Each child could contribute to each category once.

It is possible that EUROCAT underestimates the total prevalence of CAs because the age range for data capture is capped at or before age 1 for 61% of the full registries and only for 28% does it go to age 5 years or beyond. By comparison, the inclusion of primary care linkage in the present sample means I have included cases that are diagnosed in participants up to their early-/mid-20s and it is notable that

primary care linkage provides the highest proportion of ALSPAC cases. Using just the primary care linked data in ALSPAC shows an increase in new cases of CAs after age 1, with the rate of increase with age slowing but continuing up to early 20s (**Figure 2.3A**), with a similar illustration for CHDs (**Figure 2.3B**). Previous analyses in the BiB cohort also demonstrated a marked increase in numbers of CA cases diagnosed after 1 year of age through record linkage to primary care data up to when participants were aged 5 years 5 (**Figures 3C & 3D**). It is possible that the liberal definition that I have used here, defining a case as being from any of the five data sources, may mean I have overestimated the prevalence in ALSPAC. However, as can be seen from the description of the different data sources above and summarised in **Table 2.1**, the different data sources cover different geographical regions at diagnosis, time periods and have different sources of missing data. If I were to exclude a particular data source, I would have missed some true cases. It is also possible that other factors that influence the risk of CAs differ between pregnancies in the early 1990s in the Southwest of England and EUROCAT data for pregnancies for the same time period across the whole of Europe.

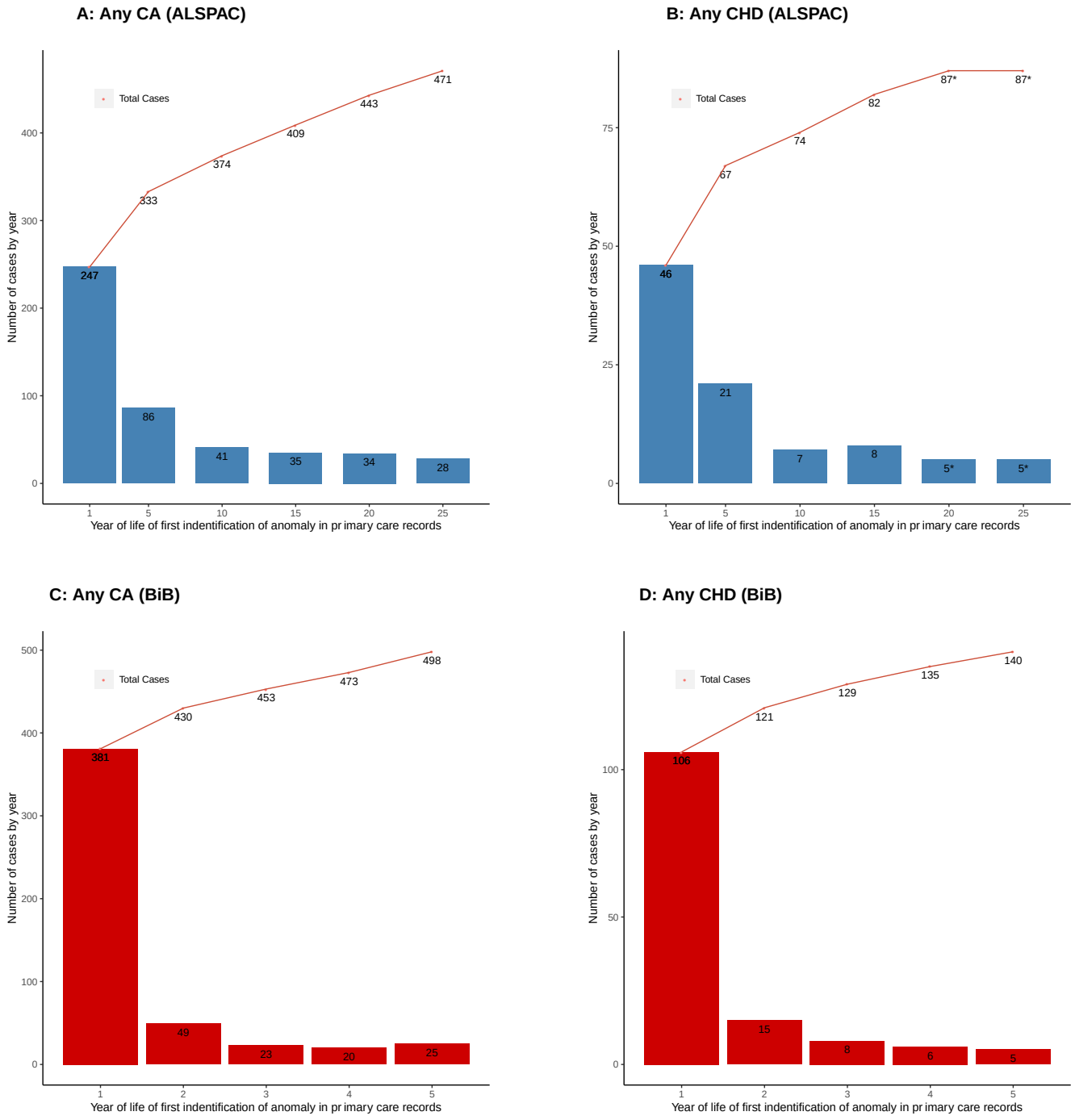


Figure 2.3. Showing the number of congenital anomaly (CA) (A) and congenital heart disease (CHD) (B) cases at different ages using linked primary care data in ALSPAC.

Age cut-offs are diagnoses in first year of life and then up until age 5, 15, 20 and 25. The age-25 column includes all diagnoses from the 2016 primary care extraction; therefore, some participants may be slightly older than 25, but younger than 26. Numbers are presented at the child level, so if a child had multiple anomaly diagnoses, they would only be counted once (at the time of their first diagnosis). For comparison, (C) and (D) show corresponding estimates for any CA and any CHD respectively from the Born in Bradford cohort primary care extraction up until age 5 (Adapted with permission from Bishop et al. (2014) [70]. Bars show the number of cases in each age category and points show the cumulative number of cases. *Cell values <5 are suppressed for disclosure control purposes (may include 0).

CHDs are the commonest form of CA and in **Table 2.3**, I report numbers for CHD subtypes. As expected, septal defects make up a large proportion of cases with 82 (65%) CHD cases having a septal defect, slightly higher than recent global estimates of around 55% [121]. Of the 127 CHD cases, 35 (28%) were classed as severe, which is higher than found in the Norwegian National birth cohort, which recruited pregnancies between 1999 and 2008 and found that 19% of CHD cases were defined as severe using a similar classification system [24]. The prevalence of CHDs in ALSPAC is similar to other European birth cohorts. In recent work involving 7 European birth cohorts, I have shown that the prevalence of CHD was close to 1% in most cohorts, with the lowest with 0.4% and the highest with 1.4% [122]. Differences in case ascertainment could be one of a number of possible explanations for the slight differences in prevalence estimates.

Table 2.3. Congenital heart disease subtypes.

CHD subtypes	N
Severe CHD [a]	35
Non-severe CHD	92
Any septal defect	82
Atrial septal defect	20
Conotruncal [b]	6
Isolated CHD [c]	110
CHD with other CAs [d]	17
CHD associated with syndrome [e]	13
Any CHD	127

Abbreviations: CHD, congenital heart disease; CA, congenital anomaly. [a] According to EUROCAT. See Table S2.4 for ICD codes. [b] Tetralogy of Fallot, transposition of great arteries, truncus arteriosus, double outlet right ventricle. [c] Those diagnosed with a CHD (or multiple CHDs) and no other congenital anomalies. [d] CHDs cooccurring with other congenital anomalies. [e] CHDs diagnosed with other syndromes (see Table 2.2 above).

2.5. Strengths and limitations of the data

A key strength of this dataset is the combination of multiple sources of data to identify cases. This enabled the capture of additional cases that might have otherwise been missed. That said, the present results indicate a strong reliance on record linkage to primary care data for case ascertainment. I have not restricted diagnoses to a particular age and here, as in other cohorts [70,123], linkage to primary care data has been essential for identifying large numbers of cases that were diagnosed after infancy. This is of particular importance for CHD diagnoses. Although CHD detection rates have improved in recent years in line with screening programs and technological advancements [124], there are still a proportion of cases that remain undiagnosed throughout early life and even into adulthood [36]. These are likely to be less severe

cases than those diagnosed antenatally or in infancy, but are important for unbiased studies of the causes, natural history and consequences of CHD. Linkage to primary care data in the UK (as in other countries) has been restricted until recently. It is appropriate that any such linkage is carefully controlled, for example through the use of a Trusted Research Environment for data storage and access, as I did through the use of ALSPAC's UK Secure eResearch Platform (SeRP). However, this research shows the importance of being able to link to these data in just one field (CHDs). I have demonstrated the importance of linking original cohort data to external data sources such as primary health records to further strengthen the platform. A further advantage is that researchers can now link the CA data that I have identified and coded to information collected on the ALSPAC participants from preconception through to adulthood and beyond. This includes, but is not limited to parental characteristics, childhood health and wellbeing, social and educational background and future outcomes that may differ between those with and without CAs. These data will provide unique opportunities to a multitude of researchers involved with CA research. In addition to this, the second generation of the ALSPAC cohort (ALSPAC-G2) is now underway [114] providing scope for future linkage and unique research opportunities, including exploring secular and birth cohort trends in the incidence and prognosis of CAs, as well as intergenerational causes [114]. CAs are prospectively collected in ALSPAC-G2 through extractions of data in antenatal, labour, neonatal and health visitor (children to age 5 years) records, parental questionnaires, linkage to ONS for deaths data and linkage to primary care data.

One limitation of this dataset is that I have not been able to successfully link to NHS Hospital Episode Statistics (HES) due to project restrictions that were in place by NHS digital at the time of data collation. An overhaul of the data sharing agreement was required, which is still ongoing at the time of writing. HES contains the records of all hospital admissions, outpatient appointments and Accident and Emergency department attendances at NHS hospitals in England [125]. This database might have provided additional cases of CAs, though given the primary care linkage I may not have identified many additional cases via HES. There are currently (March 2020) 14,819 singletons and twins enrolled in ALSPAC, who were alive at 1 year and have not subsequently withdrawn from the study. I have linked 11,810 (80%) of these participants and so may have missed some cases. At least some of those who were not eligible to be linked because of dying should have been captured by other data sources. Participants who refuse data linkage could differ notably from those who do not, but the proportion of these (~3%) is too small to notably influence any analyses with these data. Failure to link to some of the eligible (for linkage) participants will mostly reflect those who are living outside the BNSSG area and/or registered with a practice that does not use the EMIS or Apollo clinical records system. As primary care data 'follows the

patient', should any of these missing participants register with an eligible practice, then I may be able to link to additional records. Data on these participants (and any new CA diagnoses in later adulthood) would be obtained with future extractions. Furthermore, there are efforts to coordinate primary care record linkage for all cohorts across the UK. Thus, it may be possible for ALSPAC to extend linkages to additional participants as the infrastructure for primary care record linkage in the UK matures.

Another limitation is that ALSPAC-G1 participants were born before the start of transition between paper and digital health records, and that fetal anomaly screening using ultrasound scans at 18–20 weeks was not yet advanced enough to capture most cases of CAs. Therefore, antenatal and early life health data that is available now was not available for this cohort. However, I have attempted to address this in my multi-source approach to defining cases, which includes data from antenatal, labour and postnatal data extractions by ALSPAC employed research midwives. Whilst contemporary cohorts, including ALSPAC-G2 are able to benefit from the availability of advances in the governance around linking cohorts to health records and the existence of extensive electronic health data, I believe the effort to collate and code the CA data in ALSPAC-G1 participants makes a key contribution to that study; given the extensive data available on these participants this provides a valuable research resource for ALSPAC-G2. Related to this, the enrolment period for ALSPAC-G1 participants (early 1990's) predates the Southwest Congenital Anomaly Register (SWCAR) which began in 2002. The SWCAR was part of the British Isles Network of Congenital Anomaly registers and is now a member of Public Health England's National Congenital Anomaly and Rare Diseases Registration Service (NCARDRS). Future data collections (e.g., in ALSPAC-G2 participants) should be cross-validated with these registers.

The descriptions above of each data source highlight their different coverage in terms of geography and time (participant age). They also vary between linkage to mortality and coded information in health records, detailed scrutiny and extraction of data from health records and a search of text entered by parents in questionnaires about their child. I have constructed the ALSPAC-G1 CA dataset by bringing all of these data together in an attempt to have not missed any cases whilst being as transparent as possible around the methods and data sources used. I feel that combining data in the way that I have provides the best estimate of CAs in ALSPAC-G1. However, data are available with codes that clearly indicate their source, which enables any researcher who wanted to restrict main analyses to selected data sources only and/or undertake sensitivity analyses to explore whether results change if some datasets are not included. Researchers can also access and undertake analyses including (or comparing to) the 608 participants who I have defined as having 'possible' CA based on text in just one ALSPAC questionnaire.

To conclude, I have identified CAs in ALSPAC-G1 from multiple sources that are described here. The CAs have all been coded according to ICD-10 and are available to researchers. The linkage of these data to participants who are now in their late 20s and have a wealth of data from when they were in utero to the current time, including on their children as they start to become parents, makes this a powerful resource for CA research. The effort to obtain these should not be required for most contemporary birth cohorts given improved linkage systems and screening for CAs. However, it remains the case that CAs are under-researched, and some birth cohorts exclude known CAs at recruitment. This may reflect concerns that within any single cohort cases may be too few for meaningful analyses. However, with birth cohorts increasingly collaborating and sharing data, for example as in the LifeCycle collaboration [126], the potential to generate sufficient numbers for analyses is possible and I would recommend cohorts do not exclude such patients and existing (older) cohorts like ALSPAC who have not previously tried to identify all cases do so.

Chapter 3. The effect of maternal pre-/early-pregnancy BMI and pregnancy smoking and alcohol on congenital heart diseases: a parental negative control study

3.1. Chapter summary

This chapter has been published:

Taylor K, Elhakeem A, Thorbjørnsrud Nader JL, Yang TC, Isaevska E, Richiardi L, Vrijkotte T, Pinot de Moira A, Murray DM, Finn D, Mason D, Wright J, Oddie S, Roeleveld N, Harris JR, Nybo Andersen A, Caputo M & Lawlor DA. Effect of Maternal Prepregnancy/Early-Pregnancy Body Mass Index and Pregnancy Smoking and Alcohol on Congenital Heart Diseases: A Parental Negative Control Study. *The Journal of the American Heart Association* (2021). https://doi.org/10.1161/JAHA.120.020051.

In **Chapter 1**, I described study designs that can be used to explore causal inference. These included negative control and Mendelian randomisation studies and I put forward the case for these to be used in aetiological CHD research. In this Chapter (**Chapter 3**), I used parental negative exposure control analyses to explore the intrauterine effects of maternal BMI, smoking and alcohol consumption on offspring congenital heart disease (CHD). I used this approach to try and determine whether these exposures might cause CHD via intrauterine mechanisms and to explore possible confounding. Seven European birth cohorts including 232,390 offspring (2,469 CHD cases [1.1%]) were included. I used logistic regression adjusting for confounders and the other parent's exposure and combined estimates using a fixed-effects meta-analysis. Overall, I found evidence of an intrauterine effect for maternal smoking on offspring CHDs, which appeared to be driven by non-severe CHD cases. I found similar positive associations for maternal and paternal overweight and obesity categories suggesting that maternal results may be as a result of confounding. Results for alcohol were less clear as paternal data were limited. Emphasising the potential adverse effect of smoking on offspring CHD might help in supporting women of reproductive age not to start smoking and women who are smoking at the start of pregnancy to be encouraged to quit. Furthermore, understanding the mechanisms through which maternal smoking influences congenital heart disease risk could identify novel targets for prevention beyond smoking cessation.

3.2. Introduction

Congenital heart diseases (CHDs) are the most common congenital anomaly (CA), affecting 6-8 per 1000 live births and 10% of stillbirths, and are the leading cause of death from CAs [127]. Many CHD patients present with sequela from surgical intervention and late complications related to the anomaly, resulting in health problems that persist into adulthood [128,129]. The causes of CHDs are largely unknown, but intrauterine mechanisms may play a role in their underlying pathophysiology [130]. Identifying modifiable risk factors for CHDs is important for improving etiological understanding and developing preventive interventions.

Several modifiable maternal characteristics have been found to be associated with increased risk of CHDs, including maternal pre/early pregnancy body mass index (BMI) [43,65,66], smoking [55] and alcohol [41] consumption in pregnancy. Whether these are causal is unclear. A recent systematic review and meta-analysis of the association of BMI with CHDs found that risk of CHDs was higher in those whose mothers were overweight or obese at the start of pregnancy, compared with those who were normal weight. Results for underweight mothers were not reported [43], but a large cohort study consisting of >2,000,000 singletons found no clear association for maternal underweight status and CHDs [66]. These results from conventional multivariable approaches may be explained by residual confounding due to incomplete identification or adjustment for confounders. Maternal active smoking [55] and maternal exposure to alcohol [41] were both associated with offspring CHDs in recent meta-analyses. However, 68% and 69% of the studies within the meta-analyses (for maternal smoking and alcohol, respectively) did not adjust for confounders. Therefore, those studies showing associations for smoking and alcohol cannot determine whether these reflect the magnitude of a causal effect or are biased by confounding.

Negative control studies are widely used in laboratory science and in recent years have become increasingly used to explore causal effects in epidemiology [131]. The idea behind negative control studies is that either the exposure or the outcome in the real experiment is substituted for a negative control exposure (or outcome) that is not a plausible risk factor but would have similar sources of bias or confounding as in the main experiment. In epidemiology this approach has been primarily used for determining the extent to which hypothesised intrauterine and early life exposures might be associated with outcomes as a result of residual confounding [99,131]. Negative parental exposure control studies are used for this purpose. This involves comparing the confounder adjusted associations of maternal pregnancy exposures with the offspring outcome of interest to similarly adjusted associations of the same characteristics (negative controls) in the father. The assumptions of this approach are that: (i) measured

and unmeasured confounders influence the exposures in the same direction and with a similar magnitude in mothers and fathers and (ii) there is no plausible reason why the exposure in the father would affect the offspring outcome (or at a minimum the paternal association would be much weaker than in the mother). In the present study I am assuming that paternal BMI, smoking and alcohol cannot causally influence offspring CHDs through intrauterine mechanisms. Under these assumptions, if there is a causal intrauterine effect of any of the maternal pregnancy exposures, I would expect to see a maternal-specific association, with no (or a much weaker) association with the equivalent paternal exposure. Similar associations in mothers and fathers would suggest that these are largely driven by residual confounding. It is plausible that passive smoking from fathers could Influence offspring outcomes via intrauterine exposure, however, I would expect a much weaker association for fathers. As proof-of-concept maternal smoking relates strongly to lower birthweight (a known causal intrauterine effect) whereas paternal smoking has a much weaker association and when the two are mutually adjusted, the maternal remains strong whereas the weak paternal association attenuates to the null [90,131].

I aimed to explore the causal intrauterine effects of maternal pregnancy BMI, smoking and alcohol on CHDs using data from the Horizon 2020 LifeCycle project [126]. As well as the negative parental control study providing scope to explore residual confounding, the use of a large existing collaboration of birth cohorts adds benefit to this study in comparison to previous studies. First, both offspring with and without CHDs are from the same underlying populations and have been selected for inclusion and assessed in identical ways. Second, most studies of risk factors for CHDs are case control studies and these dominate meta-analysis results. These have advantages in that they have large numbers of CHD cases and hence greater statistical power than most cohorts, but they are prone to selection bias as response rates in controls are commonly low, and in some studies controls are selected from hospitals or clinics and do not reflect exposure status in the population from which the cases came [67]. Furthermore, case control studies are susceptible to information bias due to differential recall and reporting of the exposure between cases and controls [67]. Third, I have harmonised data on all exposures, confounders and outcomes. Fourth, I have large numbers, with 232,390 participants in total and 2,469 CHD cases. Lastly, the ethos of the LifeCycle collaboration is that all studies contribute to each research question unless they do not have data on either exposure or outcome, meaning publication bias is minimised.

3.3. Methods

3.3.1. Inclusion criteria and participating cohorts

This study was part of the Horizon2020 LifeCycle Project. LifeCycle is a collaboration of largely European birth cohorts that aims to determine the impact of early-life stressors on risk of developing adverse cardio-vascular/-metabolic, respiratory, cognitive and mental health outcomes (http://lifecycle-project.eu) [126]. A LifeCycle cohort was eligible for inclusion if it had information on CHD in the offspring ascertained by any method and data on at least one of the following: i) mother's pre-/early-pregnancy BMI, ii) maternal smoking during pregnancy iii) maternal alcohol consumption during pregnancy, iv) the same exposures (i-iii) measured in the father at a similar time to their pregnant partners. Eligible LifeCycle cohorts could be from any geographical area and with participants from any ethnic background. In total, seven cohorts were eligible and all participated: The Amsterdam Born Children and their Development Study (ABCD) [132], Avon Longitudinal Study of Parents and Children (ALSPAC) [111,112], Cork SCOPE BASELINE Study (BASELINE) [133], Born in Bradford (BiB) [134], Danish National Birth Cohort (DNBC) [135], Norwegian Mother, Father and Child Cohort Study (MoBa) [136,137] and Nascita e INFanzia: gli Effetti dell'Ambiente (NINFEA) [138,139]. Individual cohort descriptions can be found in the Appendices (Text S3.1). I excluded multiple births from the study population since they differ from single births for CA outcomes, with the exact cause of this being unknown, but placental dysfunction being one hypothesized mechanism [140,141]. As well as this, removing multiple births improves the external validity of findings since the majority of studies performed in aetiologic CHD research are performed in singletons (e.g., the largest study for maternal overweight and obesity) [65]. Some previous studies have excluded infants with any known chromosomal or genetic defects on the assumption that modifiable risk factors are unlikely to contribute in the presence of known causes. I have not made these exclusions in my main analyses since it is plausible that CHD in children with these complex syndromes are also influenced by the modifiable exposures I explore here. Furthermore, from a public health and clinical perspective I believe it is useful to know effects for all CHD cases. In additional analyses I explore whether their removal alters my main results.

3.3.2. BMI, smoking and alcohol measurements

I used harmonised LifeCycle data for exposure and confounder data, with the exclusion of paternal alcohol consumption which had not been harmonised by LifeCycle when I started this project [142]. ABCD

and BASELINE were not part of the core LifeCycle cohorts and therefore not part of phase 1 data harmonised data that I used here. I harmonised the data for these cohorts to resemble the harmonised LifeCycle variables. Cohort-specific information on methods of data collection can be found in Appendices (Table S3.1).

LifeCycle harmonised maternal BMI used measured or self-reported pre-/early-pregnancy weight and height. Pre-pregnancy weight was prioritised and if not available the earliest pregnancy measures were used. Paternal BMI was similarly reported (by the father or their pregnant partner) or measured and I prioritised the timing to be pre- or as early as possible in their partners pregnancy. BMI was used as a continuous variable for the main analyses. In cohorts that had >100 CHD cases, I also categorised BMI as underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5 to <25 kg/m^2), overweight (BMI 25 to <30 kg/m^2) and obese (BMI ≥30 kg/m^2). ALSPAC, BiB, DNBC and MoBa contributed to these analyses.

I used two LifeCycle smoking variables for maternal and paternal smoking at the time of pregnancy: (i) smoking in the first trimester (yes/no) where this was available, otherwise any smoking during pregnancy (yes/no) and (ii) categorised into non-smokers, light (< 10 cigarettes smoked per day) and heavy (≥ 10 cigarettes per day) throughout the entire pregnancy. Paternal smoking was categorised as 'any smoking (yes/no)' at the time of their partners pregnancy.

I used two LifeCycle variables for maternal alcohol consumption: (i) binary (yes/no), which like smoking prioritised the first trimester if available but was otherwise any alcohol intake during pregnancy and (ii) categorised into non-drinkers (none), light (>0 and <3 units per week) and moderate/heavy (≥3 units per week) drinkers during pregnancy. Two studies (ALSPAC and MoBa) had data on paternal alcohol consumption in pregnancy and thus were able to harmonize variables relating to paternal alcohol for this project. I generated one variable, categorised as: non-drinkers, light (>0 and <7units per week) or moderate/heavy (≥7 units per week) drinkers (Text S3.2).

The rationale for prioritizing maternal pregnancy smoking and alcohol during the first trimester is because fetal cardiac development starts early in pregnancy and much of the development occurs in the first trimester [143]. 47% and 96% of mothers had measures specifically in the first trimester for smoking and alcohol, respectively.

3.3.3. Congenital heart disease outcomes

Information on CHDs was retrieved from a variety of sources depending on the cohort. ALSPAC, BiB, DNBC and NINFEA had International Classification of Diseases v10 (ICD-10) coded data. BASELINE had

individual CHD diagnoses assigned by a cardiologist based on echocardiography. For ABCD and MoBa, I had a non-specific CHD diagnosis (yes/no). Data in ABCD, BASELINE, DNBC, and NINFEA were restricted to liveborn infants, whereas other cohorts included stillbirths.

In the ABCD cohort, data on CHDs in liveborn children were obtained from three different sources: (i) the infant questionnaire, which was filled out by the mother at an average infant age of 12.9 weeks, (ii) the questionnaire filled out by the mother at an average child age of 5.1 years, and (iii) clinical data of the Youth Health Care Registration. In the ALSPAC cohort, cases were obtained from a range of data sources, including health record linkage and questionnaire data up until age 25 years following European Surveillance of Congenital Anomalies (EUROCAT) guidelines [35]. In BASELINE, at 2 months, mothers were asked of any medical problems and/or referrals. If a baby had been referred to a specialist, it was checked by a cardiologist to see if they had results from an echocardiogram with exact diagnoses reported. Further diagnoses up until age 12 years were identified through records from the echocardiogram. In the BiB cohort, there were two separate sources to identify CAs. Both sources were used in this study: (i) CAs up to 5 years of age, identified in primary care records by Bishop et al [144] following EUROCAT guidelines. ICD-10 codes were mapped to clinical term (CT)-V3 codes prior to extraction from primary care records. (ii) Data extracted from the Yorkshire and Humber CAs register database. Data were ICD-10 coded. All of these were confirmed postnatally. In the DNBC, all diagnoses of congenital anomalies (according to EUROCAT guide 1.4 section 3.2 and 3.3) up until the age of 15 years were extracted from the Danish National Patient Register (DNPR) which is linked to the cohort data [145,146]. Diagnoses were ICD-coded. These data were restricted to children born alive. In MoBa, information on whether a child had a CHD or not was obtained though linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN. In the NINFEA cohort, CHDs were reported in the second questionnaire compiled 6 months after birth. Mothers compiled a checklist that included pre-specified anomalies. If the child died or had any surgery performed in the first 6 months, the cause of death and type of surgery were also checked to see if any CA was reported. Data were coded using ICD-10 codes by an experienced paediatrician and were reassessed by an independent physician. Further details of the sources of data for CHDs in each cohort are provided in the Appendices (Text S3.3).

In all studies, the main outcome was any CHD. Where data allowed (i.e., when I had full ICD-codes), any CHD was defined according to EUROCAT, which excludes isolated patent ductus arteriosus (PDA) and peripheral pulmonary artery stenosis in preterm births (gestational age <37 weeks) (Table S3.2). I also categorised cases into severe CHD (heterotaxia, conotruncal defect, atrioventricular septal defect, anomalous pulmonary venous return, left ventricle outflow tract obstruction, right ventricle outflow tract

obstruction, other complex defects) and the remainder as non-severe CHD (PDA [in full term infants], valvular pulmonary stenosis, ventricular septal defect [VSD], atrial septal defects [ASD], unspecified septal defects, isolated valve defects, other specified heart defects, unspecified heart defects) [24,25] (Table S3.2).

3.3.4. Confounders

Analyses were adjusted for a number of confounders based on their known or plausible influence on one or more of the maternal pregnancy exposures and on CHD: Maternal age (all exposures), parity (all exposures), ethnicity (all exposures), socioeconomic position (SEP; all exposures), smoking (for BMI and alcohol analyses), alcohol use (for BMI and smoking analyses). In the paternal negative control analyses confounders were similar: fathers' age (all exposures), number of children (all exposures), ethnicity (all exposures), SEP (all exposures) smoking (for BMI and alcohol), alcohol use (for BMI and smoking). I also adjusted for offspring sex in all adjusted analyses. I used educational attainment for both parents' measures of SEP. Full details of my selection and harmonization of confounders is provided in the Appendices (Text S3.4).

3.3.5. Statistical analysis

Analyses were conducted in either R (version 3.6.1) or Stata (version 16). An analysis plan was written and published in October 2019, with any subsequent changes and their rationale documented in the publication [147]. All associations between exposures and CHDs were performed within participating studies using logistic regression (binary for main analyses and multinomial for CHD severity analyses). In the two largest cohorts (DNBC and MoBa), I assessed deviation from linearity in the models in the BMI analyses by running the main confounder adjusted model with BMI split into fifths. I ran regression models with these fifths as four indicator variables (non-linear) and compared this model with one in which the fifths were treated as a continuous (score) variable. I used a likelihood ratio comparison to compare these two models. All analyses were run (i) unadjusted, (ii) adjusted for maternal/paternal age, SEP, parity, ethnicity, smoking and/or alcohol (depending on exposure) and offspring sex and (iii) adjusted for all confounders (as in (ii)) as well as the other parents exposure. In the adjusted models, studies were asked to adjust for as many of the confounders as possible. All analyses were performed with maximal numbers (i.e. numbers included in each model will vary due to missing data on exposure/outcome or confounders). In a sensitivity analysis, I repeated the main analyses using complete-case data to assess whether missing data were influencing the results.

For the main negative control analyses – i.e., where I directly compared maternal to paternal exposure-CHD associations – I used multivariable logistic regression in which both maternal and paternal exposures were adjusted for the other parent's exposure. This produces a maternal association that adjusts for maternal confounders as well as the paternal exposure, and similarly a paternal association adjusting for paternal confounders and the maternal exposure. The rationale for mutually adjusting for the other parent's exposure is that parental BMI, smoking and alcohol may relate to each other through assortative mating and/or convergence of behaviours that occurs over time in couples [148]. Causal structural graphs together with simulated data show failure to undertake this mutual adjustment will bias the negative control analysis results [149]. Also, paternal exposures may have some intrauterine impact, for example via passive smoking or paternal support for the mother to reduce alcohol and have a normal BMI during her pre-conceptual period or in pregnancy [150]. Mutual adjustment for maternal and paternal confounders was necessary for ensuring both parental results were fully adjusted. Comparisons between maternal and paternal associations from this model were assessed by visually comparing the two results. In addition, statistical evidence of any differences was obtained by calculating differences in log odds of CHD between the fathers' and mothers' associations and report the corresponding P-value (P_{diff}), under the null hypothesis that there is no difference between the maternal and paternal estimate.

Analyses were conducted separately in each study and then meta-analysed using the *meta* package in R [151]. All the data used in the present study originated from European birth cohorts, with broadly similar methods and therefore, I assumed that they were each estimating an association from the same underlying populations and used a fixed-effects meta-analysis. To explore this assumption, differences between studies were assessed using I^2 and Cochrane Q P-values for heterogeneity [152].

3.3.6. Additional Analyses

I repeated the main, and subgroup (by CHD severity) analyses after excluding infants with any known chromosomal/genetic or maternal drug effects. Methods of data collection and definition of these variables can be found in Appendices (Table S3.3). I also repeated analyses in mothers only including those with smoking data in the first trimester. Folic acid supplementation has been shown to lower risk of birth defects and adverse pregnancy outcomes [48,153]. I repeated the adjusted maternal analyses with additional adjustment for first trimester folic acid supplementation (yes/no).

3.4. Results

3.4.1. Participant characteristics

Figures S3.1-S7 in the Appendices show flowcharts designating the assignment of participants into analysis groups for each cohort. In total, 7 cohorts including 232,390 offspring with 2,469 CHD cases (1.1%) were included. The prevalence of CHD was close to 1% in most cohorts, with the lowest being in ABCD (0.4%) and the highest in DNBC (1.4%) (**Table 1**). **Table 1** shows the distributions of maternal and paternal characteristics for each cohort. Mean maternal age across the cohorts was broadly similar (all late 20s to early 30s). Mean BMI was also similar across the cohorts but proportions in different categories varied, with the lowest prevalence of pre-/early-pregnancy obesity seen in NINFEA (5%) and the highest in BiB (21%). There was also variation in maternal smoking and alcohol consumption across the cohorts, with notably high levels of both smoking (25% and 26%, respectively) and alcohol (55% and 45%, respectively) in ALSPAC and DNBC. Fathers were generally older than mothers and more likely to smoke and drink alcohol, with the overall patterns of between study differences being similar to those for the mothers. There were differing levels of missing data in each cohort (summarised in Table S3.4 and also illustrated in cohort specific flow charts (Figures S3.1-S3.7). To check whether missing data influenced any of the results, I report complete-case analysis results for our main analyses in the Appendices. Overall, complete-case results from meta-analyses were comparable (Tables S3.5-S3.8). Below, I present the main results separated by exposure. I include supplementary results for BMI (Figures S3.8-S3.20 & Tables S3.9-3.10), smoking (Figures S3.21-S3.27) and alcohol (Figures S3.28-S3.32 & Table S3.11) analyses in the Appendices.

Table 3.1. Characteristics of the participating cohorts.

	Category	ABCD *N* = 8,131	ALSPAC *N* = 13,049	BASELINE *N* = 1,436	BiB *N* = 12,799	DNBC *N* = 89,107	MoBa *N* = 101,975	NINFEA *N* = 5,893
Country		Netherlands	UK	Ireland	UK	Denmark	Norway	Italy
Recruitment period		2003-2004	1991-1992	2008-2011	2007-2011	1996-2002	1999-2008	2005-2016
Offspring								
CHD	Any	34 (0.4)	103 (0.8)	10 (0.7)	145 (1.1)	1264 (1.4)	879 (0.9)	34 (0.6)
CHD severity in those with CHD	Non-severe	-	73/103 (70.9)	-	93/145 (64.1)	896/1264 (70.9)	-	27/34 (79.4)
	Severe	-	30/103 (29.1)	-	52/145 (35.9)	368/1264 (29.1)	-	7/34 (20.6)
Chromo/Genetic defects [a]		26 (0.3)	58 (0.4)	-	198 (1.5)	698 (0.8)	169 (0.2)	7 (0.1)
Maternal								
Age, years		30.7 (5.3)	28.9 (4.8)	30.7 (4.4)	26.0 (5.7)	29.9 (4.3)	30.2 (4.6)	33.1 (4.3)
BMI, kg/m^2		23.1 (4.1)	22.6 (4.4)	24.4 (4.1)	26.0 (5.7)	23.6 (4.3)	24.0 (4.3)	22.5 (3.8)
BMI categories	Underweight (<18.5)	360 (4.9)	1271 (11.6)	23 (1.6)	444 (4.4)	3861 (4.5)	3077 (3.2)	501 (8.5)
	Normal (18.5 to <25)	5270 (71.8)	7426 (67.7)	914 (63.6)	4586 (45.4)	57894 (67.8)	63706 (65.4)	4156 (70.5)
	Overweight (25 to <30)	1245 (17.0)	1537 (14.0)	345 (24.0)	2952 (29.2)	16578 (19.4)	21280 (21.8)	826 (14.0)
	Obese (≥30)	467 (6.4)	736 (6.7)	154 (10.7)	2127 (21.0)	7017 (8.2)	9337 (9.6)	286 (4.9)
Pregnancy smoking	Yes^	769 (9.5)	3147 (24.7)^	357 (24.9)^	1788 (16.4)	22514 (26.0)^	9650 (9.6)	472 (8.1)^
	Light	-	1684 (15.7)	-	1362 (12.5)	15777 (17.9)	7856 (7.7)	438 (7.5)
	Heavy	-	1096 (10.2)	-	426 (3.9)	7431 (8.5)	1587 (1.6)	30 (0.5)
Pregnancy alcohol	Yes^	1686 (20.8)	6894 (54.6)^	527 (36.7)	-	38733 (44.7)^	22799 (27.7)^	1508 (25.8)^
	Light	-	3044 (46.8)	-	-	46774 (52.9)	10461 (12.4)	1416 (24.4)
	Mod/Heavy	-	871 (13.4)	-	-	3717 (4.2)	509 (0.6)	230 (3.9)
Parity	Nulliparous	4500 (55.3)	5645 (45.0)	1436 (100)	4912 (39.8)	42203 (47.4)	46988 (46.9)	4070 (72.4)
Education	Low	4035 (49.6)	2374 (20.0)	-	5717 (56.9)	22225 (27.6)	2735 (2.9)	278 (4.8)
	Medium	2225 (27.4)	7985 (67.1)	208 (14.6)	1563 (15.6)	17756 (22.0)	31430 (33.1)	1892 (32.4)
	High	1871 (23.0)	1538 (12.9)	1219 (85.4)	2769 (27.6)	40675 (50.4)	60847 (64.0)	3677 (62.9)

Folic acid supp	Yes	5677 (70.7)	1070 (8.5)	-	-	56998 (69.0)	74466 (74.3)	4741 (82.5)
Paternal								
Age, years		35.1 (5.8)	30.9 (5.8)	32.2 (4.8)	30.4 (6.6)	32.2 (5.2)	32.7 (5.4)	36.2 (5.2)
BMI, kg/m^2		25.0 (3.5)	25.2 (3.3)	26.8 (3.6)	26.8 (4.7)	25.2 (3.2)	25.8 (3.3)	24.8 (3.2)
BMI categories	Underweight (<18.5)	28 (0.8)	41 (0.5)	2 (0.2)	53 (1.9)	271 (0.4)	242 (0.2)	43 (0.75)
	Normal (18.5 to <25)	1966 (54.8)	4308 (53.3)	345 (30.9)	953 (35.0)	33502 (53.5)	42952 (44.4)	3332 (58.4)
	Overweight (25 to <30)	1372 (38.2)	3111 (38.5)	594 (53.3)	1137 (41.7)	24529 (39.2)	43888 (45.3)	1977 (34.6)
	Obese (≥30)	223 (6.2)	616 (7.6)	174 (15.6)	582 (21.4)	4335 (6.9)	9759 (10.1)	355 (6.2)
Smoking	Yes	-	3459 (37.9)	277 (24.9)	1021 (32.0)	26242 (30.9)	27803 (27.3)	-
Alcohol	None	-	449 (5.5)	-	-	-	2963 (4.1)	-
	Light drinking	-	4251 (51.8)	-	-	-	59577 (82.3)	-
	Mod/heavy drinking	-	3505 (42.7)	-	-	-	9882 (13.6)	-
Education	Low	190 (8.5)	2959 (25.9)	-	4299 (52.9)	17069 (21.8)	4245 (4.4)	956 (16.6)
	Medium	398 (17.9)	6391 (55.9)	-	1115 (13.7)	28230 (36.0)	43576 (45.1)	2464 (42.8)
	High	1670 (73.9)	2079 (18.2)	-	2709 (33.3)	33118 (42.2)	48782 (50.5)	2335 (40.6)

Data are means ± SD or *n* (%). Study N's are based on singletons with data on at least one outcome and one exposure. '-' indicates data were not available. Light smoking, <10 cigarettes per day; heavy smoking, ≥10 cigarettes per day; maternal light drinking, >0 and <3 units per week during pregnancy; maternal moderate/heavy drinking, ≥3 units per week during pregnancy; paternal light drinking, >0 and <7 units per week; paternal moderate/heavy drinking, ≥7 units per week. Abbreviations: ABCD, The Amsterdam Born Children and their Development Study; ALSPAC, The Avon Longitudinal Study of Parents and Children; BiB, The Born in Bradford Study; DNBC, The Danish National Birth Cohort; MoBa, the Norwegian Mother, Father and Child Cohort Study; NINFEA, (Nascita e INFanzia: gli Effetti dell'Ambiente; Birth and Childhood: Effects of the Environment); BMI, body mass index; kg, kilogram; m, meters; mod, moderate; supp, supplementation; CHD, congenital heart disease; CA, congenital anomaly.

[a] Chromosomal/genetic/teratogenic anomalies with a cause thought to be already known (see Table S3.2 for classifications).

^ Denotes that the study had data specifically during the first trimester.

Numbers in the moderate/heavy columns for smoking and alcohol do not add up to the number of any smoking/alcohol because some studies used trimester-specific data for the binary data, whereas the moderate/heavy is an assessment of the exposure throughout pregnancy. E.G. For DNBC "light drinking" has higher numbers than "yes any" because the "yes" data are for first trimester only whereas light drinking was any light drinking across the entire pregnancy.

3.4.2. BMI and CHDs

In confounder and other parent BMI adjusted analyses, there was no difference in the odds of offspring CHD per 1kg/m^2 difference in maternal BMI (OR: 1.00, 95%CI: 0.99, 1.02) or paternal mean BMI (OR: 1.01, 95%CI: 0.99, 1.03) (P_{diff} = 0.43), with both being close to the null (**Figure 3.1A**). Unadjusted and confounder only adjusted results did not differ notably from those presented in **Figure 3.1** (Figure S3.8). The odds of CHD did not clearly increase linearly in mothers or fathers in DNBC or MoBa (Figures S3.9 and S3.10 and accompanying supplementary text). Analyses of continuously measured BMI with CHD cases separated into non-severe and severe showed similar null associations for both mothers and fathers (Figure S3.11).

In analyses of BMI categories, there were increased odds of offspring CHD in overweight and obese mothers and fathers compared with those of a normal BMI, with similar magnitudes of association in both parents (P_{diff} overweight = 0.65 & P_{diff} obese = 0.83) (**Figure 3.1B**). Underweight mothers had an increased odds of offspring CHD, whereas underweight fathers had a decreased odds of offspring CHD. Because of very small numbers of underweight parents, particularly fathers, however, results were imprecise with wide confidence intervals and there was no statistical evidence for between parental differences for underweight (P_{diff} underweight = 0.27). Individual study results for BMI categories are shown in Figures S3.15-S3.17). Positive parental associations of overweight and obesity were also observed for both non-severe (**Figure 3.1C)** and severe (**Figure 3.1D)** CHDs, with similar magnitudes of association in mothers and fathers. Individual study results for BMI categories and CHD severity are shown in Figures S3.18-S3.20.

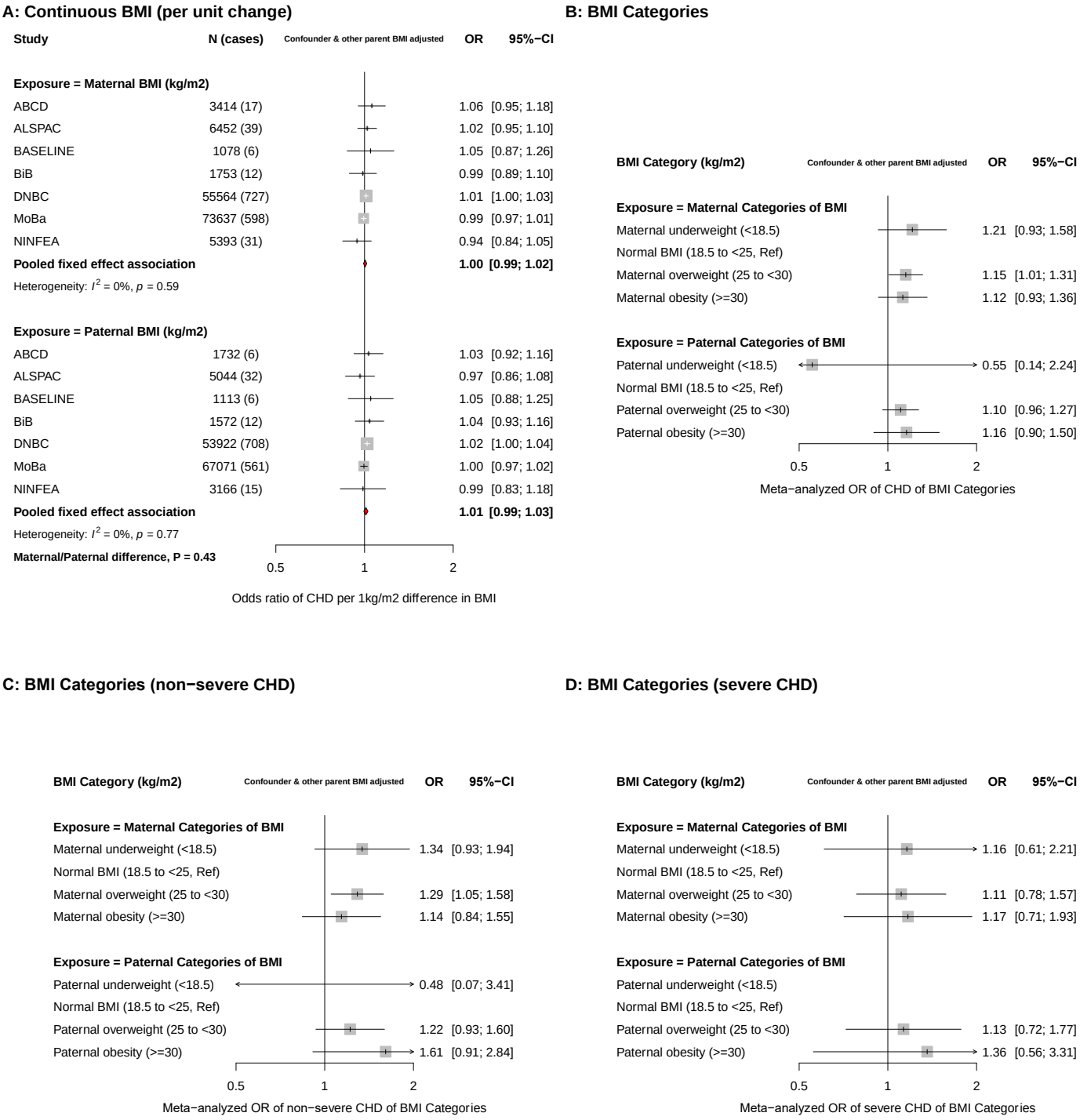


Figure 3.1. Associations between maternal and paternal pre/early pregnancy body mass index (BMI) and offspring congenital heart disease (CHD).

Figure 3.1A shows odds ratios of CHD for a one-unit (1kg/m^2) difference in maternal BMI (top graph) and paternal BMI (bottom graph) in each study and pooled across studies. Figure 1B shows the pooled (across ALSPAC, BiB, DNBC, MoBa) results for maternal (top) and paternal (bottom) BMI categories. Results are odds ratios of CHD in comparison to normal BMI. Figures 3.1C and 3.1D show odds ratios of non-severe CHD and severe CHD respectively for BMI categories in comparison to normal BMI (pooled across ALSPAC, BiB, DNBC, MoBa). All results are adjusted for confounders (depending on cohort: maternal and paternal age, education, ethnicity, smoking, alcohol, maternal parity and offspring sex) as well as the other parents BMI. The study specific results for BMI categories are shown in Appendices Figures S3.15-S3.20. In Fig.

3.1D, there were too few cases with paternal BMI data to report results. Abbreviations: BMI, body mass index; kg, kilogram; m, meter; ABCD, The Amsterdam Born Children and their Development Study; ALSPAC, Avon Longitudinal Study of Parents and Children; BASELINE, Cork SCOPE BASELINE Study; BiB, Born in Bradford; DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother, Father and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente.

3.4.3. Smoking and CHDs

In confounder and other parental smoking adjusted analyses maternal smoking in pregnancy was associated with increased odds of CHD (OR: 1.11, 95%CI: 0.97, 1.25), whereas paternal smoking at the time of their partners pregnancy did not increase odds of offspring CHD (OR: 0.96, 95%CI: 0.85, 1.07) (P_{diff} = 0.09) (**Figure 3.2A**). When removing offspring with a chromosomal/genetic defect, there was stronger statistical evidence of a difference between maternal and paternal smoking (P_{diff} = 0.02) (**Figure 3.2B)**. Results for unadjusted analyses were consistent with the confounder and mutual parent smoking adjusted result, whereas confounder only analyses were slightly attenuated for maternal smoking (Figure S3.21). Maternal smoking results were similar when analyses were restricted to studies with confirmed first trimester smoking (Figure S3.22). A positive association between maternal smoking and offspring CHD was also seen with non-severe CHDs (OR: 1.22, 95%CI: 1.04, 1.44), though not with severe CHDs (OR: 0.90, 95%CI: 0.69, 1.17) (**Figures 3.2C & 3.2D** & Figure S3.23). When I analysed maternal smoking frequency categories (i.e. none, light and heavy smoking), the results did not support an effect of heaviness over and above what I saw with any smoking (Figure S3.24). The maternal and paternal associations for these categories were statistically consistent (P_{diff} = 0.25 & 0.38 for light and heavy smoking, respectively).

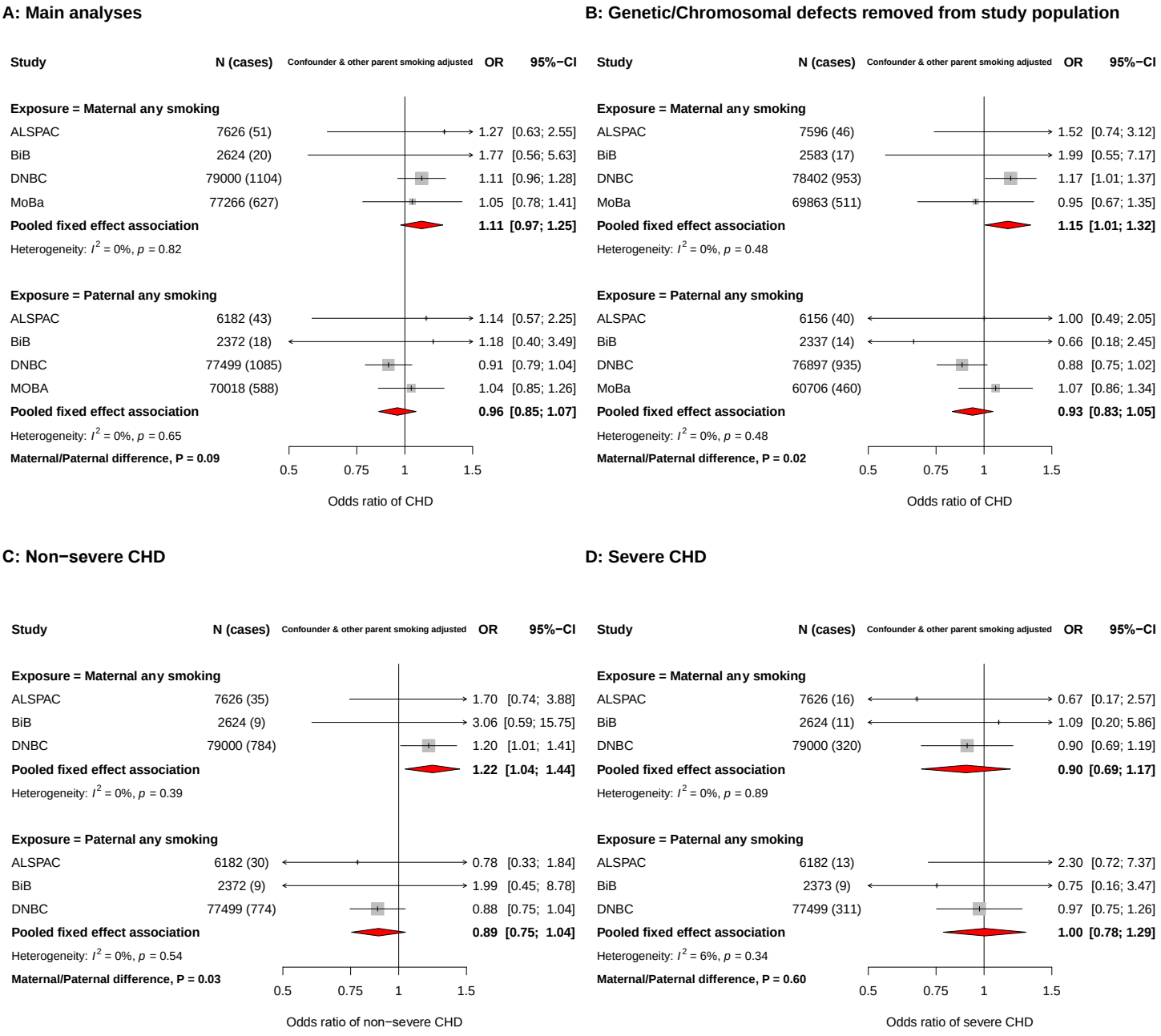


Figure 3.2. Associations in each study and pooled across studies for maternal and paternal pregnancy smoking and offspring congenital heart disease (CHD).

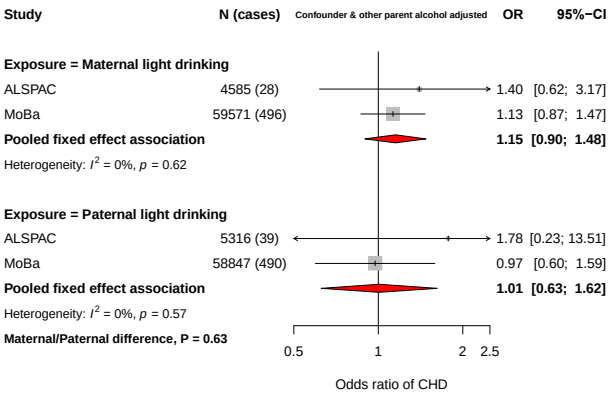
Maternal first trimester smoking was prioritised and used where possible. Figure 3.2A shows odds ratios of any CHD for maternal smoking during pregnancy (top graph) and paternal smoking (bottom graph). Figure 3.2B shows odds ratios of any CHD after removing those with a chromosomal/genetic defect from the study population. Figures 3.2C and 3.2D show odds ratios of non-severe CHD and severe CHD respectively. All results are adjusted for confounders (depending on cohort: maternal and paternal age, education, ethnicity, alcohol, maternal parity and offspring sex) as well as the other parents smoking. Abbreviations: ABCD, The Amsterdam Born Children and their Development Study; ALSPAC, Avon Longitudinal Study of Parents and Children; BASELINE, Cork SCOPE BASELINE Study; BiB, Born in Bradford; DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother, Father and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente.

3.4.4. Alcohol and CHDs

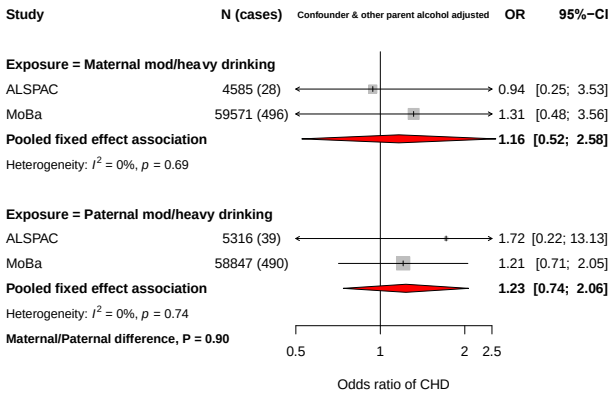
Due to lack of relevant paternal data, I was unable to undertake negative control analyses for any first trimester alcohol consumption. Maternal only associations for that exposure are presented here followed by the negative control analyses for levels of alcohol intake at any time in pregnancy. With adjustment for all confounders, any maternal first trimester alcohol consumption was not associated with odds of offspring CHD in meta-analyses from 5 cohorts (OR: 1.03, 95%CI: 0.94, 1.13) (Figure S3.28). There was a small increase in risk when restricting these analyses to non-severe CHD (OR: 1.07, 95%CI: 0.93, 1.22) although confidence intervals included the null. Associations for severe CHD were null (OR: 0.91, 95%CI: 0.73, 1.12) (Figure S3.29).

In confounder and other parental alcohol adjusted analyses, there was weak evidence of an association between maternal light alcohol intake and CHDs (OR: 1.15, 95%CI: 0.90, 1.48), which appeared stronger that than seen for paternal alcohol (OR: 1.01, 95%CI: 0.63, 1.62), though with no strong statistical support for a difference (P_{diff} = 0.63). Associations for moderate/heavy intake were consistent for maternal and paternal alcohol (P_{diff} = 0.90) with point estimates showing weak positive associations, but with wide confidence intervals that included the null (**Figure 3.3A and 3.3B**). I did not test associations between levels of alcohol intake and CHD severity due to small numbers. Due to the small number of cohorts having paternal alcohol data, I also show confounder adjusted models (without mutual paternal adjustment) for maternal alcohol intake (**Figure 3.3C**). The point estimate for maternal light drinking was very close to the null and that for heavy drinking suggested it resulted in increased risk of offspring CHD. However, both of these estimates had wide confidence intervals due to relatively few women reporting drinking (particularly heavily) during pregnancy. Results in unadjusted analyses were unchanged (Figure S3.30).

A: Parental light drinking negative control analyses



B: Parental mod/heavy drinking negative control analyses



C: Maternal alcohol (confounder adjusted without paternal adjustment)

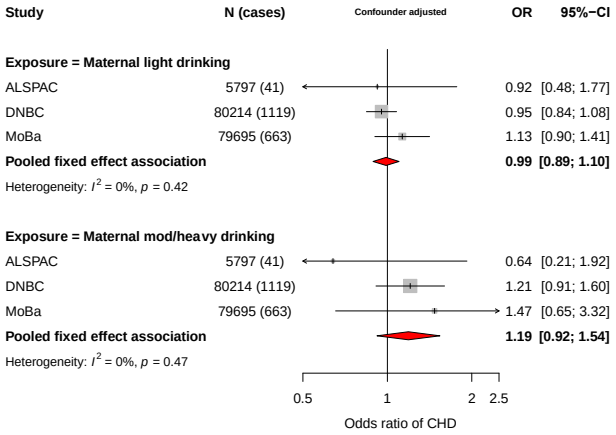


Figure 3.3. Associations in each study and pooled across studies for maternal and paternal pregnancy alcohol intake and offspring CHDs.

Figure 3.3A shows confounder and other parent's alcohol adjusted odds ratios of any CHD for maternal light drinking during pregnancy (top graph) and paternal light drinking (bottom graph). Figure 3.3B shows confounder and other parent's alcohol adjusted odds ratios of any CHD for maternal moderate/heavy drinking during pregnancy (top graph) and paternal moderate/heavy drinking (bottom graph). Figure 3.3C shows confounder adjusted odds ratios of any CHD for maternal light drinking during pregnancy (top graph) and maternal mod/heavy drinking (bottom graph). Confounders (depending on cohort): maternal and paternal age, education, ethnicity, smoking, maternal parity, offspring sex (and other parental alcohol intake in panels A & B). Definitions for maternal/paternal alcohol intake are described in the methods section. Abbreviations: ABCD, The Amsterdam Born Children and their Development Study; ALSPAC, Avon Longitudinal Study of Parents and Children; BASELINE, Cork SCOPE BASELINE Study; BiB, Born in Bradford; DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother, Father and Child Cohort Study; NINFEA, Nascita e INFanzia: gli Effetti dell'Ambiente.

3.4.5. Between study heterogeneity and additional analyses

I have included heterogeneity statistics (I^2 and $P_{heterogenity}$) in all figures. Analyses of continuously measured BMI and severe CHDs in additional analyses (Figure S3.14) and BMI analysed as categories with severe CHDs (Figures S3.19-3.20) were the only results where I found any statistical evidence of heterogeneity. Across the remaining analyses for all exposures there was no strong evidence of between study heterogeneity. Removal of those with any known genetic/chromosomal defects from the study population did not notably alter any main or severity subgroup analyses for BMI and alcohol consumption. However, for smoking, removal of offspring with a chromosomal/genetic defect increased the magnitude of the association for maternal smoking and CHDs (OR: 1.15, 95%CI: 1.01, 1.32), and slightly decreased that for paternal smoking (OR: 0.93, 95%CI: 0.83, 1.05) (P_{diff} = 0.02) (**Figure 3.2B**). Further, the positive association between maternal smoking and non-severe CHDs was slightly stronger when removing those with chromosomal/genetic defects from the study population (OR: 1.25, 95%CI: 1.05, 1.49) (Figure S3.26). All maternal results were materially unchanged after additional adjustment for folic acid supplementation (Figures S3.12, S3.27 & S3.32).

3.5. Discussion

In this large multi-cohort study, I found evidence that maternal pregnancy smoking may increase offspring CHD risk via intrauterine mechanisms and that this may be driven by a specific effect on non-severe CHDs. I did not find robust evidence to suggest a causal intrauterine effect of higher maternal pre-/early-pregnancy mean BMI or overweight or obesity on offspring CHD risk. Nor did I find evidence of an intrauterine effect of alcohol consumption on offspring CHD risk, although I acknowledge that for alcohol, I had less data and limited statistical power. To my knowledge, this is the first study to use a parental negative control method to explore whether maternal exposures have a causal intrauterine effect on offspring CHDs or whether associations are explained by residual confounding, which would generate a similar association for parental exposures.

I found increased odds of offspring CHD in mothers who were overweight and obese. This is consistent with the most recent systematic review and meta-analysis, which included 2,416,546 participants (57,172 with offspring CHD), from 19 studies and reported increased risk of any offspring CHD in women who were overweight or obese during pregnancy [43]. However, adjustment for confounders was poor, with 10 of the 19 included studies not providing information on confounder adjustment or not

adjusting for any confounders. With more stringent confounder adjustment and the findings from a negative control study, my results suggest that the increased risk of offspring CHD in overweight and obese mothers is largely the result of residual confounding. I also found that mothers who were underweight at the start of pregnancy were at increased risk of having offspring with CHD, whereas underweight in fathers appeared to be protective of offspring CHD. There were 9,537 underweight mothers (4.4%) but only 680 underweight fathers (0.4%) in the present study population, making the paternal analyses imprecise and the negative control analyses lacking in power to reliably identify parental differences. The recent systematic review mentioned above did not report on associations of underweight with CHDs because too few studies looked at this.

A large Swedish linkage study of over 2 million singleton live born infants (born between 1992 to 2012 with 28,628 CHD cases), has explored associations with maternal underweight, as well as overweight and three grades of obesity [65]. It is difficult to directly compare the results from that study with ours as I only present results for any CHD (and CHD stratified by severity), whereas they only present associations of maternal BMI with specific subtypes of CHDs. The fact that I lack statistical power in my study to explore associations with specific sub-types is a limitation. However, magnitudes of associations of BMI categories and non-severe CHDs in the present study appear to be broadly consistent with several non-severe defects in the large Swedish study, including ASDs and isolated valve defects. In their study, risks of offspring CHD were similar in underweight compared to normal weight women for all types of CHD (analysed individually), except for mitral to tricuspid valve defects (14 cases), pulmonary valve defects (24 cases) and right ventricular defects (5 cases), where there was some evidence of increased prevalence with underweight. However, these estimates were based on small numbers and hence imprecise, with confidence intervals including the null. Whilst my findings suggest maternal underweight might increase offspring risk of CHDs, I lacked power to rule out residual confounding in the negative control analyses, and as noted above the large Swedish study had limited power to determine precise effects in relation to maternal underweight for specific types of CHD where point estimates suggested potentially important magnitudes of increased risk. Other studies that I am aware of have not explored associations of maternal underweight. Thus, any possible effect of maternal underweight on CHD risk remains unclear. As the prevalence of CHD in some low- and middle-income countries is high [154], and these countries currently experience the double burden of under- and over-nutrition I would argue that further exploration of any possible impact of maternal underweight is warranted.

Consistent with my findings, a recent meta-analysis of >8 million participants (137,575 CHD cases) from 125 studies reported positive associations between maternal pregnancy smoking and offspring CHDs

[55]. There was substantial heterogeneity (I^2 = 89%) in their pooled results and only 68% of the included studies report adjustment for confounders. The authors also report positive associations between maternal passive smoking and paternal active smoking with offspring CHDs, both of which (somewhat unexpectedly) had stronger magnitudes of association than results from maternal active smoking. My results, including the negative control study, add to the previous research findings by providing more robust evidence that these associations for maternal smoking are unlikely to be explained by residual confounding and are potentially causal. Other research has shown that pregnancy smoking is a risk factor for orofacial clefts [155]. The prevalence of CHD is around 1% in the general population, as shown in the present study, yet in those with orofacial clefts, CHD prevalence rates of up to 20% have been reported [156]. Both the heart and the palate develop during early pregnancy around weeks 5 to 9. Therefore, it is plausible that smoking in early pregnancy could disturb common biological pathways in these conditions. I found that the associations for maternal smoking were possibly largely driven by an effect in non-severe CHDs, with the association strengthening when those with chromosomal or genetic defects were removed. Previous research has reported positive associations between maternal smoking and septal defects, in particular for ASDs [157–159] which are defined as non-severe according to the classification system used in the present study. However, caution is needed in interpreting results by subgroups based on severity. First, one of the largest studies (MoBa) did not have information on case severity and so the severity subgroup analyses are based on different participants and have lower statistical power than in the main analyses. Second, even had all studies been included in the severity analyses, by definition subgroup analyses have limited power in comparison to main analyses. Third, and importantly, caution is required with any subgroup analyses as it is common for multiple characteristics to differ between subgroups in addition to the subgroup defining feature (here CHD severity).

In confounder adjusted analyses maternal alcohol consumption in the first trimester of pregnancy was not associated with offspring CHD. There was some evidence that maternal moderate or heavy alcohol consumption any time in pregnancy was associated with increased risk of offspring CHD. Whilst associations between mothers and fathers light, moderate and heavy alcohol consumption, compared with none, were statistically consistent, only 2 cohorts (80,627 participants, 703 with offspring CHD) had alcohol information on fathers around the time of their partners pregnancy. Associations for fathers in particular were imprecise with wide confidence intervals. Two recent meta-analyses found consistent modest increases in risk of offspring CHD in mothers reporting alcohol consumption in pregnancy (OR: 1.11 (95%CI: 0.96, 1.29) [160] and 1.16 (1.05, 1.27)) [41]. Although the first of these concluded 'no association' it can be seen that the results for the two are consistent, and the larger sample size of the second has

increased precision. Of note, the second of these studies also explored paternal consumption and found increased risk of offspring CHD related to fathers' alcohol consumption (1.44 (1.19, 1.74)) [41]. Although the odds ratio for fathers' consumption suggests a stronger effect, the confidence intervals are wide, and the result is statistically consistent with that for mothers' alcohol consumption. As in the present study there were fewer studies with data on paternal alcohol consumption around the time of their partners pregnancy. Taken together with the findings presented here, these suggest that positive associations of maternal alcohol consumption with offspring CHD may be due to residual confounding rather than a causal intrauterine effect.

The key strengths of this study are its large sample size, the use of a negative paternal exposures control study and the pooling of results from several cohort studies that are less prone to selection bias that can occur in case control studies and are not selected based on publication, but on being part of an existing collaboration. The latter reduces the risk of publication bias as studies were included if they had data and not on the basis of (published) results. This also allowed me to explore replication across studies and the consistency of findings between studies in the main analyses adds confidence to my conclusions.

The use of harmonised data from LifeCycle is a strength that limits between study heterogeneity. However, harmonizing data across several studies, as I have done in LifeCycle, can mean that some variables lose detail. Here that is particularly relevant for the exposure and confounding variables. For example, I was not able to explore pack weeks of smoking across the entire pregnancy. Simplified confounder measurements, such as Western versus non-Western for ethnicity could result in residual confounding if more specific ethnic groups have strong influences on exposure and outcome. Furthermore, there were other confounders that I considered, including type-1 / pre-existing diabetes and physical activity, but had too few numbers (diabetes) across all cohorts or too few studies with data (physical activity) to include. However, I aimed to address any form of residual confounding in the paternal negative control analyses. Under the assumption that adjusted for but poorly measured (e.g. ethnicity) or unadjusted for (e.g. physical activity) confounders influence paternal exposures in the same direction and to the same extent as in mothers, observing parental consistency of association implies that the maternal association is influenced by residual confounding.

I was not able to fully harmonize outcome data with the key differences between studies being the extent to which they only included cases that were diagnosed antenatally or at birth or whether they included cases later in life. MoBa (N = 101,975 participants and N = 879 cases) only had cases diagnosed antenatally or around the time of birth, with the remaining cohorts having diagnoses beyond antenatal care, ranging from 6 months to 25 years. Many previous studies have only included cases diagnosed at

birth or early infancy. They, and the cohorts included here that only have these early life cases, may be biased by outcome misclassification (i.e., the offspring who would have been diagnosed later in life are treated as not having CHD). This is an important point for consideration because although most CHDs are identified in utero or at birth, many are diagnosed after discharge from hospital during childhood or even adulthood [69]. Therefore, it is reassuring that the main results are largely consistent across studies. In confounder and other parent adjusted smoking analyses, the weakest association was found in the MoBa cohort. It is likely that I missed some non-severe cases in MoBa which were diagnosed later in life. Given that I demonstrate the smoking results were largely driven by non-severe CHDs, this could have biased MoBa (and therefore meta-analysis) results towards the null.

The negative control analyses assume that factors that would confound the maternal exposure-offspring CHD associations would have a similar magnitude and direction of confounding for the equivalent paternal associations, irrespective of whether the confounders are measured or if measured how accurately and precisely they are measured. This is likely to be true for paternal negative control exposure studies, as used here [99,131]. Both maternal and paternal BMI, smoking and alcohol consumption could have pre-conceptual effects via influences on gametes, including epigenetic changes. Any such effects would plausibly differ between mothers and fathers, and for the mother would be in addition to potential intrauterine effects, such that I may still expect stronger maternal associations. Furthermore, there is little conclusive evidence of effects of factors, such as smoking, on gametes that do not render them infertile but are sufficient to influence embryo development and hence CHDs, as such studies are difficult in humans. Heart development occurs in utero (specifically in early pregnancy), and I would expect passive paternal smoke inhalation to expose the fetus to a lower level of exposure, than active maternal smoking. As proof-of-concept paternal smoking does not associate with offspring birth weight or fetal growth parameters (assessed by repeat ultrasound), in contrast to maternal smoking, which has marked effects [90]. It is possible that potential differences in misreporting smoking and alcohol consumption between mothers and fathers could produce spurious parental differences. Pregnant women are likely to underreport whether they smoke or drink alcohol and the amount they smoke or drink, because of the social stigma of these, particularly in recent decades. As the report of alcohol and smoking in the LifeCycle cohorts was collected early in pregnancy it is likely to be random in relation to an offspring CHD as the vast majority would not have been diagnosed. Hence, this underreporting would be expected to attenuate any true effect of smoking/alcohol on CHD towards the null. This misclassification is less likely in fathers. Thus, the specific positive association of maternal smoking on CHDs and its difference to the paternal association may be underestimated.

Finally, only 47% of mothers with smoking data in the present study had this specifically during the first trimester. Paternal smoking was defined as smoking around the time of pregnancy with no specific trimester measurements. However, whilst amount smoked may change across pregnancy, it is highly likely that any smoking in later trimesters is a strong proxy for smoking in the first trimester. Importantly, I have shown that my results using only maternal first trimester smoking are consistent with the main results. Similarly, paternal smoking at any time during pregnancy is likely to be a good proxy for smoking in early pregnancy. Though I acknowledge it would be useful to have more detailed data on both parents across all trimesters to explore whether association magnitudes vary by trimester.

In summary, I found evidence to support a causal intrauterine effect of maternal smoking on any CHD, particularly with non-severe CHDs, but did not find robust evidence for a causal effect of maternal BMI or alcohol on offspring CHD risk. Whilst everyone should be encouraged not to smoke, and all clinical guidelines advocate not starting smoking, and if women do smoke, to quit before becoming pregnant, there are still high rates of smoking in some groups, particularly those from deprived backgrounds. In the studies included in this paper, two contemporary cohorts, BASELINE (Ireland), with births occurring between 2008 and 2011 and BiB (UK), with births occurring between 2007 and 2011, smoking prevalence rates were 25% and 16% respectively. The prevalence in BiB masks the high rate in white British women (33%) who are from socioeconomically deprived backgrounds, as over 50% of births in that cohort are to Pakistani women who have low rates of smoking (3%) [134]. It is possible that emphasizing the potential adverse effect on CHDs in specific groups might help in supporting women of reproductive age not to start smoking and women who are smoking at the start of pregnancy to be encouraged to quit. Furthermore, understanding the specific mechanisms that link maternal smoking to increased offspring CHD risk could identify targets for interventions for its prevention.

Chapter 4. The effect of maternal BMI, smoking and alcohol on congenital heart diseases: a Mendelian randomisation study

4.1. Chapter summary

This Chapter has been published as a pre-print and submitted to a peer-reviewed journal:

Taylor K, Wootton R, Yang Q, Oddie S, Wright J, Yang TC, Magnus M, Andreassen OA, Borges MC, Caputo M & Lawlor DA. The effect of maternal BMI, smoking and alcohol on congenital heart diseases: a Mendelian randomization study. *medRxiv* (2022). https://doi.org/10.1101/2022.01.27.22269962.

In **Chapter 3**, I used parental negative exposure control analyses to explore the effects of maternal pre-pregnancy BMI, and pregnancy smoking and alcohol on offspring congenital heart disease (CHD). Negative control analyses attempt to address the issue of residual confounding in observational studies but have strong assumptions that are difficult to prove. In this Chapter (**Chapter 4**), I used Mendelian randomisation (MR) to examine the same exposures as in **Chapter 3**. Exploring the same causal question using complimentary study designs that have differing sources of bias can improve causal inference. Three birth cohorts, including 38,662 mother/offspring pairs (N = 319 CHD cases) were included. I found no robust evidence of a causal effect of higher maternal BMI on offspring CHD which corroborated findings from negative control analyses. Using MR, I did not replicate the positive association for smoking seen in **Chapter 3**. However, I did find some evidence of a potential causal effect of maternal alcohol on offspring CHD. The effect of maternal alcohol intake on offspring CHDs using MR needs to be replicated in larger study populations.

4.2. Introduction

Congenital heart diseases (CHDs) are the most common congenital anomaly, affecting 6-8 per 1000 live births and 10% of stillbirths [127]. CHDs are a leading cause of childhood mortality and many CHD patients experience health problems that persist into adulthood [128,129]. The causes of CHDs are largely unknown, but the pregnancy environment (intrauterine factors) may play a role in the underlying pathophysiology [130]. Identifying modifiable risk factors for CHDs is important for improving aetiological understanding and developing preventive interventions to reduce disease burden.

Several modifiable maternal characteristics have been found to be associated with increased risk of CHDs, including maternal pre/early pregnancy body mass index (BMI) [43,65,66], smoking [55] and alcohol [41] consumption in pregnancy. The causal relevance of the results from meta-analyses is unclear, due to many studies not controlling for key confounders and for the risk of residual confounding. Previously, using parental negative exposure control analyses, I found that positive associations between maternal overweight and obesity with offspring CHDs may be being driven by confounding factors [122]. This work found some evidence of an intrauterine effect of maternal smoking on offspring CHDs. For alcohol consumption, results were inconclusive due to limited data [122]. Negative control analyses attempt to address the issue of residual confounding in observational studies [122,131], but have strong assumptions that are difficult to prove.

Mendelian randomization (MR) uses genetic variants as instrumental variables (Ivs) to test causal effects in observational data [100]. The key assumptions for MR are: (i) relevance assumption – the genetic instruments are robustly associated with the exposure, (ii) independence assumption – there is no confounding of the genetic instrument-outcome association, (iii) exclusion restriction criteria – the genetic variant is not related to the outcome other than via its association with the exposure [161]. Genetic variants are less likely to be confounded by the socioeconomic and environmental factors that might bias causal estimates in conventional multivariable regression [162], but may be biased by violation of their assumptions due to weak or irrelevant instruments, population stratification (causing confounding of the genetic instrument-outcome association) and a path from the genetic instrument to CHD not mediated by the exposure, for example via horizontal pleiotropy or fetal genotype [89]. Triangulating results from negative control and MR analyses, whereby the key sources of bias differ can help improve the causal understanding of maternal risk factors on CHDs [96]. Consistent results from both would increase confidence that the relationship is causal. The recent acquisition of genotype information on a large number of maternal-offspring dyads means that I now have relevant data to further test the potential effects of BMI,

smoking and alcohol with a complementary method to those used previous. The objective of this study was therefore to explore associations between genetically predicted maternal BMI, smoking and alcohol on offspring CHD.

4.3. Methods

4.3.1. Inclusion criteria and participating cohorts

To be eligible for inclusion in this study, cohorts and participants were required to have genome-wide data in mothers and CHD data in the offspring. From previous work with large consortia, including MR-PREG [163] and LifeCycle [126], I identified three cohorts meeting these criteria: The Avon Longitudinal Study of Parents and Children (ALSPAC), Born in Bradford cohort (BiB), and the Norwegian Mother, Father and Child Cohort Study (MoBa). ALSPAC is a UK prospective birth cohort study which was devised to investigate the environmental and genetic factors of health and development [111–113]. Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. BiB is a population-based prospective birth cohort including 12,453 women across 13,776 pregnancies who were recruited at their oral glucose tolerance test at approximately 26–28 weeks' gestation [134]. Eligible women had an expected delivery between March 2007 and December 2010. MoBa is a nationwide, pregnancy cohort comprising family triads (mother-father-offspring) who are followed longitudinally. All pregnant women in Norway who were able to read Norwegian were eligible for participation. The first child was born in October 1999 and the last in July 2009 [136,137]. One singleton pregnancy per mother in each cohort were included in analyses. **Figure 4.1** shows the inclusion of participants, after excluding those with missing maternal genotype data and those that did not pass genetic quality control (QC). A total of 38,662 mother-offspring pairs contributed to the main analyses and 28,485 to the adjusted (for fetal genotype) analyses.

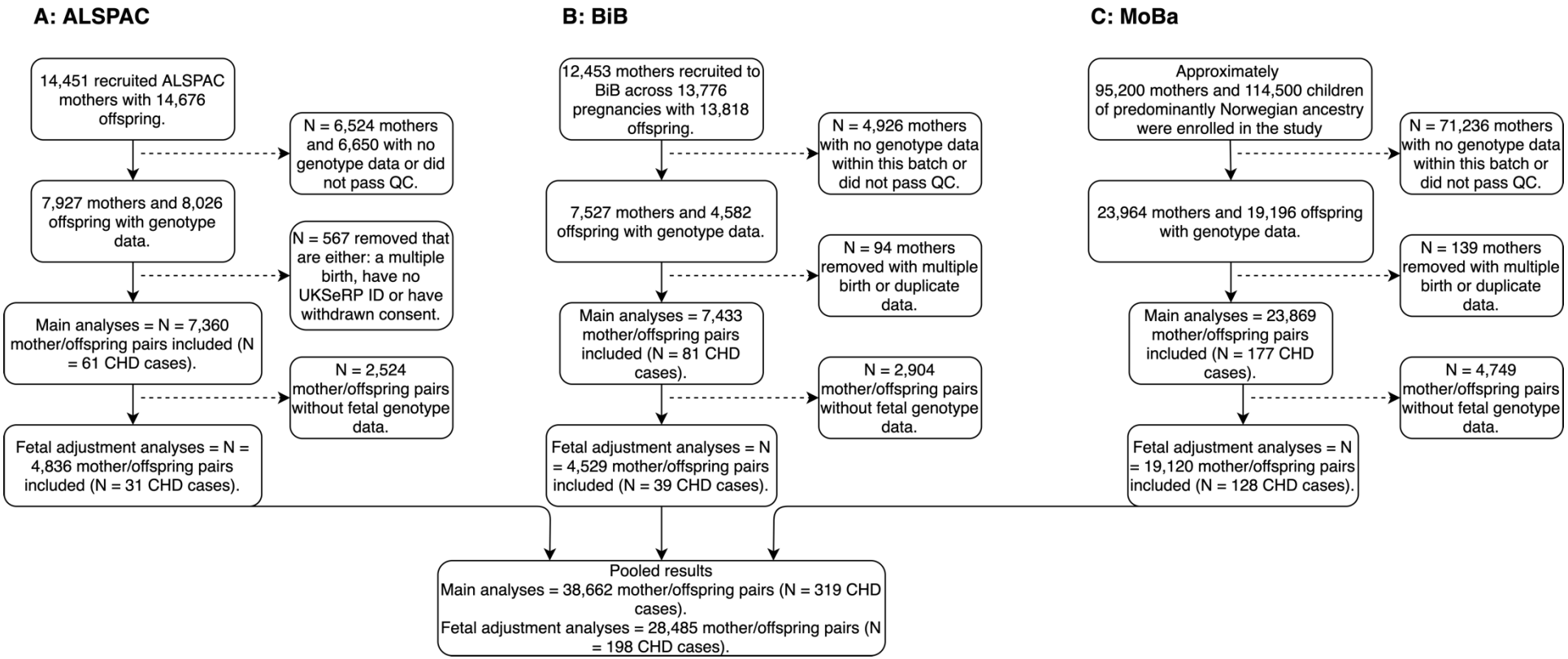


Figure 4.1. An overview of included cohorts and selection of study participants.

Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort; QC, quality control; UKSeRP, the secure research platform containing CHD data for ALSPAC; CHD, congenital heart disease; GWAS, genome-wise association study

4.3.2. Genetic data

4.3.2.1. Genotyping in each cohort

ALSPAC mothers were genotyped using Illumina human660K quad single nucleotide polymorphism (SNP) chip, and ALSPAC children were genotyped using Illumina HumanHap550 quad genome-wide SNP genotyping platform. Genotype data for both ALSPAC mothers and children were imputed against the Haplotype Reference Consortium v1.1 reference panel, after performing the QC procedure (minor allele frequency (MAF) ≥1%, a call rate ≥95%, in Hardy-Weinberg equilibrium (HWE), correct sex assignment, no evidence of cryptic relatedness, and of European descent). The samples of the BiB cohort (mothers and offspring) were processed on three different type of Illumina chips: HumanCoreExome12v1.0, HumanCoreExome12v1.1 and HumanCoreExome24v1.0. Genotype data were imputed against UK10K + 1000 Genomes reference panel, after a similar QC procedure (a call rate ≥99.5%, correct sex assignment, no evidence of cryptic relatedness, correct ethnicity assignment). In MoBa, blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth [164]. Genotyping has had to rely on several projects – each contributing with resources to genotype subsets of MoBa over the last decade. The data used in the present study was derived from a cohort of genotypes samples from four MoBa batches. The MoBa genetics QC procedure involved MAF ≥1%, a call rate ≥95%, in HWE, correct sex assignment, and no evidence of cryptic relatedness. Further details of the genotyping methods for each cohort are provided in Appendices (Text S4.1).

4.3.2.2. GWAS data and SNP selection

I selected SNPs from the largest and most relevant GWAS of European ancestry participants for each exposure (further information for each GWAS shown in Table S4.1 in Appendices). Selected SNPs were those with a p-value below a p-value threshold used to indicate genome-wide significance after accounting for multiple testing. Of those reaching this threshold I ensured that I only took forward independent SNPs to create the genetic risk scores (GRSs). This was done either by methods used in the GWAS or by applying my own criteria if the GWAS did not report independent SNP associations. For BMI, there were 941 near-independent SNPs in a combined GWAS of ~700,000 individuals as reported in Yengo et al [165] (near-independent SNPs defined as SNPs with a $P < 1x10^{-8}$ after a conditional and joint multiple SNP analysis to take into account linkage disequilibrium (LD) between SNPs at a given locus). For smoking

analyses, there were 126 independent SNPs (genome-wide significant ($p<5x10^{-8}$) SNPs that achieved independence at LD $r^2 = 0.001$ and a distance of 10,000 kb). The study was a GWAS of a lifetime smoking index (which combined smoking initiation, duration, heaviness and cessation), conducted in a sample of 462,690 current, former and never smokers in UK Biobank [166]. For the alcohol weighted GRS, there were 99 conditionally independent SNPs (MAF ≥1% and $P<5x10^{-8}$), measured as number of alcoholic drinks per week [167]. This GRS has also previously been shown to be associated with alcohol consumption during pregnancy as well as the general population [168]. The ALSPAC cohort was included within the original GWAS for alcohol by Liu et al, accounting for 8,913 participants out of a total sample size of 941,280 (0.9%). Previous work has suggested any bias introduced by this level of overlap would be minimal [169]. Furthermore, a recent study explored this by excluding ALSPAC from the summary statistics and results were unbiased and largely unchanged [168]. Therefore, I proceeded using the full summary data for generating the alcohol GRS. All GRSs were generated using summary GWAS data that was derived in both men and women. I was unable to obtain female-specific summary data for these GWAS data. However, I perform checks to ensure the GRSs are robustly associated with the maternal exposure during pregnancy.

4.3.2.3. Genetic risk score generation

Weighted GRSs were calculated for BMI, smoking and alcohol consumption by adding up the number of risk factor increasing alleles among the selected SNPs after weighting each SNP by its effect on the corresponding risk factor:

$$GRS = w1 \times SNP1 + w2 \times SNP2 + \cdots wn \times SNPn$$

where w is the weight (i.e., the beta-coefficient for the SNP-exposure association reported from the published GWAS) and SNP is the genotype dosage of exposure-increasing alleles at that locus (i.e., 0, 1, or 2 exposure-increasing alleles). Selected SNPs were extracted from the imputed genotype data in dosage format using QCTOOL (v2.0) and VCF tools (v 0.1.12b) in ALSPAC and BiB, respectively. PLINK (v1.9) was then used to construct the GRS for each exposure coded so that an increased score associated with increased exposure. In MoBa, I constructed the GRSs from the QC'd data in PLINK format. Further information on GRS construction for each cohort is shown in Text S4.2 (Appendices).

4.3.3. Phenotype data

4.3.3.1. CHD data

In the ALSPAC cohort, cases were obtained from a range of data sources, including health record linkage and questionnaire data up until age 25 following European surveillance of congenital anomalies (EUROCAT) guidelines [35]. In BiB, cases were identified from either the Yorkshire and Humber congenital anomaly register database, which will tend to pick up most cases that diagnosed antenatally and in the early postnatal period of life, and through linkage to primary care (up until aged 5), which will have picked up any additional cases, in particular those that might have been less severe and not identified antenatally/in early life [70]. All these cases were confirmed postnatally and were assigned international classification of disease Version 10 (ICD-10) codes. ICD-10 codes were used to assign CHD cases according to EUROCAT guidelines. In MoBa, information on whether a child had a CHD or not (yes/no) was obtained through linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN, and information on malformations are reported to the registry up to 12 months postpartum [170]. Further details on defining CHDs including ICD codes used (in ALSPAC and BiB) are shown in Text S4.3 and Table S4.2 (Appendices).

4.3.3.2. Pregnancy phenotype data

As noted above, the SNP selection and weights for the GRS were taken from GWAS in women and men [165–167]. To determine their relevance in women during pregnancy I examined the associations of the GRS with pre/early pregnancy BMI, and pregnancy smoking and alcohol consumption in each cohort. In ALSPAC and MoBa, pre-pregnancy weight and height were self-reported during the first pregnancy questionnaires. In BiB, weight and height were measured at the recruitment assessment as this was at the time of the oral glucose tolerance test (~24-28 weeks). Because of this, maternal weight would be influenced by pregnancy fat deposition, amniotic fluid and fetus. Therefore, I used weight extracted from the first antenatal clinic (median 12 weeks) in calculating BMI. As the timing of questions and the details requested for smoking during pregnancy differed across the three cohorts [90,171,172] I was only able to generate a simple binary variable of any smoking in pregnancy versus none. There was insufficient data and/or power across the cohorts to be able to generate a measure of smoking heaviness in pregnancy. As with smoking, the aim for alcohol was to determine whether the GRS was robustly associated with drinking status during pregnancy. I used questionnaire data in each cohort and used binary variables

(yes/no) for whether women consumed any alcohol during pregnancy or not. Further details regarding these phenotype data, including questionnaire information are described in Text S4.4 (Appendices).

4.3.4. Statistical analysis

This study is reported using the Strengthening the Reporting of Observational studies in Epidemiology using Mendelian randomisation (STROBE-MR) guidelines (see Appendices: STROBE-MR Checklist) [173,174]. Analyses were performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). I undertook MR in each of the 3 cohorts, including all ALSPAC, BiB and MoBa participants, with maternal genetic data and offspring CHD data. Logistic regression was used to estimate the odds ratio (OR) of CHD per 1 standard deviation (SD) change in GRS, with adjustment for the first 10 genetic principal components (PCs) with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. Statistical analyses in relation to the verification of MR assumptions are described below.

The key assumptions of MR are: (i) relevance assumption, (ii) independence assumption and (iii) exclusion restriction criteria and are described above in the introduction. Regarding the first assumption, to explore the relevance of the GRS to each exposure in pregnancy, I undertook linear (BMI) and logistic (smoking and alcohol) regression to derive the difference in mean BMI and OR of pregnancy smoking and pregnancy alcohol consumption per 1SD higher GRS in each cohort. Results are presented with 95% confidence intervals (CI) in each cohort. For BMI, instrument strength was assessed with F-statistics and R^2. For smoking and alcohol, instrument strength was assessed using the area under the ROC curve and pseudo-R^2 by the Nagelkerke method [175].

To minimise the potential for confounding of the GRS-CHD association due to population stratification (second assumption), I adjusted for the first 10 ancestry-informative principal components [176]. I also repeated the MR analyses without the inclusion of BiB, given that BiB has a unique ethnic structure of South Asians and White Europeans. GRS-CHD association results were pooled using a random effects meta-analysis for all three cohorts and fixed-effect meta-analyses when excluding BiB in sensitivity analyses (i.e., ALSPAC and MoBa). Between study heterogeneity was assessed using the Cochrane Q-statistic and I^2 [152].

The third assumption may be violated when the genetic instruments influence other risk factors for the outcome independently of the exposure of interest (horizontal pleiotropy) [103]. To explore horizontal pleiotropy, I checked the association of GRSs with known risk factors for CHD that I had data on. I explored the relationship between 1 SD increase in the GRS with risk factors for CHD (education,

parity and diabetes) using linear or logistic regression. I describe methods for these variables in each cohort in Appendices (Text S4.4). If any of the GRSs were associated with a risk factor, I considered that a potential pleiotropic effect. I then performed multivariable MR (MVMR) analyses if GWAS data for the potential pleiotropic variable was available [177]. Methods for these GRSs and the rationale for selecting these risk factors are described in Text S4.5 (Appendices). In this work, I am asking whether BMI, smoking and alcohol are risk factors for CHDs. Therefore, I also explored the relationship between the GRS's for the different exposures of interest. I acknowledge that I am unable to tease apart horizontal from vertical pleiotropy from these analyses. In sensitivity analyses to explore potential bias via fetal genotype I repeated the PC (and batch) adjusted GRS-CHD association in the subsample of participants with fetal genome wide data (**Figure 4.1**) and then compared those results with the same associations additionally adjusted for the fetal GRS.

4.4. Results

4.4.1. Participant characteristics

MR analyses included 38,662 mother-offspring pairs, of which 319 offspring had CHD (**Figure 4.1**). The distributions of offspring and maternal characteristics for these analyses in ALSPAC, BiB and MoBa are displayed in **Table 4.1**. The prevalence of any CHD, mean maternal age and pre-/early-pregnancy BMI were similar in the three cohorts. Women in ALSPAC were more likely to smoke during pregnancy in comparison to those in BiB and MoBa although, the overall prevalence in BiB masks marked differences between the two largest ancestral groups, with 3.4% of South Asian women reporting smoking during pregnancy compared to 34% of White European women. Women in ALSPAC and BiB were more likely to consume alcohol to those in MoBa, although, in BiB, there are limited data available on alcohol consumption with very few South Asians responding to questions relating to alcohol in questionnaires.

Table 4.1. Participant characteristics for the 3 studies included in Mendelian randomisation analyses.

Characteristic	Category	ALSPAC (N = 7,360)	BiB (N = 7,433)	MoBa (N = 23,869)
Offspring				
CHD	Yes	61 (0.8)	81 (1.1)	177 (0.7)
	CHD sex stratify	35 Male (57%) / 26 Female (43%)	35 Male (43%) / 46 (57%) Female	84 Male (47%) / 93 Female (53%)
Sex	Male	3,703 (50.3)	3,818 (51.4)	12,139 (50.9)
	Female	3,657 (49.7)	3,615 (48.6)	11,704 (49.0)
Maternal				
Age, years		29.2 (4.6)	27.4 (5.6)	30.1 (4.5)
Parity	Primiparous	3,257 (46.6)	2,963 (40.1)	11,288 (47.3)
BMI, kg/m^2		22.5 (4.2)	26.2 (5.7)	24.1 (4.3)
Ethnicity	White European	7,360 (100.0) [a]	3,084 (42.6)	NA [b]
	South Asian	-	3,503 (48.4)	-
	Other	-	656 (9.1)	-
Any smoking during pregnancy	Yes	1,679 (26.1)	1,175 (18.1)	1,814 (8.6)
Any alcohol during pregnancy	Yes	4,866 (79.9)	1,040 (49.3)	6,209 (31.5)

Data are means ± SD or n (%) unless stated. % are based on data available (data were not complete).

[a] All non-white European women with ethnicity data were not included in the analysis.

[b] MoBa participants believed to be of primarily European origin.

Abbreviations: BiB, Born in Bradford; ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort Study; CHD, congenital heart disease; BMI, body mass index; kg, kilograms; m, meters.

4.4.2. MR results

There were similar statistically strong positive associations of the BMI GRS with pre-pregnancy BMI and the smoking GRS with pregnancy smoking in all three cohorts (**Table 4.2**). The alcohol GRS also associated positively with alcohol consumption during pregnancy in all three cohorts with a somewhat weaker association in BiB and MoBa in comparison to ALSPAC.

Table 4.2. Relevance and strength of the genetic risk scores with exposures in pregnancy.

Study	N participants	N SNPs in GRS	Coefficient (95% CI)[a]	P-Value	R^2/ pseudo R^2 [b]	F statistic [c]	AUC
Association of GRS for BMI with pre-/early-pregnancy BMI							
ALSPAC	6,253	941	0.24 (0.21, 0.26)	1×10^{-80}	5.6%	372	-
BiB	6,196	939	0.20 (0.18, 0.23)	5×10^{-59}	4.1%	268	-
MoBa	22,533	868	0.25 (0.24, 0.27)	$< 1 \times 10^{-100}$	6.5%	1,555	-
Association of GRS for a lifetime smoking index with any smoking during pregnancy							
ALSPAC	6,428	126	1.27 (1.20, 1.35)	1×10^{-16}	1.6%	-	0.56
BiB	6,482	126	1.36 (1.27, 1.45)	2×10^{-20}	2.2%	-	0.59
MoBa	20,981	119	1.23 (1.17, 1.29)	7×10^{-17}	0.8%	-	0.56
Association of GRS for drinks per week with any alcohol consumption in pregnancy							
ALSPAC	6,087	98	1.14 (1.07, 1.21)	3×10^{-5}	0.4%	-	0.53
BiB	2,110	99	1.08 (0.99, 1.18)	0.09	0.2%	-	0.52
MoBa	19,737	73	1.02 (0.99, 1.05)	0.13	0.02%	-	0.51
MoBa sensitivity [d]	19,737	73	1.06 (1.01, 1.10)	0.01	0.07%	-	0.52

[a] Effect estimates (coefficient) are difference in mean (BMI) or odds ratio (smoking or drinking yes/no during pregnancy) per SD increase in genetic risk score.

[b] for the binary outcomes (smoking and alcohol) pseudo-R^2 are presented

[c] for BMI F-statistic is presented; for binary outcomes (smoking and alcohol) AUC is presented.

[d] in MoBa 7,356/23,784 consumed any alcohol during pregnancy. However, 4,754 of the 7,356 consumed alcohol "less than once per month" based on the questionnaire data. In the sensitivity analysis shown above, I re-coded the variable so that those that consumed alcohol less than once per month were classed as non-drinkers (N.B. due to the small numbers in each individual category, I were not able to analyse these separately). This was performed as an additional check to ensure the GRS was associated with pregnancy alcohol consumption in MoBa.

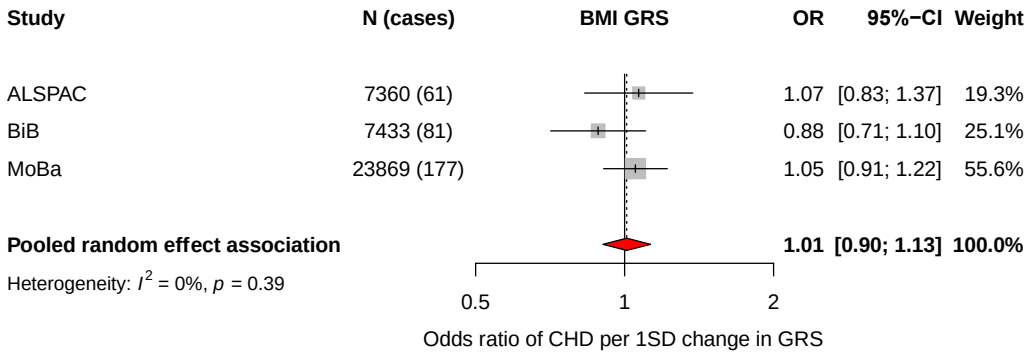
Abbreviations: SNP, single nucleotide polymorphism; GRS, genetic risk score; CI, confidence interval; AUC, area under the curve; ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort.

The MR effects in each study and pooled across studies of each exposure and offspring CHDs are shown in **Figure 4.2**. There was no strong evidence that the maternal GRS for BMI influenced offspring CHD (OR (95%CI) per 1SD higher GRS: 1.01 (0.90, 1.13), with no statistical evidence of between study heterogeneity (**Figure 4.2A**). When excluding BiB from these analyses, the pooled point estimate showed a weak positive effect, although confidence intervals spanned the null (OR: 1.06 (0.93, 1.20); Figure S4.1B Appendices). The BMI GRS associated with smoking, education, and diabetes across all three cohorts (Table S4.3). Results were unchanged in MVMR models including GRSs for education and smoking (Figures S4.1C & S4.1D). When further adjusting for offspring genotype, the pooled result attenuated to below the null, although this may be explained by the low number of CHD cases in BiB and should therefore be treated with caution. In offspring genotype adjusted analyses excluding BiB, the pooled result was null (OR: 0.97 (0.83, 1.14)) (Figures S4.1E-S4.1H).

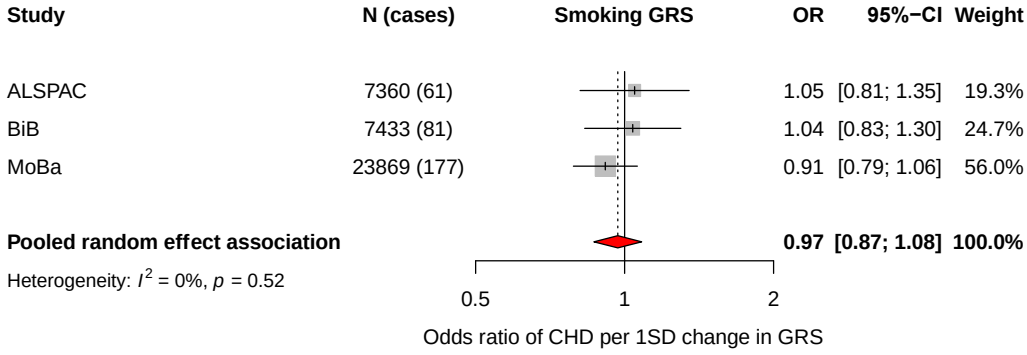
The maternal GRS for maternal lifetime smoking index was also not associated with offspring CHD (OR (95%CI) per 1SD higher GRS: 0.97 (0.87, 1.08), with no statistical evidence of between study heterogeneity (**Figure 4.2B)**. The smoking GRS associated with BMI and education across the cohorts (Table S4.4). Results were consistent and unchanged in additional analyses excluding BiB (Figure S4.2B), MVMR analyses adjusting for education or BMI (Figures S4.2C & S4.2D) and in offspring genotype adjusted analyses (Figures S4.2E-S4.2H).

There was weak evidence of a positive association between maternal GRS for alcoholic drinks per week and offspring CHDs, with the strongest associations seen in MoBa (pooled OR: 1.09 (0.98, 1.22)) (**Figure 4.2C**). In analyses excluding BiB, the pooled estimated was consistent with main analyses (OR: 1.10 (0.97, 1.25)). Although there was no statistical evidence of heterogeneity between ALSPAC and MoBa, results suggest this estimate is largely being driven by MoBa (Figure S4.3B). The alcohol GRS showed consistent association with smoking across the cohorts (Table S4.5). The positive association remained in MVMR analyses adjusting for a GRS of smoking (Figure S4.3C) and in analyses adjusting for offspring genotype (Figures S4.3D-S4.3G).

A: Genetically predicted maternal BMI and offspring CHDs



B: Genetically predicted maternal smoking and offspring CHDs



C: Genetically predicted maternal alcohol consumption and offspring CHDs

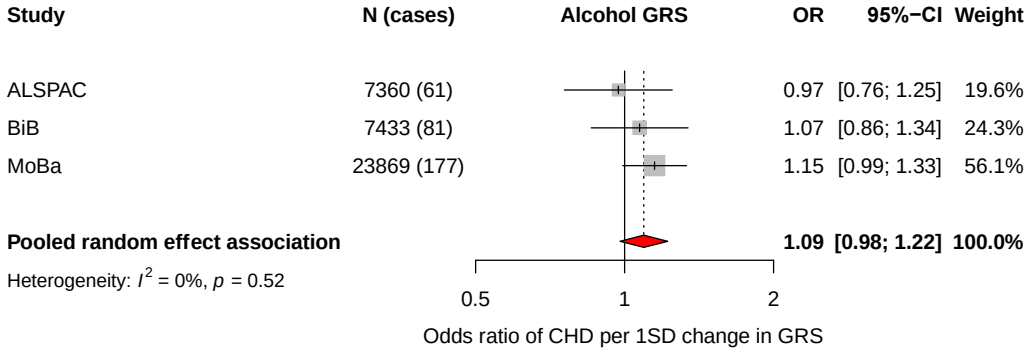


Figure 4.2. Forest plots showing the mendelian randomisation results for genetically predicted maternal body mass index (Panel A), smoking (GRS of a lifetime smoking index: Panel B), and alcohol consumption (GRS of drinks per week: Panel C) with offspring congenital heart disease.

Odds ratios (ORs) of CHD for a 1SD difference in maternal GRS in each study and pooled across studies using random effects meta-analysis. Adjusted for top 10 genetic principal components in all cohorts with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort Study; BMI, body mass index; CI, confidence interval; CHD, congenital heart disease; SD, standard deviation; GRS, genetic risk score.

4.5. Discussion

In the current study, using MR across three birth cohorts, I found no strong evidence for an effect of genetically predicted maternal BMI or smoking on risk of offspring CHD, but did find evidence of a potential causal effect of genetically predicted greater alcohol consumption on odds of offspring CHD. However, for alcohol there may have been weak instrument bias given that the GRS had somewhat weaker associations for pregnancy alcohol consumption, in comparison to GRSs of BMI and smoking. In one sample MR, a weak instrument would be expected to bias results toward the confounded observational result. To the best of my knowledge this is the first MR of these maternal exposures with offspring CHD, and it complements my previous negative paternal control study [122]. The findings from MR analyses of BMI are consistent to what I saw in the negative control study, with both suggesting that higher maternal BMI may not causally influence offspring CHD. I have not replicated my previous result for smoking, which suggested an increased risk of offspring CHD in women who smoked in pregnancy, whereas here there was no strong evidence for this. Conversely the possible effect of alcohol consumption on CHD seen here was not conducted in my previous multivariable adjusted observational analyses as lack of paternal data on alcohol consumption meant I was unable to explore paternal negative control analyses [122].

Results from this study using a GRS from GWAS of BMI produced comparable null results from linear BMI analyses presented in previous work [122]. Other work including large record linkage studies and pooled results from meta-analyses suggest that increasing maternal obesity severity increases offspring CHD risk [43,65]. However, parental negative control analyses suggested that these increased risks could be a result of confounding [122]. Exploring non-linear effects using MR with the data that were available was beyond the scope of this paper due to data availability. Nevertheless, recent work has used MR to explore non-linear effects [178] and future work of this nature could help further disentangle the causal relationship between maternal exposures on offspring CHDs. Overall, with the negative control analyses providing evidence that associations of higher maternal BMI are due to confounding, and the lack of an effect in the MR analyses presented here, the evidence suggests that previous associations between maternal BMI and any offspring CHD are unlikely to be causal.

The present results from a GRS derived from a lifetime smoking GWAS found no strong evidence of an association for genetically predicted maternal smoking and any offspring CHD. Despite this, there is still a considerable body of evidence (e.g., a meta-analysis of >8 million participants (137,575 CHD cases) that found maternal smoking increase offspring CHD risk. This, coupled with the evidence from parental

negative control analyses provides good evidence that these results may be causal. The MR results from this study do not definitively rule out an effect. Larger datasets are needed to increase the precision of these findings. One possible reason that could have biased the MR results to the null is using "any CHD" grouped as one outcome. In previous work, I showed that effects of maternal smoking on offspring CHD could mainly be being driven by non-severe CHDs [122]. Sub-categorising CHDs was not possible in the present study due to data availability and the numbers required for meaningful analyses. Another reason that may explain the null finding could be the use of a lifetime smoking index to instrument smoking. I believe that this was the best option for the MR analyses presented here as CHDs are rare meaning I was unable to stratify into current smokers or smoking heaviness. However, a GRS of lifetime smoking is different to e.g., a GRS of smoking heaviness because it also includes smoking initiation SNPs, which tend to capture personality traits related to initiating smoking such as impulsivity [166] and these are unlikely to be causal for CHD. Future work exploring the effects of maternal smoking on offspring CHDs should include MR analyses in larger datasets to increase the precision of the findings I present here as well as exploring the possibility of including two-sample MR analyses which would require publicly available GWAS datasets for CHD [179].

The current results, based on GRS derived from an alcohol GWAS, suggest a possible causal relationship between maternal alcohol consumption and offspring CHDs. Recent meta-analyses found consistent modest increases in risk of offspring CHD in mothers reporting alcohol consumption in pregnancy, however, many of the included studies did not adjust for confounders [41,160], meaning that it is difficult to determine whether the association is as of a result of alcohol or other characteristics that are related to alcohol and offspring CHDs. Results from parental negative control analyses had limited data and were thus inconclusive [122]. Results from this present study found a positive association between a maternal GRS derived from a GWAS of drinks per week (which is different from any alcohol consumption) and offspring CHDs. Although this is not definitive evidence for a causal relationship between maternal alcohol consumption and offspring CHDs, these results contribute to the overall body of evidence. The possibility remains that previous observational studies finding an effect of maternal alcohol consumption could be due to confounding, given that parental negative control analyses were inconclusive. In the present MR study, it is possible that the MR effects for alcohol could have been biased by weak instrument which would bias results towards the confounded observational estimate. Genetic instruments explain a small proportion of the exposure, despite using GRSs (as opposed to single SNPs) to improve statistical power [180]. Therefore, going forward, research should incorporate alcohol data in large numbers in mums and fathers (for negative control analyses to robustly explore residual confounding) and include larger MR

studies, in particular two-sample MR for which weak instrument bias would bias estimates in the opposite direction towards the null.

There are several strengths of the results presented in the current study. To date, few studies have used an MR approach to investigate the role of the pregnancy environment in the aetiology of offspring CHDs. The inclusion of 3 cohorts to maximise numbers and explore heterogeneity improves the robustness of the findings. I was able to adjust for offspring genotype in a large subsample of each cohort, which is important in attempting to separate the influence of genetic inheritance from a possible intrauterine effect [89]. A limitation of this study is that despite a relatively large sample size (N = 38,662) the effect estimates were often imprecise due to CHD being a rare condition and for alcohol may have been biased by the weak instrument. The inclusion of BiB increases the risk of confounding due to population stratification in MR. However, I tried to address this by adjusting for ancestry principal components and exploring consistency of results without BiB. In relation to this, the cohorts and the GWAS data used to construct the GRSs both aimed to test potential causal effects in a largely European population. Therefore, the results may not be generalisable to other populations. Next, I have only explored the effects of any CHD and therefore could have missed potential effects of these exposures on specific CHD subtypes. Related to this, the MoBa cohort only had cases diagnosed antenatally or around the time of birth (first year of life) which would increase the chances of outcome misclassification by assigning CHD cases which were diagnosed later in life as non-CHD cases. This is particularly pertinent for smoking analyses, given that I previously showed stronger potential effects in non-severe cases which would be more likely to suffer from misclassification in the case described here. Lastly, the results could have been affected by selection bias [181,182], although, I anticipate that by including multiple different birth cohorts and exploring consistency would help mitigate this.

Identifying causal risk factors is important for developing public health preventive interventions and to understand the mechanisms that link maternal lifestyle factors to offspring CHDs. The analysis steps taken in this work aimed to explore the presence of a causal effect of maternal BMI, smoking and alcohol on offspring CHDs. In summary, I found no robust evidence of an effect for maternal genetically determined BMI or smoking on offspring CHD. I did observe a weak relationship between genetically predicted maternal alcohol intake on offspring CHDs, but this may be explained by weak instrument bias. Many of the results, such as those for smoking, produced imprecise estimates. Future larger studies that employ a range of causal methods with information on CHD subtypes are warranted to further interrogate maternal gestational risk factors for offspring CHDs.

Chapter 5. The relationship of maternal gestational mass spectrometry-derived metabolites with offspring congenital heart disease: results from multivariable and Mendelian randomisation analyses

5.1. Chapter summary

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It is plausible that maternal pregnancy metabolism influences risk of offspring of congenital heart disease. In this Chapter (**Chapter 5**) I use the mass spectrometry-derived pregnancy data to explore the relationship between maternal gestational metabolites and offspring CHD. I sought to explore this through a systematic approach using different methods and data. I found 44 metabolites suggestively associated with offspring CHD in BiB including those from the following super pathways: amino acids, lipids, co-factors and vitamins, xenobiotics, nucleotides, energy, and several unknown molecules. I then took additional steps to explore these further. Firstly, I repeated the analysis within the BiB cohort for any metabolite that was measured by nuclear magnetic resonance (NMR) or clinical chemistry in larger numbers than the initial analysis. Then, I used genetic risk scores (GRS: weighted genetic risk scores of single nucleotide polymorphisms that were genome-wide significantly associated with each metabolite) in Mendelian randomisation (MR) analyses. MR analyses were performed in BiB and two additional birth cohorts. Of the 44 metabolites suggestively associated with CHD, 2 were available (isoleucine and leucine) in larger numbers via the NMR platform, and results for these were validated showing a potential protective effect of higher levels of amino acids. MR analyses were possible for 27/44 metabolites and for 11 there was consistency with multivariable regression results. In summary, I have used complimentary data sources and statistical techniques to construct layers of evidence. I found that amino acid metabolism during pregnancy, several lipids (more specifically androgenic steroids), and levels of succinylcarnitine could be important contributing factors for CHD.

5.2. Introduction

Congenital heart diseases (CHDs) are the most common congenital anomaly affecting approximately 6-8 per 1000 live births and 10% of stillbirths. They are the leading cause of death from congenital anomalies [127]. Approximately 20% of CHD cases can be attributed to known chromosomal anomalies, gene disorders or teratogens [8]. The causes of the remaining cases are unknown. Identifying causes of CHDs is important for improving aetiological understanding and developing potential targets for intervention.

Metabolomics technologies have enabled the quantification of a large number of metabolites in a biological sample. Metabolites are small-molecule intermediates and products of metabolism. The metabolome, the complete set of metabolites in biological tissues/fluids, is influenced by both genotype and environment, and dynamically responds to environmental influences. Analyses of maternal metabolomic profiles could identify causal mechanisms leading to CHDs [205]. Because the metabolome reflects interactions of genomic, environmental (e.g., air pollution), behavioural (e.g., smoking) and pathophysiological states (e.g., body composition), examining associations of it with CHDs could help elucidate modifiable upstream risk factors and/or potential molecular targets for intervention to prevent CHDs.

Studies have explored maternal molecular markers and found that offspring of women with a compromised vitamin D status (defined as 25-hydroxyvitamin D < 50 nmol/l in comparison to adequate defined as > 75 nmol/l) [85] and lipid profile [86,87] have an increased risk of CHDs. Other work has shown that poor glucose control and diabetes during pregnancy can increase CHD risk [206–208]. However, these studies focus on single or few biomarkers. Exploring the wider metabolome could provide opportunities to improve our understanding of the molecular mechanisms that underpin CHDs [205]. Previous work has explored metabolomics in maternal serum as a predictor of offspring CHDs and uncovered potentially relevant biological pathways [209]. The study found more than 100 metabolites that differed between CHD cases and non-cases concluding that abnormal lipid metabolism was an important feature of CHD pregnancies. Other research has explored potential biomarkers of maternal urine metabolomics with offspring CHDs (N = 70 CHD cases and 70 controls) [83]. Their results indicated that short chain fatty acids and aromatic amino acid metabolism may be relevant to CHDs. Replication of these results are warranted. A recent retrospective study in a Chinese population performed metabolomic analyses using maternal amniotic fluid and found that two metabolites (uric acid and proline) were elevated in CHD affected pregnancies [84]. In summary, there have been studies uncovering potentially important biological pathways

associated with offspring CHDs. However, pregnancy metabolomic studies are still relatively novel with scope for future research to provide new insights and seek replication of previous findings.

The aim of this study was to explore associations of the maternal metabolome quantified by an untargeted mass spectrometry (MS) platform and the odds of CHD in the offspring. To address this aim I searched for relevant studies within The LifeCycle Project-EU Child Cohort Network [126] to identify any study with detailed untargeted maternal gestational metabolomic data and offspring CHD information. I identified only one cohort with relevant data in a subgroup: the Born in Bradford (BiB) cohort (N = 2,605 participants; 46 CHD cases) [134,210]. Recognising that these novel data were potentially underpowered, I sought internal validation of metabolites suggestively associated with CHD, by repeating the multivariable regression analysis within the BiB cohort for any metabolite that was measured by nuclear magnetic resonance (NMR) or clinical chemistry in larger numbers (N = 7,296, 87 CHD cases). I subsequently searched the MR-PREG consortia studies [90,163] for cohorts with maternal genome-wide data and offspring CHD information that could be used for Mendelian randomization (MR) analyses of associations of genetic instruments for maternal metabolites. I performed pooled MR analyses across three cohorts (N = 38,663, 319 CHD cases) for any metabolites that were: (i) suggestively associated with CHD in BiB (P<0.05 in confounder adjusted analyses) and (ii) had summary data in the most recent metabolomic genome-wide association study (GWAS).

5.3. Methods

5.3.1. Study design and participants

A schematic overview of the study design is illustrated in **Figure 5.1**. I excluded children of multiple births because they differ from single births for congenital anomaly outcomes [211,212]. For multivariable metabolomic analyses, I used data from the BiB cohort as this was the only cohort that had measures of a substantial number of metabolites reflecting a wide range of metabolic paths assessed during pregnancy and CHD outcomes [210]. I also explored internal validation of any findings with a p-value < 0.05 within the BiB study where equivalent (or near equivalent) measures to any on the MS platform markers are available from other sources. BiB is a population-based prospective birth cohort, including 12,453 women across 13,776 pregnancies who were recruited at their oral glucose tolerance test (OGTT) at approximately 26–28 weeks' gestation [134]. Eligible women had an expected delivery between March 2007 and December 2010. The use of a multivariable p-value threshold of <0.05 to take associations forward into further

validation analyses is appropriate as an initial screen, for a relatively rare outcome, to avoid missing potential causal effects.

To be included in MR analyses, studies and participants had to have genome-wide data in mothers and CHD data in the offspring. Three cohorts contributed to MR analyses: BiB, the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother, Father and Child Cohort Study (MoBa). ALSPAC is a UK prospective birth cohort study which was devised to investigate the environmental and genetic factors of health and development [111–113]. Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. MoBa is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health [136,137]. Participants were recruited from all over Norway from 1999-2008. The women consented to participation in 41% of the pregnancies. The cohort includes approximately 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on 12 of the quality-assured data files released for research in 2019. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is currently regulated by the Norwegian Health Registry Act. The current study was approved by The Regional Committees for Medical and Health Research Ethics (2018/1256).

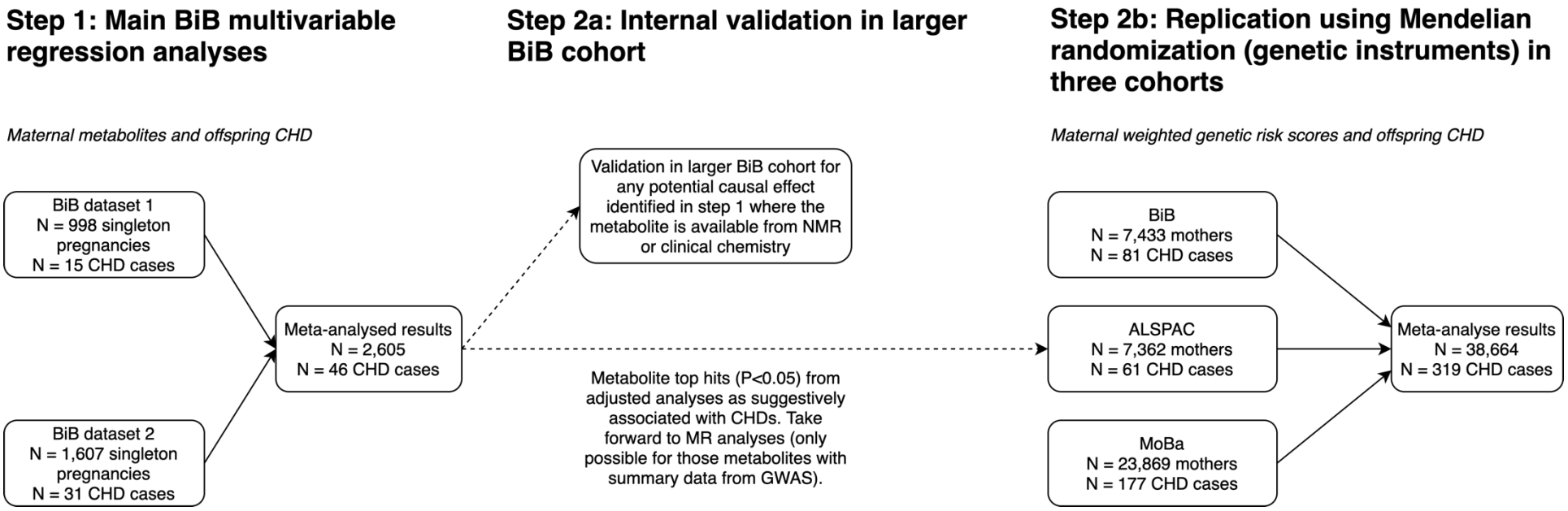

Figure 5.1. An overview of the study design.

BiB has pregnancy mass spectrometry derived metabolomics in two separate datasets. Dataset 1 was completed in December 2017 and included 1,000 maternal pregnancy samples. Dataset 2 was completed in December 2018 and consisted of 2,000 maternal pregnancy samples within a case cohort design. The selection of participants into the two MS metabolomic datasets are shown in flowcharts in Figure S5.1. Abbreviations: CHD, congenital heart disease; BiB, Born in Bradford; NMR, Nuclear Magnetic Resonance; MR, Mendelian Randomization; GWAS, genome-wise association study; ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort.

5.3.2. Sample collection and metabolomic profiling in BiB

Of the 13,776 pregnancies in the BiB cohort, 11,480 had a fasting blood sample taken during the OGTT (n = 10,574 [92%] between 26–28 weeks' gestation, with the remaining women being within 11–39 weeks' gestation). Samples were taken by trained phlebotomists working in the antenatal clinic of the Bradford Royal Infirmary and sent immediately to the hospital laboratory. The metabolomics data in the BiB cohort has previously been described in detail [210]. In brief, metabolomics analysis was performed on ethylenediamine tetraacetic acid (EDTA) plasma samples around 26-28 weeks' gestation. The untargeted MS metabolomics analysis of over 1,000 metabolites was performed at Metabolon, Inc. (Durham, North Carolina, USA). Quality control of the metabolite data was conducted by Metabolon. The classes of metabolites include amino acids, carbohydrates, cofactors and vitamins, energy, lipids, nucleotides, partially characterised molecules, peptides, and xenobiotics. These super-pathways, as defined by Metabolon, are also further subdivided into ~80 sub-pathways. Metabolite concentrations were quantified using area under the curve of primary MS ions and were expressed as the multiple of the median (MoM) value for all batches processed on the given day. The MoM more closely reflects the biological variation rather than technical variation between samples or analysis platform [185]. Due to the timing of funding acquisition, samples were sent to Metabolon in two separate batches. Dataset 1 was completed in December 2017 and included 1,000 maternal pregnancy samples. Dataset 2 was completed in December 2018 and consisted of 2,000 maternal pregnancy samples within a case cohort design. Over-sampled cases were removed to obtain a representative sample. The selection of participants into the two MS metabolomic datasets are shown in flowcharts in Figure S5.1 (Appendices) and have been described in detail previously [210].

5.3.3. Confounders

In multivariable regression analyses in BiB, I adjusted for the following maternal characteristics based on their known or plausible influence on maternal metabolites and on CHD: age, ethnicity, parity, residential neighbourhood Index of Multiple Deprivation (IMD), body mass index (BMI), smoking, and alcohol consumption. Details of the methods for how confounders were assessed are provided in Appendices (Text S5.1).

5.3.4. Congenital heart disease outcomes

In BiB, cases were identified from either the Yorkshire and Humber congenital anomaly register database, which will tend to pick up most cases that were diagnosed antenatally and in the early postnatal period of life, or through linkage to primary care (up until aged 5), which will have picked up any additional cases, in particular those that might have been less severe and not identified antenatally/in early life [70]. All BiB cases were confirmed postnatally and were assigned ICD-10 codes. I used ICD-10 codes to assign CHD cases according to the European surveillance of congenital anomalies (EUROCAT) guidelines. In the ALSPAC cohort, cases were obtained from a range of data sources, including health record linkage and questionnaire data up until age 25 following European EUROCAT guidelines [35]. In MoBa, information on whether a child had a CHD or not (yes/no) was obtained through linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN, and information on malformations are reported to the registry up to 12 months postpartum [170]. Further details on defining CHDs are shown in Text S5.2 and Table S5.1 (Appendices).

5.3.5. Genetic data

The rationale for performing MR analyses was to explore replication using a different method with two additional independent studies and to explore causation. Metabolites are affected by multiple disease processes as well as numerous environmental exposures; therefore, understanding the metabolic pathways implicated in CHD is nontrivial. MR can help discriminate causal from non-causal metabolites because genetic variants are less likely to be confounded by the socioeconomic and environmental factors that might bias causal estimates in conventional multivariable regression [162], but may be biased by a path from the metabolomic genetic score to CHD, for example via horizontal pleiotropy or fetal genotype [89]. Consistent results from both increase confidence that the result is causal.

5.3.5.1. Genotyping in each cohort

ALSPAC mothers were genotyped using Illumina human660K quad single nucleotide polymorphism (SNP) chip, and ALSPAC children were genotyped using Illumina HumanHap550 quad genome-wide SNP genotyping platform. Genotype data for both ALSPAC mothers and children were imputed against the Haplotype Reference Consortium v1.1 reference panel, after performing the QC

procedure (minor allele frequency (MAF) ≥1%, a call rate ≥95%, in Hardy-Weinberg equilibrium (HWE), correct sex assignment, no evidence of cryptic relatedness, and of European descent). The samples of the BiB cohort (mothers and offspring) were processed on three different type of Illumina chips: HumanCoreExome12v1.0, HumanCoreExome12v1.1 and HumanCoreExome24v1.0. Genotype data were imputed against UK10K + 1000 Genomes reference panel, after a similar QC procedure (a call rate ≥99.5%, correct sex assignment, no evidence of cryptic relatedness, correct ethnicity assignment). In MoBa, blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth [164]. Genotyping has had to rely on several projects - each contributing with resources to genotype subsets of MoBa over the last decade. The data used in the present study was derived from a cohort of genotypes samples from four MoBa batches. The MoBa genetics QC procedure involved MAF ≥1%, a call rate ≥95%, in HWE, correct sex assignment, and no evidence of cryptic relatedness. Further details of the genotyping methods for each cohort are provided in Appendices (Text S5.3) including flow charts showing selection of participants (Figure S5.2).

5.3.5.2. GWAS data and SNP selection

I aimed to construct weighted GRSs for metabolites that had a p-value <0.05 (referred to throughout as "suggestively associated" with CHDs) in the multivariable regression analyses using BiB data. To do this, I cross-referenced suggestive associations with large relevant GWAS. I used summary data from two GWAS. In the first, the authors explored the genetic effects of 174 metabolites (compared with the 923 included in our study) [203]. To ensure associations were independent, SNPs used from the first GWAS were selected at $p < 5 \times 10^{-8}$ and were clumped to ensure independence at linkage disequilibrium (LD) $r^2 = 0.001$ and a distance of 10,000 kb using the TwoSampleMR package [213]. In the second (unpublished), the authors performed a GWAS of metabolon metabolite levels using samples from the EPIC-Norfolk [214] and INTERVAL studies [215]. 14,296 participants were included in a discovery set (5,841 from EPIC-Norfolk; 8,455 from INTERVAL) and 5,698 from EPIC-Norfolk in a validation set. The authors performed exact conditional analyses to identify independent associations. A total of 913 metabolites were taken forward for their GWAS analysis.

5.3.5.3. Genetic risk score generation

GRSs were calculated using SNPs previously associated in largescale GWAS with metabolites (described above) by adding up the number of metabolite increasing alleles among the selected SNPs after weighting each SNP by its effect on the corresponding metabolite:

$$GRS = w1 \times SNP1 + w2 \times SNP2 + \cdots wn \times SNPn$$

where w is the weight (i.e., the beta-coefficient of association of the SNP with the exposure from the published GWAS) and SNP is the genotype dosage of exposure-increasing alleles at that locus (i.e., 0, 1, or 2 exposure-raising alleles). After matching metabolites suggestively associated with CHDs at P<0.05 from multivariable regression analyses and removing indels, selected SNPs were extracted from the imputed genotype data in dosage format using QCTOOL (v2.0) and VCF tools (v 0.1.12b) in ALSPAC and BiB, respectively. PLINK (v1.9) was then used to construct the GRS for each exposure coded so that an increased score associated with increased levels of metabolite. In MoBa, I constructed the GRSs from the QC'd data in PLINK format. If a SNP was missing, a proxy SNP was used where available based on $r^2 > 0.8$ using the European reference panel in the LDLink R package [216].

5.3.6. Statistical analysis

Analyses were performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). An analysis plan was written and uploaded to the Open Science Framework before analyses commenced, where any subsequent changes to analyses were documented along with the rationale [217]. I used scaled imputed data (in which missing data have been imputed and the multiple of median values transformed to standard deviation (SD)- scores) which was log transformed. Any metabolite (in either dataset) where there was too little variation for meaningful analyses (defined as < 440 unique values) was excluded [218]. Transformed metabolite values were converted to standard deviation SD units. There were 1,100 and 1,150 quantified metabolites included in dataset 1 and 2, respectively, with 923 of these present in both datasets.

5.3.6.1. Multivariable regression (metabolomic) analyses

I used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of any CHD per SD higher metabolite, with and without adjustment for confounders. As I am interested in potential causal effects, I present confounder adjusted results throughout. Analyses were done separately

in the two BiB datasets and results pooled using fixed-effects meta-analyses. Given that CHD is rare and binary, I accepted an uncorrected P<0.05 (from meta-analyses) for the metabolite being suggestively associated with CHD in the offspring (but requiring further validation). I took these metabolites forward to MR analyses.

The MS-platform used in BiB includes measures of xenobiotics which are synthetic chemicals that are not synthesised by humans. Their presence in the circulation usually reflects endogenous exposures, such as medications and supplements. Given that these metabolites would not be present in all participants (and therefore have high missingness), many were removed (86/154 (56%)) from the dataset given the metabolite inclusion criteria of 440 unique observations mentioned above. Therefore, I performed an exploratory additional analysis using xenobiotics (N = 154) as binary variables (1 = yes; metabolite is detected in the sample, 0 = no; metabolite is not detected in the sample). I present adjusted ORs of these binary variables (any presence vs none) with CHDs.

I sought to internally validate any of the metabolites suggestively associated with CHDs that were also measured in BiB in larger numbers using different methods. After matching suggestive associations, I used data from the NMR platform (N = 2 metabolites) and did not use any data from the clinical chemistry measurements. More information on the BiB NMR data including methods, QC and participant information has been described in detail previously (Appendices) [210].

5.3.6.2. Mendelian randomisation analyses

I undertook MR in each of the 3 cohorts, including all BiB, ALSPAC, and MoBa participants with maternal genetic data and offspring CHD data. Logistic regression was used to estimate the OR of CHD per SD change in GRS, with adjustment for the first 10 genetic principal components (PCs) with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa.

The key assumptions for MR are: (i) relevance assumption - the genetic instruments are robustly associated with the exposure and relevant to the population being studied (i.e., here pregnant women). I tested the association of the GRS of each metabolite with metabolite levels during pregnancy in BiB dataset 2. (ii) Independence assumption - The IV outcome association is not confounded. Such confounding could occur as a result of population stratification. To minimise this, I adjusted GRS-CHDs associations for the first 10 genetic PCs. I also repeated the MR analyses without the inclusion of BiB, given that BiB has a unique ethnic structure of South Asians and White Europeans. (iii) Exclusion restriction criteria - The genetic variant is not related to the outcome other than via its association with the exposure.

I assessed pleiotropy by estimating the variance explained in all metabolites by each of the GRSs by undertaking the linear regression of every metabolite measured in BiB on each GRS. If the variance explained in other metabolites was similar or greater than to that explained in the candidate risk metabolite, this would suggest that there is low metabolite-specificity for the GRS and potential horizontal pleiotropic bias via the other metabolite(s). Importantly, however, this approach of testing GRS specificity does not distinguish between vertical pleiotropy (e.g., the GRS influences the candidate metabolite which is the precursor of another metabolite that affects CHD) and horizontal pleiotropy (e.g., the GRS influences two metabolites that affect CHD independently). I also check consistency of MR results when additionally adjusting for fetal genotype [89]. I performed MR analyses separately in BiB, ALSPAC and MoBa and report pooled results from random-effect meta-analyses for all three cohorts and fixed-effect meta-analyses for MR analyses excluding BiB (i.e., ALSPAC and MoBa).

5.4. Results

5.4.1. Main BiB multivariable regression analyses

Table 5.1 shows the distributions of characteristics for the women in both BiB datasets. In total, there were 2,605 mother-offspring pairs with 46 CHD cases included in the BiB multivariable regression metabolomic analyses. N.B. for consistency and clarity, I refer to metabolites here by their super-pathways (as defined by Metabolon). A metabolite might have a different super-pathway and chemical group. For example, N-Acetylcarnosine is a metabolite that is part of the amino acid super-pathway, but it is not an amino acid itself. The super-pathways that included the largest proportions of the 923 metabolites were lipids (38%), unknown (22%), amino acids (18%) and Xenobiotics (8%), with other super-paths having ≤ 3% of the total (**Table 5.2)**.

Of the 923 metabolites quantified in both BiB datasets, 44 (4.8%) were associated with any CHD, at $P < 0.05$, in confounder adjusted pooled analyses (**Figure 5.2**). I observed suggestive effects (i.e., confounder adjusted associations reaching the p-value threshold <0.05) with several amino acids, lipids and co-factors and vitamins. There were also suggestive effects for two xenobiotics, one nucleotide, one energy metabolite and some partially characterised and unknown metabolites (**Figure 5.2**). None of the 22 peptide or 19 carbohydrate-related metabolites associated with CHD at this p-value threshold. Of the 18 lipid-related metabolites associated with CHD, 13 were positively associated (i.e., increased odds) (e.g., Glycolithocholate Sulfate: adjusted odds ratio (aOR) per SD increase in metabolite: 1.73 95% CI (1.21,

2.48)) and 5 were negatively associated (decreased odds) (e.g., Phosphocholine: aOR 0.65 (0.47, 0.90)). All but one (N−Acetylcarnosine) of the 10 amino acid-related metabolites were negatively associated with CHDs (e.g., isoleucine: aOR: 0.67 (0.49, 0.92)). 3 of the 4 co-factors and vitamins were negatively associated, whereas 1 (biliverdin) was positively associated (aOR 1.41 (1.07, 1.86)). The one nucleotide was negatively associated (inosine 5'−Monophosphate (Imp): aOR 0.59 (0.36, 0.99)) and the one energy related metabolite positively associated (succinylcarnitine (C4): aOR 1.42 (1.02, 1.97)). Benzoate and Saccharin were the two xenobiotics associated with CHDs in main analyses both showing positive associations. Results for associations of all metabolites (irrespective of p-value) in unadjusted and confounder adjusted analyses from the pooled datasets, and each dataset separately are provided in Appendices (Tables S5.5-S5.7).

In the analysis treating xenobiotics as binary variables, after removal of metabolites with no exposed cases, there were 6 xenobiotic metabolites suggestively associated with offspring CHDs (Appendices: Table S5.2). 2 out of the 6 showed positive associations: saccharin, which was also associated in main analyses (adjusted odds ratio (aOR) for the presence of metabolite vs not: 2.16 95% CI (1.02, 5.13)) – an artificial sweetener) and salicyluric glucuronide (aOR: 2.27 (1.16, 4.29)) – a metabolite involved in aspirin metabolism). The remaining 4 showing negative associations are all part of the food component/plant metabolite sub pathway (Table S5.2).

5.4.2. Internal validation using NMR or clinical chemistry measures of suggestive associations from main multivariable regression analyses

It was possible to explore 2 of the 44 metabolites suggestively associated with CHDs in the larger BiB sample. In comparable confounder adjusted analyses, NMR measured amino acids isoleucine and leucine were available on 7,296 mothers, with 87 having an offspring with CHD. Results for these two amino acids were highly consistent between the two samples/assay methods (aOR per SD increase in MS isoleucine 0.67 (0.49, 0.92) vs 0.65 (0.50, 0.84) for NMR isoleucine and aOR per SD increase in MS leucine 0.69 (0.51, 0.94) vs 0.67 (0.53, 0.85) for NMR leucine).

Table 5.1. Participant characteristics for the Born in Bradford metabolomic analyses.

Characteristic	Category	BiB dataset 1 (N = 998)	BiB dataset 2 (N = 1,607)
Offspring			
CHD	Yes	15 (1.6)	31 (1.9)
Sex	Male	510 (51.1)	844 (52.5)
	Female	488 (48.9)	763 (47.5)
Maternal			
Age, years		27.5 (5.7)	27.3 (5.6)
Parity	Nulliparous	358 (37.0)	616 (36.8)
	Multiparous	610 (63.0)	991 (63.2)
BMI, kg/m^2		26.7 (6.0)	26.5 (5.8)
Ethnicity	White British	500 (50.0)	733 (45.6)
	Pakistani	498 (50.0)	874 (54.4)
Neighbourhood deprivation (IMD)	Quintile 1 (most deprived)	654 (65.5)	1084 (67.5)
	Quintile 2	175 (17.5)	281 (17.5)
	Quintile 3	112 (11.2)	175 (10.9)
	Quintile 4	38 (3.8)	40 (2.5)
	Quintile 5 (least deprived)	19 (1.9)	27 (2.7)
Smoking	Yes	176 (17.7)	311 (19.2)
Alcohol	Yes	338 (33.9)	496 (30.8)
Gest age at blood sampling, weeks		26.2 (2.0)	26.2 (2.0)

Data are means ± SD or n (%) unless stated. Abbreviations: BiB, Born in Bradford; CHD, congenital heart disease; BMI, body mass index; kg, kilogram; m, meter; IMD, Index of Multiple Deprivation (taken from 2010 national quintiles); gest, gestational.

Table 5.2. Showing the breakdown of metabolites in the dataset (N = 923) into the 10 super-pathways as defined by Metabolon.

Super pathway	N (%) for all metabolites (N = 923)	N (%) for metabolites suggestively associated with CHDs (N = 44)
Amino Acid	170 (18.4%)	10 (22.7%)
Lipid	354 (38.4%)	18 (40.9%)
Cofactors and Vitamins	27 (2.9%)	4 (9.0%)
Partially Characterised Molecules	3 (0.3%)	1 (2.3%)
Unknown	201 (21.8%)	7 (15.9%)
Xenobiotics	86 (9.3%)	2 (4.5%)
Nucleotide	33 (3.6%)	1 (2.3%)
Energy	8 (0.9%)	1 (2.3%)
Carbohydrate	19 (2.1%)	0
Peptide	22 (2.4%)	0

Abbreviations: CHD, congenital heart disease.

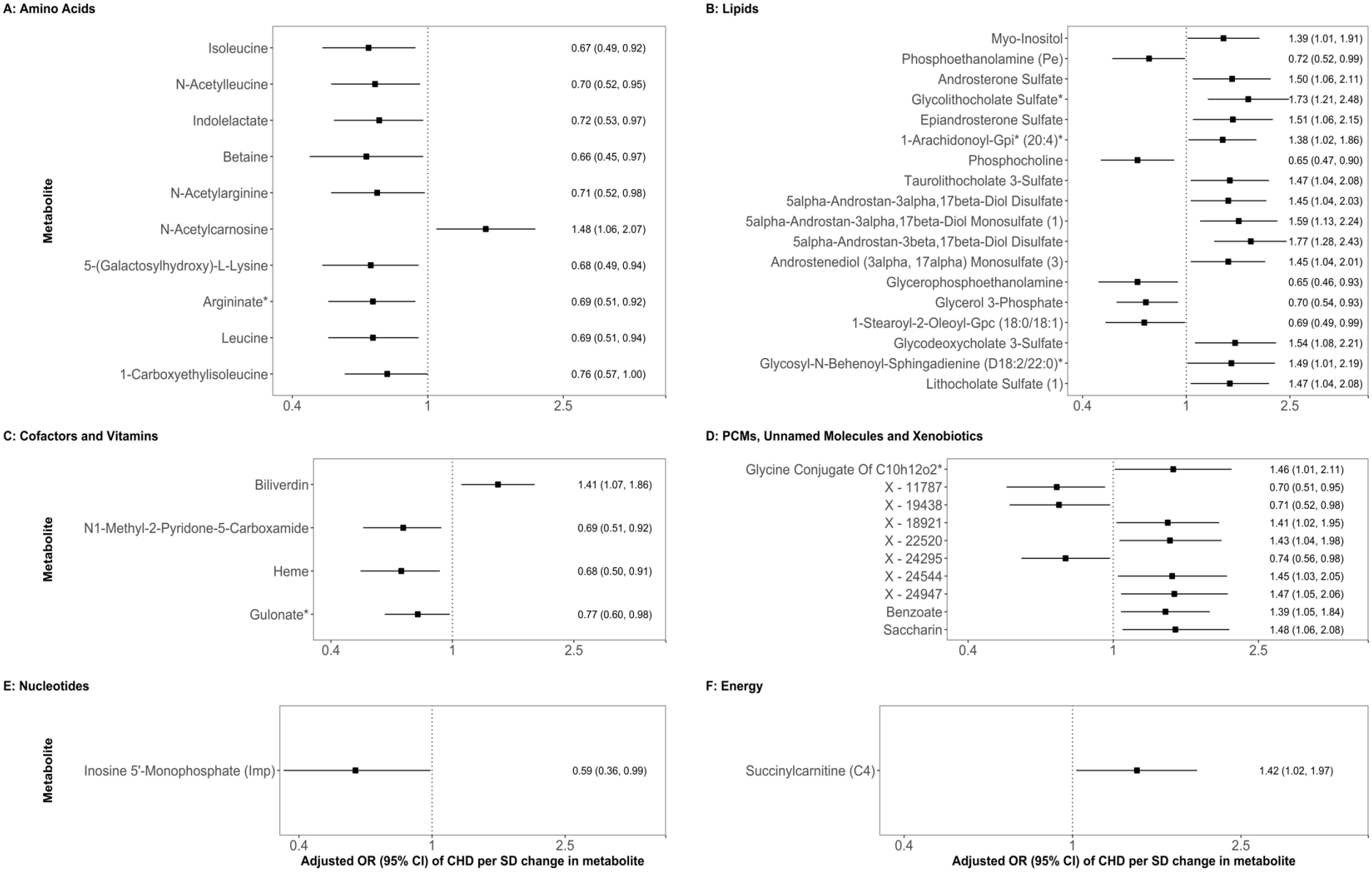

Figure 5.2. Pooled confounder adjusted associations of maternal pregnancy metabolites with offspring congenital heart disease in the Born in Bradford cohort (N = 2,391 & N CHD cases = 42).

The associations show confounder adjusted odds ratios of CHD per standard deviation change in log-transformed metabolite levels for the 44 (out of 923) metabolites that associated with CHD at p-value <0.05 separated by super pathways as defined by Metabolon. Metabolites were measured at ~26-28 weeks' gestation. Heterogeneity statistics and separate associations for datasets 1 and 2 are reported in Supplementary Tables S5.5-S5.7. Associations were adjusted for maternal age, ethnicity, parity, Index of Multiple Deprivation, body mass index, smoking and alcohol intake. Abbreviations: PCMs, partially characterised molecules; OR, odds ratio; CHD, congenital heart disease; SD, standard deviation.

5.4.3. Validating findings with Mendelian randomisation

The distributions of offspring and maternal characteristics for MR analyses in BiB, ALSPAC and MoBa are displayed in Table S5.3 (Appendices). It was possible to explore MR replication for 27 of the 44 metabolites that associated with CHD in multivariable analyses (the other 17 were either not available in the GWAS or had genetic variants not available in the cohorts (see flowchart in Figure S5.3)). All but 3 of the GRSs (24/27 (89%)) were associated with the corresponding metabolite during pregnancy in BiB (with R^2 values ranging from 0.3% to 34%, for the remaining 3 the associations were wide with confidence intervals that included the null (Table S5.4). Of the 27 GRSs, 3 were specific for the metabolite they were instrumenting (i.e., had the strongest association with it and little evidence of associations with other metabolites; N-acetylcarnosine, phosphocholine and succinylcarnitine). 18 GRSs were associated with the metabolite they were instrumenting and several others that were correlated with that metabolite (e.g., the biliverdin GRS was associated with it and also similarly with other hepatic-related metabolites). 6 GRSs were more strongly associated with other (uncorrelated) metabolites than the one they were instrumenting (scatter plots for all 27 GRSs are shown in Figure S5.4). The 6 non-specific GRS were for indolelactate, glycolithocholate sulfate, isoleucine, leucine, myo-inositol and taurolithocholate 3-sulfate (MR results for these should be treated with caution and are denoted in **Figure 5.3B** by white-filled points).

MR analyses replicated and provided causal evidence for a potential protective effect of higher levels of the amino acids leucine, indolelactate and isoleucine on CHD, but for the other amino acids MR results were either very close to the null or in the opposite direction (**Figure 5.3**). Seven of the lipid-related metabolites that were positively associated in multivariable regression were also replicated in MR analyses (6 of which were highly correlated androgenic steroid metabolites), as was the energy related metabolite succinylcarnitine (**Figure 5.3**). For the 11 metabolites where I consider the MR GRS analyses providing some evidence of replication and a potential causal effect, 7 of the GRSs were specific for the metabolite alone and/or also for its correlates. Individual study results and P-values for heterogeneity are included in shown Table S5.8. MR results were largely unchanged when excluding BiB from analyses (Table S5.8) and when adjusting for offspring genotype (Table S5.9).

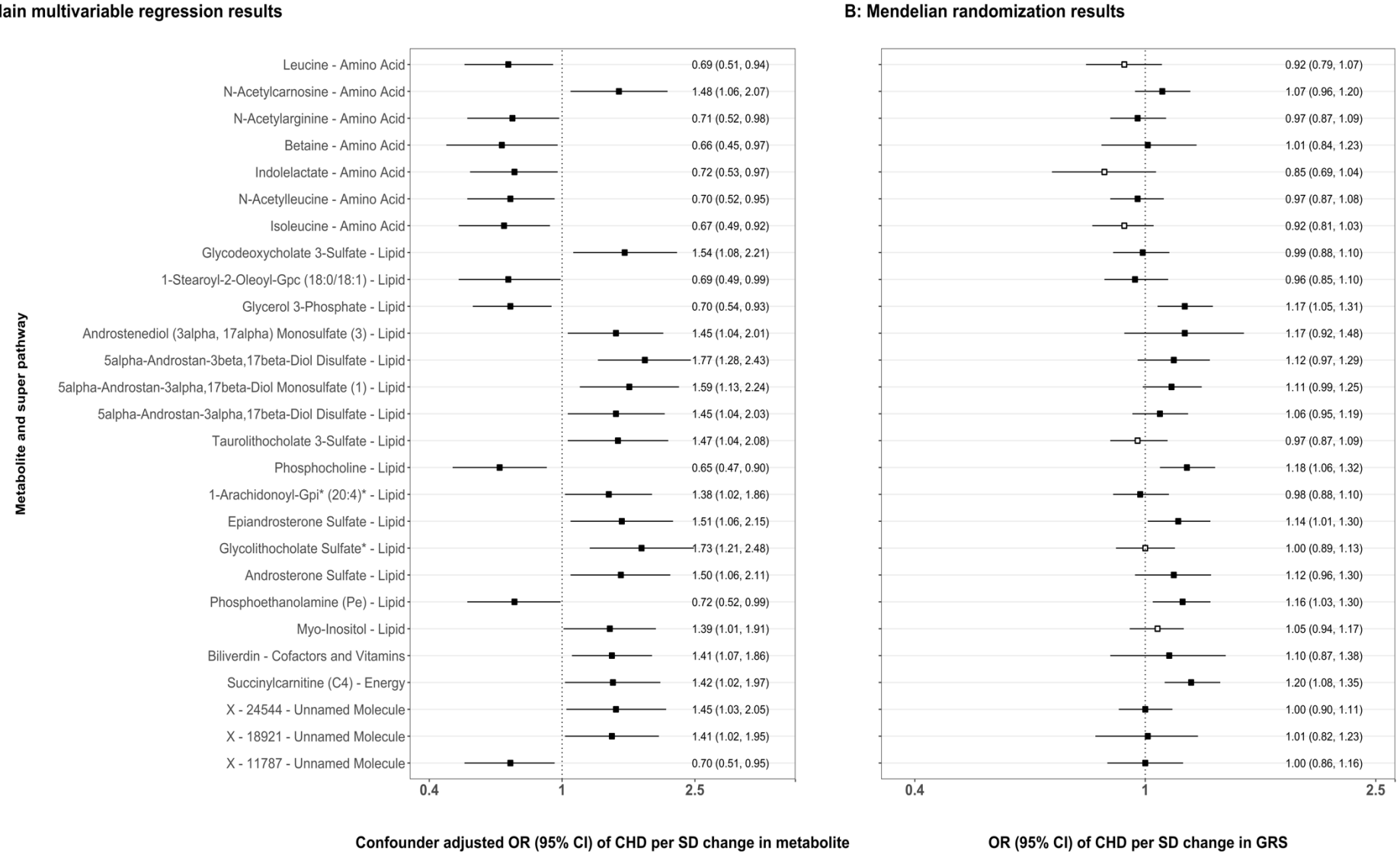

Figure 5.3. Showing results comparing the main confounder adjusted associations of maternal metabolites with offspring CHDs (Panel A: N = 2,391 & N CHD cases = 42) to the Mendelian randomisation analyses of maternal genetic risk scores and offspring CHDs (Panel B: N = 38,662 & N CHD cases = 319).
N.B. results from each analysis are presented on different scales; I am not attempting to quantify estimates in the MR analyses, the aim is to compare the direction of effect. The confounder adjusted associations are as above in Figure 5.2. The MR analyses are adjusted for the top 10 genetic principal components and genetic batches in MoBa. In Panel B, the metabolite genetic risk scores filled with white appeared to be non-specific for the metabolite I was trying to

instrument. The metabolites filled in black were either metabolite-specific or specific to the metabolite and other correlated metabolites (see scatter plots in Figure S5.4). The results were pooled using random effects meta-analyses; individual study results and P-values for heterogeneity are shown in Supplementary Table S5.7. Abbreviations: BiB, Born in Bradford; CHD, congenital heart disease; GRS, genetic risk score; MR, Mendelian randomisation; OR, odds ratio; CI, confidence interval.

5.5. Discussion

Maternal metabolism is important for healthy fetal growth and development. To my knowledge no previous study has examined the association of detailed maternal metabolites with risk of CHD within a causal framework and BiB is the only cohort that I was aware of with relevant data. In this novel study I found 44 metabolites (of 923) suggestively associated with CHD. These included metabolites related to amino acids, lipids, co-factors and vitamins, unknown molecules, xenobiotics, nucleotides and energy. In separate xenobiotics analyses, there was some evidence that metabolites related to aspirin and saccharine may increase odds of CHD, whereas metabolites related to plant food components may reduce odds. Two of the amino acids were validated in BiB in larger numbers using an alternative metabolomics platform. In MR analyses, there was directional consistency for 11/27 metabolites. I found that amino acid metabolism during pregnancy, several lipids (more specifically androgenic steroids), and levels of succinylcarnitine could be important contributing factors.

9 out of the 10 amino acids suggestively associated with CHD were negatively associated suggesting that deficiencies in certain amino acids during pregnancy could contribute to CHD. Previous research found that amino acid concentrations measured in amniotic fluid were lower in patients with CHD [219], a similar pattern to what I found here. I was able to validate findings for isoleucine and leucine in larger numbers in BiB which improves the confidence in the findings. The MR analyses also provided evidence to support the direction of association for these metabolites. However, the GRSs for isoleucine and leucine were non-specific and so these results should be treated with caution.

18 of the 44 maternal metabolites associated with CHD were part of the lipid super pathway, which is the most common super pathway measured by the Metabolon platform. Previous work reported that an abnormal lipid profile (defined as elevated cholesterol and apolipoprotein B) [86], abnormal lipid metabolism (defined as a disturbance in phosphatidyl-choline and various sphingolipids and choline metabolism) [13] and high maternal blood lipids [87] are a feature of CHD pregnancies. I was able to take forward 15 (out of 18) of the lipid metabolites and replicated the direction of effect for 7. All except 1 of these 7 replicated metabolites were androgenic steroids and so were highly correlated. Steroids are important for numerous functions during gestation, particularly for normal placental function [220]. Here I present evidence of a potential causal effect (associated in metabolomic analyses with consistent direction of effect in MR analyses) of positive associations between maternal gestational androgenic steroid metabolites and offspring CHD.

Levels of bilirubin and biliverdin were positively associated with CHD, two compounds involved in heme catabolism which should be investigated further. MR findings for biliverdin were inconclusive with wide confidence intervals. Levels of succinylcarnitine were also positively associated with CHDs and I found good replications in MR analyses with consistent directions of effect and a GRS that appeared highly specific for succinylcarnitine. Succinylcarnitine is an acylcarnitine which are a group of metabolites responsible for beta oxidation of fatty acids and mitochondrial function [221]. It is well documented that fatty acids play an important role in embryonic and fetal development [222,223]. I included analyses of partially characterised and unknown metabolites in results as with increasing evidence from genomic studies, previously unknown metabolites are having their function identified. With future studies identifying the function of some of these unknown/partially characterised metabolites, my results could shed light on the aetiology of CHDs.

A key strength of this study is the unique data that I had in BiB to support novel analyses of associations of a wide range of maternal metabolic paths with offspring CHD risk. I was not able to identify any other study with such data. However, I realised, even before analyses, that I would have limited statistical power with just 46 CHD cases. This motivated me to think about ways of trying to replicate any findings in larger samples either through finding measures of the same metabolites available from other assays in larger samples or using GRSs as instruments for the metabolites. In the initial multivariable regression analyses I adjusted for potential confounders. I defined suggestive associations based on a p-value threshold < 0.05, i.e., not taking account of multiple testing, and when I apply a Bonferroni corrected threshold ($P < 0.0001$) none of the associations pass this. However, I felt this was appropriate for determining which associations to take forward to replication. As with any 'screening' for further analyses I wanted to ensure that I would not miss potential causal effects. I recognise that selecting results based on a p-value threshold is problematic as some associations with higher p-values might have associations of a magnitude that could be clinically important, but there would also be potential for several false positives. Also, I limited MR analyses only to those metabolites that associated with $p < 0.05$ rather than undertaking these analyses on all of the 923 metabolites. The reason for this was that having searched for all studies with maternal genome wide data and offspring CHD outcomes I identified only three cohorts and recognised that for MR analyses pooled results from these might also have limited power. The limited power in both multivariable and MR analyses also meant that I could only examine associations with any CHD and not subtypes.

MR analyses are sensitive to their assumptions that the GRS is statistically strongly associated with the metabolite in pregnancy. I examined associations of these with pregnancy metabolite levels and I am

careful in my interpretation of results in relation to this. Methods that are available for exploring potential bias due to horizontal pleiotropy in two-sample MR were not possible here. I know that many of the 923 metabolites will be biologically related to each other and with the sample size in the present study it would be difficult to robustly distinguish effects of correlated metabolites. I explored this by examining the strength of association (proportion of variation explained) of each of the 27 GRSs with all other metabolites available in BiB dataset 2 (Figure S5.4). Stronger or similar associations with other metabolites would suggest that the GRS is not a specific instrument for the metabolite that I am using it for. In this case this could be because of known biological relations. For example, many of the lipid metabolites are related to each other biologically, and I saw this with similar proportions of variation explained by the GRS of the androgenic steroid lipid metabolites with other androgenic steroid lipid metabolites. As such I would interpret results for these metabolites as supporting an effect of maternal androgenic steroid metabolites on CHD, but I cannot be specific about which ones are driving this. Similar or stronger variation of a GRS for other metabolites could be related to vertical pleiotropy, i.e., the metabolite for which the GRS is instrumenting strongly influences other metabolites that are related to CHD with the other metabolites partly mediating the effect of the focused metabolite. This would not bias the result. However, this could also occur with horizontal pleiotropy where the GRS, independently of the metabolite of interest, influences other metabolites that are risk factors for CHD. With the current data I was not able to distinguish between these two.

A further limitation of this study is that maternal plasma/serum metabolomics data were derived at a single timepoint around 26-28 weeks' gestation. Fetal cardiac development starts early in pregnancy and much of the development occurs in the first trimester [143]. Here, I am assuming that metabolite levels around 26-28 weeks' gestation are good proxies for levels in early pregnancy - when the offspring heart is forming. Previous work has shown that between person differences throughout pregnancy remain largely consistent (i.e., those with a high level of a metabolite in early pregnancy tend to have a similarly high level of a metabolite in later pregnancy) [81]. Similarly, and worth mentioning, the effects obtained from MR studies are often interpreted as the lifetime effect of the exposure (metabolites) in question [224].

In summary, I have used metabolomics data obtained during pregnancy to explore how the maternal metabolome may contribute to offspring CHDs. I found evidence that amino acid metabolism during pregnancy, several lipids (more specifically androgenic steroids), and levels of succinylcarnitine could be important contributing factors. My analysis pipeline, which involved seeking replication of metabolite associations by harnessing large-scale GWAS data, provides scope to improve the reliability of findings and should prove to be more useful as these datasets continue to grow. Metabolomics could prove to be

an important tool for identifying biological pathways that may lead to identification of prevention targets to decrease the disease burden of CHDs. To do this, future research will require international collaboration of more and larger studies with detailed metabolomics data in pregnancy, ideally with some of these having repeat measures across pregnancy and offspring CHD data.

Chapter 6. Associations between maternal gestational NMR-derived metabolic profiles and congenital heart disease in the offspring: results from multivariable and Mendelian randomisation analyses

6.1. Chapter summary

This Chapter has not yet been published. I am waiting for data from the Norwegian Mother, Father and Child Cohort (MoBa) which was delayed due to COVID-19 to perform external replication analyses before submitting for publication.

Chapter 5 examined the relationship of maternal gestational mass spectrometry (MS)-derived metabolites with offspring congenital heart disease (CHD) using multivariable regression and Mendelian randomisation (MR) analyses. In this Chapter (**Chapter 6**)**,** I undertook a similar approach to **Chapter 5** by employing multivariable regression and MR analyses. The work in this chapter examined the relationship of maternal gestational nuclear magnetic resonance (NMR)-derived metabolic traits with offspring CHD. This work complements the work in **Chapter 5** by having metabolomic data in larger numbers (~11,000 versus ~3,000) and by constructing genetic instruments for more traits using data from a larger and more powered GWAS. I found evidence that amino acid metabolism during pregnancy could be an important contributing factor which is consistent to results presented in **Chapter 5**. There was also evidence of potential effects for some fatty acid and very low-density lipoprotein (VLDL) traits, albumin, and citrate.

6.2. Introduction

Congenital heart diseases (CHDs) are the commonest form of congenital anomaly and remain one of the leading causes of childhood mortality [225]. Causes of CHDs include chromosomal abnormalities, gene disorders and a small number of known teratogens [8]. The causes of most cases remain largely unknown, although, the pregnancy environment (intrauterine factors) appears to be important. Identifying modifiable risk factors for CHDs is important for improving aetiological understanding and developing preventative interventions.

Associations between modifiable environmental exposures during pregnancy and offspring CHDs have been studied extensively [61]. Despite this, there are few well established causal risk factors for CHDs, partly due to the conventional epidemiological study designs that are commonly used and the heterogeneity in presentation/prevalence of the disorder. More recently, studies have started to explore associations between potential biomarkers during pregnancy and offspring CHDs. One study found that a compromised vitamin D status (defined as 25-hydroxyvitamin D < 50 nmol/l in comparison to adequate vitamin D status, defined as > 75 nmol/l) was associated with increased risk of offspring CHDs [85]. Fatty acids are known to play an important role in embryonic and fetal development [222,223] and there is some evidence suggesting that high maternal blood lipids are associated with increased risk of offspring CHDs [86,87]. Other work has shown that poor glucose control and diabetes during pregnancy can increase CHD risk [206–208].

These studies focus on single or few biomarkers. Exploring the wider metabolome could provide opportunities to improve our understanding of the molecular mechanisms that underpin CHDs [205]. Metabolomics is the quantification of metabolites. The metabolome is influenced by both genotype and environment, and dynamically responds to environmental influences. Information on the metabolome during pregnancy can provide information on a range of diet and lifestyle factors that are being used/adopted by the mother which can be difficult to reliably obtain via self-report. Previous work has shown how changes in pregnancy characteristics such as body mass index (BMI), gestational diabetes, hypertensive disorders of pregnancy and others are associated with changes in metabolic profiles assessed by nuclear magnetic resonance (NMR) metabolomics [197]. It is plausible that these changes in the metabolome that are caused by these exposures could influence offspring outcomes.

To date, there have been several studies that have used pregnancy metabolomics data to research offspring CHDs. Some of the studies have aimed to use metabolomics for early diagnosis rather than attempting to establish causal mechanisms. For example, one study including 27 CHD cases and 59

controls aimed to identify metabolomic markers in maternal serum during pregnancy for the detection of CHDs [82]. They found more than 100 metabolites that differed between CHD cases and non-cases concluding that abnormal lipid metabolism was a significant feature of CHD pregnancies. However, the sample size was small, and their results have not been externally validated. Other work has explored potential biomarkers of maternal urine metabolomics with offspring CHDs (N = 70 CHD cases and 70 controls) [83]. Their results indicated that short chain fatty acids and aromatic amino acid metabolism in a Chinese population may be relevant to CHDs. Recent work using an untargeted metabolomics approach using maternal amniotic fluid samples discovered that uric acid and proline were significantly elevated in CHD cases [84]. In summary, there have been promising studies uncovering potentially important biological pathways associated with offspring CHDs. However, the evidence is preliminary and there is a need for further larger prospective studies to further interrogate this research area and attempt to replicate previous findings.

The aim of this study was to explore associations of pregnancy metabolic profiles quantified by NMR (N = 148 metabolomic traits) and the odds of CHD in the offspring. I used data from the Born in Bradford (BiB) cohort (N = 11,195 participants; 127 CHD cases (1.1%)), a UK birth cohort with approximately half White European women and half South Asian women [134]. I subsequently perform Mendelian randomisation (MR) [100] analyses using genetic instruments of NMR metabolomic traits to explore replication. The rationale for MR analyses was twofold: firstly, using alternative methods that have differing sources of bias to explore the same causal question can improve confidence in findings [96]. Secondly, I was able to identify two additional cohorts to include in these analyses (total N in pooled analyses = 38,664 participants; 319 CHD cases (%)) that had maternal genotype data and offspring CHD data (but did not have NMR metabolomics data to contribute to the initial analyses).

6.3. Methods

6.3.1. Study design and participants

An overview of the study design is illustrated in **Figure 6.1**. I excluded children of multiple births because they differ from single births for congenital anomaly outcomes [211,212]. For multivariable metabolomic analyses, I used data from the BiB cohort. BiB is a population-based prospective birth cohort including 12,453 women across 13,776 pregnancies who were recruited at their oral glucose tolerance test (OGTT) at approximately 26–28 weeks' gestation [134]. To be included in MR analyses, studies and

participants had to have genome-wide data in mothers and CHD data in the offspring. Three cohorts contributed to MR analyses: BiB, The Norwegian Mother, Father and Child Cohort Study (MoBa) and The Avon Longitudinal Study of Parents and Children (ALSPAC). ALSPAC is a UK prospective birth cohort study which was devised to investigate the environmental and genetic factors of health and development [111–113]. 14,541 pregnant women with an expected delivery date of April 1991 and December 1992, residing in the former region of Avon, UK, were eligible to take part. MoBa is a nationwide, pregnancy cohort comprising family triads (mother-father-offspring) who are followed longitudinally. All pregnant women in Norway who were able to read Norwegian were eligible for participation. The first child was born in October 1999 and the last in July 2009 [136,137]. MR analyses included 38,664 mother-offspring pairs, within which 319 offspring had CHD (**Figure 6.1**).

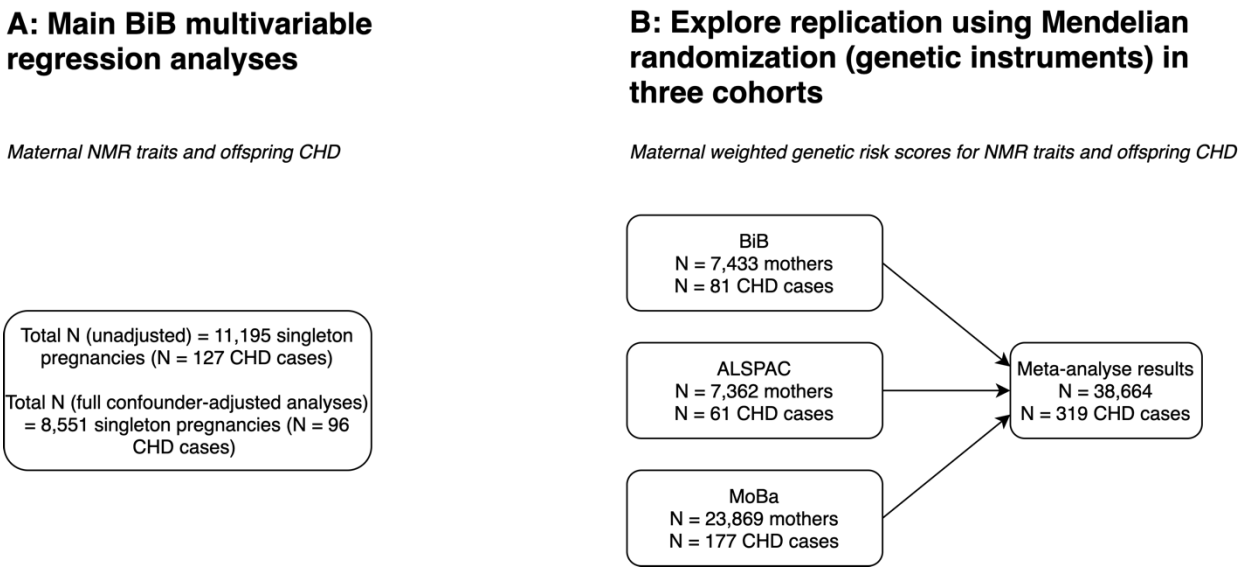

Figure 6.1. An overview of the study design.
Abbreviations: BiB, Born in Bradford; NMR, nuclear magnetic resonance; CHD, congenital heart disease; ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort Study.

6.3.2. Sample collection and metabolomic profiling

Of the 13,776 pregnancies in the BiB cohort, 11,480 had a fasting blood sample taken during the OGTT (n = 10,574 [92%] between 26–28 weeks' gestation, with the remaining women being within 11–39 weeks' gestation). The selection of participants into the BiB NMR analysis dataset is shown in a flowchart

in Appendices (Figure S6.1). Samples were taken by trained phlebotomists working in the antenatal clinic of the Bradford Royal Infirmary and sent immediately to the hospital laboratory. The metabolomics data in the BiB cohort have been described in detail previously including methods, participant selection, validation, known issues and a summary of published research (Appendices) [226]. In brief, profiling of circulating lipids, fatty acids, and metabolites was done by a high-throughput targeted NMR platform (Nightingale Health© (Helsinki, Finland)) at the University of Bristol, providing quantitative information on 227 metabolomic traits (including ratios and other traits derived from the quantified NMR spectra) [76]. After removing ratios and derived traits, I included 148 metabolomic traits for analysis in this study. Samples that were flagged from quality control for either having low glucose, high lactate, high pyruvate or low protein content were removed from analyses.

6.3.3. Confounders

In multivariable regression analyses in BiB, I adjusted for the following maternal characteristics based on their known or plausible influence on maternal metabolites and on CHD: age, ethnicity, parity, residential neighbourhood Index of Multiple Deprivation (IMD), BMI and smoking status. Methods for confounders are described in the appendices (Text S6.1).

6.3.4. Congenital heart disease outcomes

In BiB, cases were identified from two sources. Firstly, the Yorkshire and Humber congenital anomaly register database. This database will tend to pick up most cases that diagnosed antenatally and in the early postnatal period of life. Secondly, through linkage to primary care (up until aged 5), which will have picked up any additional cases, in particular those that might have been less severe and not identified antenatally/in early life [70]. All these cases were confirmed postnatally and were assigned ICD-10 codes. CHD cases were defined according to the European surveillance of congenital anomalies (EUROCAT) guidelines. In the ALSPAC cohort, cases were obtained from a range of data sources, including health record linkage and questionnaire data up until age 25 following European EUROCAT guidelines [35]. In MoBa, information on whether a child had a CHD or not (yes/no) was obtained through linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN. Further details on defining CHDs including ICD codes are shown in the Appendices (Text S6.2).

6.3.5. Genetic data

6.3.5.1. GWAS data and SNP selection

Genetic instruments for NMR metabolomic traits were selected using GWAS that were conducted in UKBiobank (UKBB) participants of European ancestry (N = up to 115,082). The details on genotyping quality control, phasing, and imputation in the UKBB have been described previously [227]. GWAS were performed using BOLT-LMM. GWAS analyses were adjusted for sex, array, and fasting time. A full description of the pipeline can be found online (https://data.bris.ac.uk/data/dataset/pnoat8cxo0u52p6ynfaekeigi). The GWAS summary data for each NMR metabolomic trait has been made publicly available via the OpenGWAS database under the batch name 'met-d' (https://gwas.mrcieu.ac.uk/datasets/). GWAS were conducted as a whole cohort and stratified by sex. I used the TwoSampleMR R package [213] to select genome-wide independent SNPs for each metabolomic trait from the full cohort analyses (associated at $P < 5 \times 10^{-8}$, $r^2 = 0.001$ and a distance of 10,000 kb) and used weights from the female-specific GWAS to construct genetic risk scores (GRS) [213].

6.3.5.2. Genotyping in each cohort

ALSPAC mothers were genotyped using Illumina human660K quad single nucleotide polymorphism (SNP) chip, and ALSPAC children were genotyped using Illumina HumanHap550 quad genome-wide SNP genotyping platform. Genotype data for both ALSPAC mothers and children were imputed against the Haplotype Reference Consortium v1.1 reference panel, after performing the QC procedure (minor allele frequency (MAF) ≥1%, a call rate ≥95%, in Hardy-Weinberg equilibrium (HWE), correct sex assignment, no evidence of cryptic relatedness, and of European descent). The samples of the BiB cohort (mothers and offspring) were processed on three different type of Illumina chips: HumanCoreExome12v1.0, HumanCoreExome12v1.1 and HumanCoreExome24v1.0. The pre-processing of samples was done separately for the three chips. Genotype data were imputed against UK10K + 1000 Genomes reference panel, after a similar QC procedure (a call rate ≥99.5%, correct sex assignment, no evidence of cryptic relatedness, correct ethnicity assignment). In MoBa, blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth. The data used in the present study was derived from a cohort of genotypes samples from four MoBa batches (N samples = 98,110). Phasing and imputation was performed using the publicly available Haplotype

Reference Consortium data. The MoBa genetics QC procedure involved MAF ≥1%, a call rate ≥95%, in HWE, correct sex assignment, and no evidence of cryptic relatedness. Further details of the genotyping methods for each cohort are provided in Appendices (Text S6.3).

6.3.5.3. Genetic risk score generation

GRSs were calculated using SNPs previously associated in large-scale GWAS with metabolites (described above) using the dose of the effect (exposure-increasing) allele at each SNP, which was first weighted by the effect size of the variant in GWAS and then summed:

$$GRS = w1 \times SNP1 + w2 \times SNP2 + \cdots wn \times SNPn$$

where w is the weight (i.e., the beta-coefficient of association of the SNP with the exposure from the published GWAS. N.B. I used females-specific beta-coefficients) and SNP is the dosage of exposure-raising alleles at that locus (i.e., 0, 1, or 2 exposure-raising alleles). Selected SNPs from the GWAS summary data were extracted from the imputed genotype data in dosage format using VCF tools (v 0.1.12b) and QCTOOL (v2.0) in BiB and ALSPAC, respectively. In MoBa, I constructed the GRS's from the QC'd data in PLINK format. PLINK (v1.9) was used to construct the GRS for each exposure coded so that an increased score associated with increased metabolic trait.

6.3.6. Statistical analysis

Analyses were performed in R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). A pre-specified analysis plan was written alongside discussions with all authors and uploaded to the Open Science Framework prior to analyses [228]. Any subsequent changes to analyses were documented along with the rationale.

6.3.6.1. Multivariable regression (metabolomic) analyses

Prior to analyses, all metabolomic traits (N = 148) were converted to standard deviation (SD) units. I used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of any CHD per SD change in metabolomic trait, with no adjustment and adjustment for cofounders. As I am interested in potential causal effects, I present confounder adjusted results throughout. I checked if missing data were

influencing results by exploring consistency between unadjusted and confounder adjusted estimates in the sub-population with full confounder data. I included an interaction term for ethnicity (White European vs South Asian women) and also visually explored ethnic-stratified results to check for ethnic-specific associations. Given that CHD is rare and binary, and to avoid missing potential causal effects, I did not apply a statistical threshold for a trait being potentially associated with CHDs. Rather, I focused on the point estimates and precision (measured by 95% CIs) and whether the association could be replicated in MR analyses to draw inference.

6.3.6.2. Mendelian randomisation analyses

I undertook MR in each of the 3 cohorts, including BiB, ALSPAC and MoBa participants with maternal genetic and offspring CHD data (N = 38,663, 319 CHD cases: flowchart shown in Figure S6.2). One singleton pregnancy per mother was included. I aimed to perform MR for: (i) all traits that were included in multivariable regression analyses and (ii) were included in the GWAS and had valid genetic instruments. In total I included 145 (of 148) metabolomic traits in MR analyses. Logistic regression was used to estimate the OR of CHD per 1 SD change in GRS, with adjustment for the first 10 genetic principal components (PCs) with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. Using this method (i.e., regressing an outcome on a genetic instrument) allows to test for the presence of a causal effect, whereas it does not quantify the causal estimate which would require a two-step instrumental variable approach with genetic, exposure (NMR data) and outcome data in all participants.

The key assumptions for MR are: (i) relevance assumption - the genetic instruments are robustly associated with the exposure and relevant to the population being studied (pregnant women). I tested the association of the GRS of each metabolomic trait with levels measured during pregnancy in all women with genome-wide and NMR data in BiB. (ii) Independence assumption - the IV outcome association is not confounded. Such confounding could occur as a result of population stratification. To minimise this, I adjusted GRS-CHD associations for the first 10 genetic PCs. I also repeated the MR analyses without the inclusion of BiB, given that BiB has a unique ethnic structure of South Asian and White European women. (iii) Exclusion restriction criteria - the genetic variant is not related to the outcome other than via its association with the exposure – the potential for pleiotropy. I assessed pleiotropy by estimating the variance explained in all metabolomic traits by each of the GRS by running linear regression of every metabolomic trait on each GRS. If the variance explained in other metabolites was similar or greater than

to that explained in the candidate risk metabolite, this would suggest that there is low metabolite-specificity for the GRS and potential horizontal pleiotropic bias via the other metabolite(s). Importantly, however, this approach of testing GRS specificity does not distinguish between vertical pleiotropy and horizontal pleiotropy. I would also expect some GRS to correlate with others. I use Pearson correlations and heatmaps to illustrate the correlation structure of the NMR and GRS datasets in BiB. I also check consistency of MR results when additionally adjusting for fetal genotype [89]. I performed MR analyses separately in BiB, ALSPAC and MoBa and report pooled results from random-effect meta-analyses for all three cohorts and fixed-effect meta-analyses for MR analyses excluding BiB (i.e., ALSPAC and MoBa).

6.4. Results

6.4.1. BiB NMR multivariable regression analyses

Table 6.1 shows the distributions of characteristics for the women included in BiB multivariable regression NMR analyses. In total, there were 11,195 mother-offspring pairs with 127 CHD cases included. Confounder adjusted associations of metabolomic traits measured during pregnancy with offspring CHDs are shown in **Figure 6.2** separated by the trait class. In **Figure 6.2**, I show a truncated version of the results (N = 63 key metabolomic traits, mostly lipoprotein subclasses removed) for clarity and include results for all traits within Appendices (Figure S6.3). For amino acids (**Figure 6.2A**), I observed negative associations for alanine, isoleucine, leucine and valine and a positive association for glutamine with the remainder having estimates around the null. There were no clear differences in odds of CHD for changes in the fatty acids docosahexaenoic acid (DHA), omega-3, omega-6, linoleic acid or polyunsaturated fatty acids (**Figure 6.2B**), whereas monounsaturated, saturated, and total fatty acids were negatively associated. Estimated degree of unsaturation was positively associated with CHD. Levels of remnant cholesterol and total cholesterol in very low-density lipoproteins (VLDL) were negatively associated with offspring CHD, with the remainder of estimates for cholesterol-related traits being around the null (**Figure 6.2C**). All glyceride and phospholipid metabolomic traits were negatively associated, with somewhat stronger negative associations seen for triglycerides (**Figure 6.2D**). There were no differences in odds of CHD for many of the lipoproteins, with exception of VLDL-related lipoproteins which were negatively associated (**Figure 6.2E)**. Levels of glucose were positively associated with offspring CHD (**Figure 6.2F**). Apolipoprotein B (ApoB), citrate, glycerol, lactate, pyruvate, albumin, glycoprotein acetyls (GlycA; a cumulative marker of inflammation) and 3-hydroxybutryare all had point estimates below the null (negative associations). There

were no notable differences in unadjusted results, apart from glucose having a stronger magnitude of association in comparison to the confounder-adjusted estimate (OR and 95% CI of CHD per SD change in metabolomic trait: 1.14 (0.95, 1.38) in confounder adjusted versus 1.23 (1.08, 1.41) unadjusted) (Figure S6.4, Appendices). Results were unchanged when repeating unadjusted and adjusted analyses in the population with full confounder data (N = 8,551 versus N = 11,195 in crude analyses) suggesting that any missing data did not influence findings.

There was no strong statistical evidence for ethnic-specific associations ($P_{interaction} > 0.05$) for associations of metabolomic traits and CHD. However, when visually examining forest plots of the associations of metabolomic traits and CHD stratified by ethnicity, there were some notable differences for creatinine, glucose and several fatty acids (Figure S6.5). Creatinine was negatively associated with CHD in South Asian women (OR and 95% CI of CHD per SD change in metabolomic trait: 0.80 (0.62, 1.05)), but positively associated with CHDs in White European women (1.12 (0.90, 1.41)). Results for the combined ethnicity cohort (including adjustment for ethnicity) were null. For glucose, there is evidence that the positive association seen in the combined ethnicity cohort was being driven by White European women (1.36 (1.00, 1.84)), with results in South Asian women being null (1.00 (0.81, 1.25)) (Figure S6.5).

Table 6.1. Participant characteristics for the Born in Bradford NMR metabolomic analyses.

Characteristic	Category	BiB (N = 11,195)
Offspring		
CHD	Yes	127 (1.1)
Sex	Male	5750 (51.4)
	Female	5444 (48.6)
Maternal		
Age, years		27.3 (5.6)
Parity	Nulliparous	4238 (40.1)
	Multiparous	6325 (59.9)
BMI, kg/m^2		26.1 (5.7)
Ethnicity	White European	4325 (40.9)
	South Asian	5329 (50.5)
	Other	911 (8.6)
Neighbourhood deprivation (IMD)	Quintile 1 (most deprived)	6490 (66.0)
	Quintile 2	1778 (18.1)
	Quintile 3	1093 (11.1)
	Quintile 4	299 (3.0)
	Quintile 5 (least deprived)	170 (1.7)
Smoking	Yes	1672 (17.0)
Gestational age at blood sampling, weeks		26.3 (2.0)

Data are means ± SD or n (%) unless stated. Not all data are complete. Abbreviations: BiB, Born in Bradford; CHD, congenital heart disease; BMI, body mass index; kg, kilogram; m, meter; IMD, Index of Multiple Deprivation (taken from 2010 national quintiles); gest, gestational.

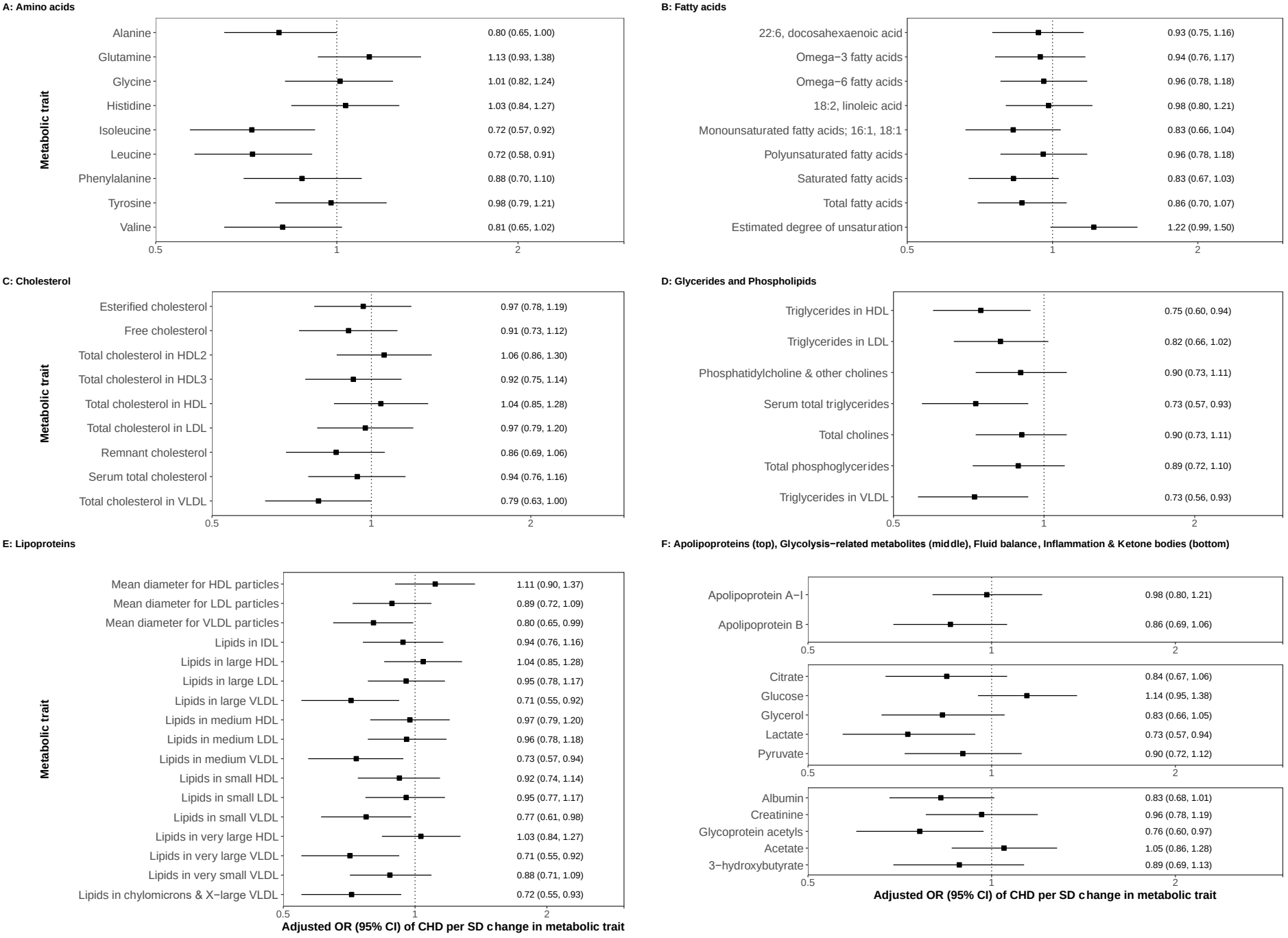
A: Amino acids
Metabolic trait
Alanine
Glutamine
Glycine
Histidine
Isoleucine
Leucine
Phenylalanine
Tyrosine
Valine
0.80 (0.65, 1.00)
1.13 (0.93, 1.38)
1.01 (0.82, 1.24)
1.03 (0.84, 1.27)
0.72 (0.57, 0.92)
0.72 (0.58, 0.91)
0.88 (0.70, 1.10)
0.98 (0.79, 1.21)
0.81 (0.65, 1.02)
0.5
1
2
B: Fatty acids
22:6, docosahexaenoic acid
Omega−3 fatty acids
Omega−6 fatty acids
18:2, linoleic acid
Monounsaturated fatty acids; 16:1, 18:1
Polyunsaturated fatty acids
Saturated fatty acids
Total fatty acids
Estimated degree of unsaturation
0.93 (0.75, 1.16)
0.94 (0.76, 1.17)
0.96 (0.78, 1.18)
0.98 (0.80, 1.21)
0.83 (0.66, 1.04)
0.96 (0.78, 1.18)
0.83 (0.67, 1.03)
0.86 (0.70, 1.07)
1.22 (0.99, 1.50)
0.5
1
2
C: Cholesterol
Metabolic trait
Esterified cholesterol
Free cholesterol
Total cholesterol in HDL2
Total cholesterol in HDL3
Total cholesterol in HDL
Total cholesterol in LDL
Remnant cholesterol
Serum total cholesterol
Total cholesterol in VLDL
0.97 (0.78, 1.19)
0.91 (0.73, 1.12)
1.06 (0.86, 1.30)
0.92 (0.75, 1.14)
1.04 (0.85, 1.28)
0.97 (0.79, 1.20)
0.86 (0.69, 1.06)
0.94 (0.76, 1.16)
0.79 (0.63, 1.00)
0.5
1
2
D: Glycerides and Phospholipids
Triglycerides in HDL
Triglycerides in LDL
Phosphatidylcholine & other cholines
Serum total triglycerides
Total cholines
Total phosphoglycerides
Triglycerides in VLDL
0.75 (0.60, 0.94)
0.82 (0.66, 1.02)
0.90 (0.73, 1.11)
0.73 (0.57, 0.93)
0.90 (0.73, 1.11)
0.89 (0.72, 1.10)
0.73 (0.56, 0.93)
0.5
1
2
E: Lipoproteins
Metabolic trait
Mean diameter for HDL particles
Mean diameter for LDL particles
Mean diameter for VLDL particles
Lipids in IDL
Lipids in large HDL
Lipids in large LDL
Lipids in large VLDL
Lipids in medium HDL
Lipids in medium LDL
Lipids in medium VLDL
Lipids in small HDL
Lipids in small LDL
Lipids in small VLDL
Lipids in very large HDL
Lipids in very large VLDL
Lipids in very small VLDL
Lipids in chylomicrons & X−large VLDL
1.11 (0.90, 1.37)
0.89 (0.72, 1.09)
0.80 (0.65, 0.99)
0.94 (0.76, 1.16)
1.04 (0.85, 1.28)
0.95 (0.78, 1.17)
0.71 (0.55, 0.92)
0.97 (0.79, 1.20)
0.96 (0.78, 1.18)
0.73 (0.57, 0.94)
0.92 (0.74, 1.14)
0.95 (0.77, 1.17)
0.77 (0.61, 0.98)
1.03 (0.84, 1.27)
0.71 (0.55, 0.92)
0.88 (0.71, 1.09)
0.72 (0.55, 0.93)
0.5
1
2
Adjusted OR (95% CI) of CHD per SD change in metabolic trait
F: Apolipoproteins (top), Glycolysis−related metabolites (middle), Fluid balance, Inflammation & Ketone bodies (bottom)
Apolipoprotein A−I
Apolipoprotein B
0.98 (0.80, 1.21)
0.86 (0.69, 1.06)
0.5
1
2
Citrate
Glucose
Glycerol
Lactate
Pyruvate
0.84 (0.67, 1.06)
1.14 (0.95, 1.38)
0.83 (0.66, 1.05)
0.73 (0.57, 0.94)
0.90 (0.72, 1.12)
0.5
1
2
Albumin
Creatinine
Glycoprotein acetyls
Acetate
3−hydroxybutyrate
0.83 (0.68, 1.01)
0.96 (0.78, 1.19)
0.76 (0.60, 0.97)
1.05 (0.86, 1.28)
0.89 (0.69, 1.13)
0.5
1
2
Adjusted OR (95% CI) of CHD per SD change in metabolic trait

Figure 6.2. Confounder adjusted associations of maternal pregnancy metabolomic traits with offspring congenital heart disease in the Born in Bradford cohort (N = 8,551 and N CHD cases = 96).

The associations show confounder adjusted odds ratios of CHD per standard deviation change metabolomic trait levels for 63 (out of 148) key traits separated by the trait class. Metabolomic traits were measured at ~26-28 weeks' gestation. Associations were adjusted for maternal age, ethnicity, parity, Index of Multiple Deprivation, body mass index, and smoking. Unadjusted associations are shown in appendices (Figure S6.4). Abbreviations: OR, odds ratio; CHD, congenital heart disease; SD, standard deviation; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

6.4.2. Validating findings with Mendelian randomisation

The distributions of offspring and maternal characteristics for MR analyses in BiB, ALSPAC and MoBa are displayed in **Table 6.2**. It was possible to explore MR replication for 145 of the 148 metabolomic traits included in multivariable analyses. 138/145 (95%) of the GRS associated with the corresponding metabolomic trait during pregnancy in the BiB cohort ($P < 0.05$) with R^2 values ranging from 0.1% to 10.4% and F-statistics ranging from 8 to 825 (Table S6.1). The seven traits that were not associated at $P < 0.05$ were acetate and six lipoprotein traits related to chylomicrons. The phenotypic and genetic correlation structure of the traits are shown in Figure S6.6 (Appendices) which illustrate that many of the traits are correlated. This is also evident in scatter plots of GRSs with all metabolomic traits with the large majority of GRSs being non-specific (i.e., were associated with many different traits, which was unsurprising given what is known about the correlation structures and how the traits relate to each other). The GRSs that were specific for the trait they were instrumenting (i.e., had the strongest association with it and little evidence of associations with other traits) were: alanine, creatinine, glucose, glutamine, glycine, pyruvate, and tyrosine (Figure S6.7, Appendices).

MR analyses replicated and showed consistent negative associations (i.e., potential protective effect of higher levels) of amino acids alanine, isoleucine, leucine and valine (**Figure 6.3)**. The GRS for alanine was strongly associated with alanine levels in the BiB cohort, whereas the GRS for isoleucine, leucine and valine were less specific (associated with different metabolomic traits than the one I was trying to instrument) (Figure S6.7, Appendices). Overall, these results provide evidence of protective effect for higher maternal amino acid on offspring CHD. MR results for estimated degree of saturation replicated the multivariable regression result showing positive associations with CHD (**Figure 6.3),** coupled with a robust association between the GRS and metabolomic trait in BiB (Figure S6.7, Appendices). There was also a consistent direction of association in MR analyses for potential protective effects for albumin and citrate. Several VLDL traits that had negative (protective) associations also had consistent directions of effect in MR analyses. Results for glucose were not replicated in MR analyses. Forest plots comparing multivariable regression and MR results for all metabolomic traits (N = 145) are shown in Figure S6.8 (Appendices). There was no statistical evidence of heterogeneity between cohorts for any metabolomic traits in pooled MR analyses ($P_{heterogeneity}$ for all > 0.05). MR results were broadly consistent when excluding BiB from analyses (Figure S6.9, Appendices) and when adjusting for offspring genotype (Figure S6.10, Appendices).

Table 6.2. Participant characteristics for the 3 studies included in Mendelian randomisation analyses.

Characteristic	Category	BiB (N = 7,433)	ALSPAC (N = 7,360)	MoBa (N = 23,869)
Offspring				
CHD	Yes	81 (1.1)	61 (0.8)	177 (0.7)
Sex	Male	3,818 (51.4)	3,703 (50.3)	12,139 (50.9)
	Female	3,615 (48.6)	3,657 (49.7)	11,704 (49.0)
Maternal				
Age, years		27.4 (5.6)	29.2 (4.6)	30.1 (4.5)
Parity	Nulliparous	2,963 (40.1)	3,257 (46.6)	11,288 (47.3)
BMI, kg/m^2		26.2 (5.7)	22.5 (4.2)	24.1 (4.3)
Ethnicity	White European	3,084 (42.6)	7,360 (100.0) [a]	NA [b]
	South Asian	3,503 (48.4)	-	-
	Other	656 (9.1)	-	-
Any smoking during pregnancy	Yes	1,175 (18.1)	1,679 (26.1)	1,814 (8.6)
Any alcohol during pregnancy	Yes	1,040 (49.3)	4,866 (79.9)	6,209 (31.5)

Data are means ± SD or n (%) unless stated. % are based on data available (data were not complete).

[a] All non-white European women with ethnicity data were not included in the analysis.

[b] MoBa primarily of white European origin.

Abbreviations: BiB, Born in Bradford; ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort Study; CHD, congenital heart disease; BMI, body mass index; kg, kilograms; m, meters.

Exploring directional consistency between phenotype (conventional multivariable regression) and genotype (Mendelian randomization) associations with metabolic traits

A: Main multivariable regression results

B: Mendelian randomization results

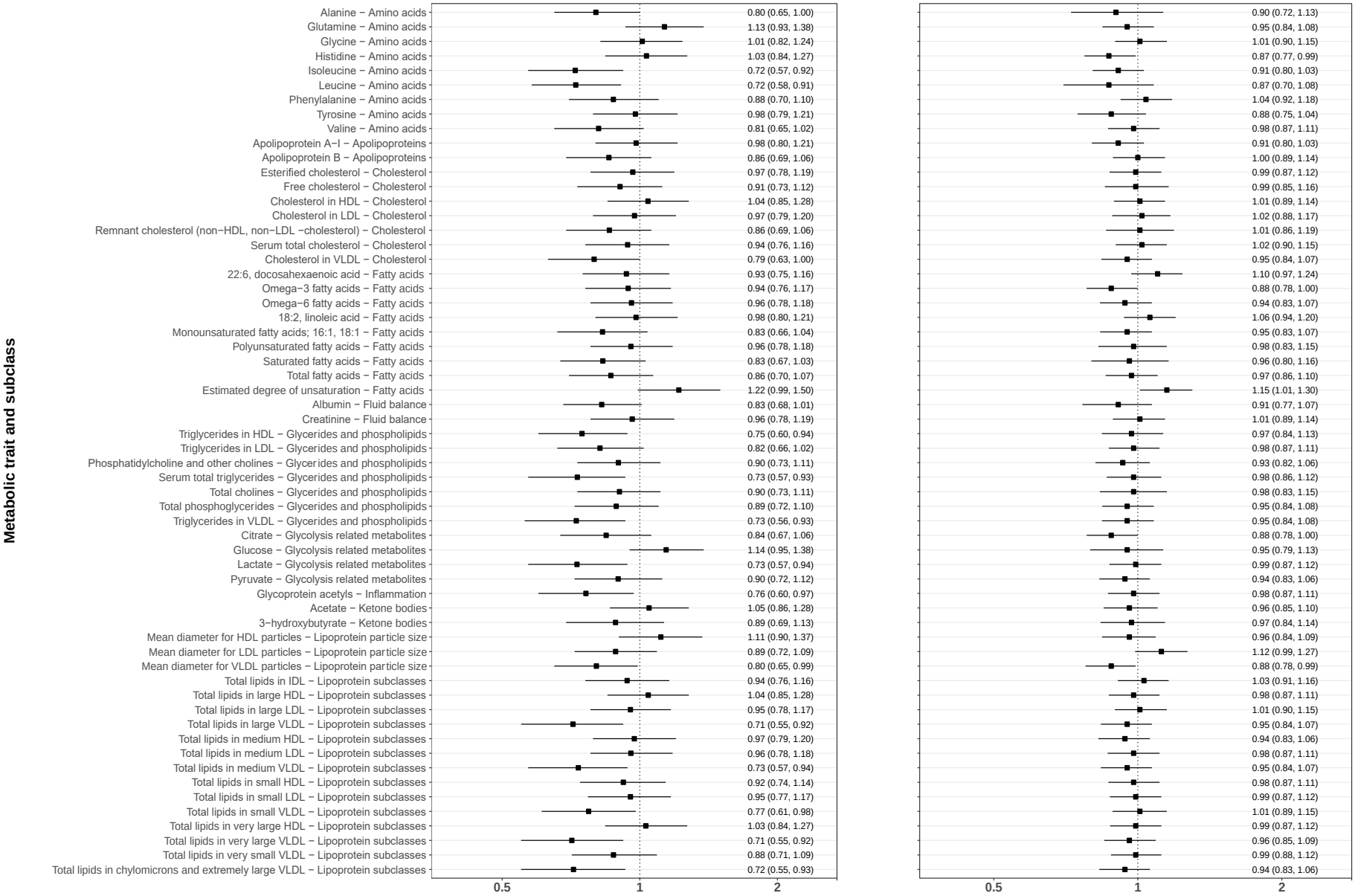

Figure 6.3. Showing results comparing the main confounder adjusted associations of maternal metabolomic traits with offspring CHDs (Panel A: N = 8,551 & N CHD cases = 96) to the Mendelian randomisation analyses of maternal genetic risk scores and offspring CHDs (Panel B: N = 38,662 & N CHD cases = 319).
N.B. results from each analysis are presented on different scales; I am not attempting to quantify estimates in the MR analyses, the aim is to compare the direction of effect. The confounder adjusted associations are as above in Figure 2. The MR analyses are adjusted for the top 10 genetic principal components and genetic batches in MoBa. The results were pooled using random effects meta-analyses. Abbreviations: BiB, Born in Bradford; CHD, congenital heart disease; GRS, genetic risk score; MR, Mendelian randomisation; OR, odds ratio; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

6.5. Discussion

Maternal metabolism dynamically responds to different exposures and disease states. During pregnancy, there are widespread metabolic changes which are required to meet the demands of the developing fetus. Here, I have performed a study to explore the association of pregnancy metabolomic traits with odds of CHD within a causal framework. Using multivariable regression, I found that high levels of serum amino acids, VLDL traits, albumin, and citrate during pregnancy could decrease odds of CHD, with results replicating in MR analyses. There was also good evidence from multivariable regression and MR analyses of a positive association for maternal estimated degree of unsaturation and offspring CHD. I replicated findings from previous research showing that higher glucose was positively associated with offspring CHD and showed that this was only seen in White European women and not South Asian. Glucose results were not replicated in MR analyses.

The results presented in this work complement those presented in Chapter 5. Here, there were less traits (N = 148 versus 923 in Chapter 5) but more participants with metabolomics data (N = 11,195 versus 2,605). Furthermore, I used summary data from a GWAS that was performed in greater numbers for MR replication analyses (N = ~115,000 versus ~15,000 in Chapter 5). In this Chapter, I found similar negative associations for several amino acids providing further evidence that low levels of amino acids during pregnancy could contribute to CHDs. I found consistent directions of effect for alanine, isoleucine, leucine and valine in MR analyses. However, the GRS s for isoleucine, leucine and valine were not specific to the intended amino acids, whereas the GRS of alanine was strongly associated with alanine levels in BiB - satisfying the MR relevance assumption. The GRS was also specific for alanine suggesting less risk of potential pleiotropic effects. Overall, the results presented here, combined with those presented in Chapter 6, and previous research finding that amino acid concentrations were lower in offspring with CHDs [219] provide evidence of a potential causal effect of amino acid concentrations and offspring CHD.

There was evidence from multivariable regression and MR analyses of a positive association of estimated degree of unsaturation (a measure of the number of double bonds found in fatty acids). There were also some potential negative associations for other fatty acid traits, although none provided robust evidence with consistent directions of effect in multivariable and MR analyses, with confidence intervals crossing the null. There was some evidence of differences of associations for fatty acids and CHD between South Asian and White European women, although it was not possible to explore this further as stratifying reduced power to detect effects. However this is complemented by previous work of mine which has

reported ethnic differences in fatty acid concentrations during pregnancy (e.g., higher levels of mono-unsaturated fatty acids in White European compared to South Asian women) [197].

The results presented here do not replicate previous findings that have found positive associations of conventional (clinical chemistry) maternal blood lipids and offspring CHD [86,87]. Smedt et al [86] found positive associations for ApoB, LDL cholesterol and total cholesterol, whereas the results presented in the current study were null for related cholesterol traits and in the opposite direction (negatively associated) for ApoB, but with confidence intervals including the null. One key difference between the study performed by Smedt et al and the present study is that they measured blood lipids 16 months after pregnancy in mothers that gave birth to a child with CHD [86]. Given that it is known that the metabolome and lipidome dynamically respond to pregnancy, and then returns to a pre-pregnancy state post-partum [78], it is difficult to directly compare their results to those presented here. In contrast, Cao et al [87] assessed lipid levels using blood samples taking during the first trimester of pregnancy in a Chinese population. They also found positive associations for maternal ApoB as well as triglycerides. The study did not have the depth of confounder data that was used in the present study. One other study reported associations of maternal triglycerides and offspring CHD and reported a non-linear association (increased CHD risk for low and high triglycerides) [229]. I found negative associations for several triglyceride traits, partially replicating these previous findings, but was not able to explore non-linearity. MR analyses of these traits did not replicate and were less clear due to the imprecision in estimates.

There is a large body of evidence suggesting that poor maternal glycaemic control and diabetes are strong risk factors for offspring CHD [207,230–232]. In this study, I found positive associations of maternal glucose during pregnancy and offspring CHD, corroborating these previous findings. When stratifying by ethnicity, there was evidence that this may be specific to White European women. MR analyses did not replicate findings for glucose. Further well-powered MR studies are warranted to explore this further. There was some evidence in multivariable regression and MR analyses of negative associations for albumin and citrate. Lower levels of albumin (hypoalbuminemia) are a significant predictor of various adverse pregnancy outcomes such as hypertensive disorders of pregnancy (HDP) as proteinuria results in urinary albumin loss [233]. Given that maternal HDP have previously been associated with offspring CHD [49,234], it is plausible that the negative association for albumin seen here could be related. For citrate, a recent review has summarised previous findings and potential uses of measuring serum citrate concentrations for clinical purposes [235]. Citrate is involved in many metabolic processes including regulating energy production and fatty acid synthesis. The findings presented in the current study suggest deficiencies in maternal citrate concentrations could be involved with the pathophysiology of CHDs with

MR providing evidence of replication. External replication of these results is warranted which could pave way to future mechanistic studies and therapeutic targets.

This study has several strengths. Firstly, >11,000 women (> 100 CHD cases) were included and not pre-selected based on having CHD. BiB has a rich resource of data which allowed for control of potentially relevant confounders. Multivariable regression associations from a single cohort without replication does not provide the most robust findings, so I attempted to seek replication in other ways. This was done by firstly attempting to use NMR data in the MoBa cohort, but due to delays caused by the COVID-19 pandemic, these data were not available. The next step was to use the novel NMR GWAS data from UKBB to generate genetic instruments of metabolomic traits for MR analyses. Exploring consistency of effects in MR analyses using data from 3 cohorts provides greater confidence in the findings where there was directional consistency between the two methods. Lastly, to my knowledge, no previous study that has used pregnancy metabolomics in relation to CHD has explored potential ethnic-specific effects. The unique structure of the BiB cohort provided an opportunity to do this. However, the ethnic-stratified results were underpowered. Despite this, it demonstrates the importance of acknowledging that results performed in homogenous ethnic populations may not be generalisable to other populations, given what we know about ethnic differences during pregnancy [197] and the results presented here. As I was able to perform MR analyses for nearly all metabolomic traits (145/148) included in multivariable regression analyses, I did not use P-value cut-offs to determine whether a trait was associated with CHD. Rather, I focused on magnitudes and directions of effects and whether results were directionally consistent in MR analyses.

A key limitation of this study is not having the ability to replicate the multivariable regression results in an independent cohort. Secondly, the NMR data was measured in maternal serum taken at a single timepoint during pregnancy around 26-28 weeks' gestation. Fetal cardiac development occurs during the first trimester [143]. The assumption in the work presented here is that metabolite levels around 26-28 weeks' gestation are good proxies for levels in early pregnancy - when the offspring heart is forming. Whilst this is an assumption which can't be directly tested here, previous work has shown that between person differences throughout pregnancy remain largely consistent (i.e., those with a high level of a metabolite in early pregnancy tend to have a similarly high level of a metabolite in later pregnancy) [81].

There are several points to consider in relation to the MR analyses presented in this study. MR analyses are sensitive to their assumptions that the GRS is statistically strongly associated with the metabolite in pregnancy. I assessed this by examining the association of each GRS with the metabolomic trait that I was trying to proxy. Most of the GRSs were robustly associated with the trait, but also other

traits, which was not unsurprising given the correlation structure. Therefore, in most instances it is difficult to draw robust conclusions for single traits, but more appropriate to conclude effects for the group of correlated traits as I have done with amino acids above. Methods that are available for exploring potential bias due to horizontal pleiotropy in two sample MR were not possible here. Similar or stronger variation of a GRS for other metabolomic traits could be related to vertical (i.e., influencing CHD via the trait of interest and other traits through mediating pathways) or horizontal (i.e., influencing CHD independently of the trait of interest) pleiotropy. I was not able to distinguish between these two.

This study provides insight into how maternal metabolism during pregnancy may influence offspring CHD. I found evidence that amino acid metabolism during pregnancy could be important to the aetiology of CHD. There was also evidence of potential effects for some fatty acid and VLDL traits, albumin, and citrate. My analysis pipeline which included exploring replication of findings using MR should prove to be more valuable as metabolomic and GWAS datasets continue to grow. Ultimately, metabolomics studies in relation to CHD are still novel and further larger studies are needed. Metabolomic studies provide scope to identify important biological pathways involved in CHD which could lead to therapeutic targets. Recommendations for future studies include having large numbers with data on multiple metabolomics platforms during pregnancy and CHD subtypes where possible, having the ability to perform external replication analyses, and to focus on using methods that can improve causal inference.

Chapter 7. Discussion

The aim of this thesis was to explore pregnancy risk factors for congenital heart disease (CHD). To do this, I explored the effects of maternal body mass index (BMI), smoking and alcohol using parental negative control analyses and Mendelian randomisation (MR). I then went on to explore potential effects of maternal metabolites on CHDs to further interrogate how the pregnancy environment might contribute to CHD pathophysiology. In this discussion chapter, I provide an overview of the primary findings for analyses included in each chapter along with strengths and limitations. I then consider the public health and clinical implications of my findings and provide recommendations for future research.

7.1. Summary of findings

Chapter 2 described the CHD data in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort which was used for analyses in subsequent chapters. I demonstrated that combining multiple sources of data including data from antenatal, delivery, primary and secondary health records, and parent-reported information can improve case ascertainment. Importantly, using linked primary care data, I showed that not all CHD cases are identified during early life which was also seen in the Born in Bradford (BiB) cohort [70]. Not only were the data described in this chapter integral to the work presented in this thesis, but I hope they will also prove to be useful to the wider research community. Limitations of this work include not all participants being able to be linked to electronic health records and not having access to hospital episode statistics (HES) data. However, these limitations were partly mitigated by the approach of combining multiple sources of data to identify cases, which is the key strength of this work.

In **Chapter 3**, I used parental negative exposure control analyses to explore the effects of maternal pre-pregnancy BMI, and pregnancy smoking and alcohol on offspring CHD. To my knowledge, this study was the first to use this method to explore the effects of potential residual confounding of maternal exposures on CHDs. With the formation of the Horizon 2020 LifeCycle cohort collaboration, I was able to include seven different European birth cohorts which maximised numbers and provided opportunities to explore heterogeneity between populations. From this work, I found no evidence of a linear effect of maternal BMI but did find positive associations between maternal overweight and obesity categories with offspring CHD, which was consistent with previous findings [43,65,66]. However, paternal negative control analyses suggested that these positive associations could be due to confounding factors. I found evidence of an intrauterine effect for maternal smoking on offspring CHD, which appeared to be driven by non-

severe CHD cases. In this work, results for alcohol were inconclusive due to a lack of paternal alcohol data around the time of the mother's pregnancy. The key strengths of this work are the large sample size, the use of a negative paternal exposures control analyses, and the pooling of results from several cohort studies that are less prone to selection bias that can occur in case-control studies. Limitations include The Norwegian Mother, Father and Child Cohort (MoBa, the largest cohort included in the study) only having CHDs diagnosed around birth and not throughout childhood which meant that some CHD cases diagnosed later in life could have been misclassified as non-CHD cases. Second, the negative control analyses assume that factors that would confound the maternal exposure-offspring CHD associations would have a similar magnitude and direction of confounding for the equivalent paternal associations. Although previous work has showed proof-of-concept [90,131], I was not able to test this assumption. There is also the possibility that maternal smoking and alcohol were underreported, which could have biased results towards the null.

Chapter 4 examined the same exposures as in **Chapter 3** using MR. I found no evidence of a causal effect of higher maternal BMI on offspring CHD which corroborated findings from negative control analyses. Using MR, I did not replicate the positive association for smoking found in **Chapter 3**. However, I did find some evidence of a potential causal effect of maternal alcohol on offspring CHD. Very few studies have used MR to explore maternal risk factors for CHD. Early work used meta-analyses to combine data and improve statistical power to explore effects of folate deficiency and the methylene tetrahydrofolate reductase C677T genetic variant [236], but to my knowledge the work presented in this chapter is the first to use MR to explore effects of BMI, smoking and alcohol on CHD. Strengths of this work are the inclusion of 3 cohorts to maximise numbers and explore heterogeneity and having the ability to adjust for offspring genotype. The limitations of this work are the relatively small number of CHD cases included as CHD is a rare outcome, meaning that estimates were often imprecise and there was potential for weak instrument bias in alcohol analyses.

Chapter 5 examined the relationship of maternal gestational mass spectrometry (MS)-derived metabolites with offspring CHD using multivariable regression and MR analyses. I found evidence that amino acid metabolism during pregnancy, several lipids (more specifically androgenic steroids), and levels of succinylcarnitine could be important contributing factors to CHD. There were two key strengths of this study. First, the unique data in BiB could support novel analyses of associations of a wide range of maternal metabolic paths with offspring CHD risk. Second, the use of complimentary data sources and statistical techniques to build layers of evidence improved the robustness of the findings. Limitations include only having 46 CHD cases in the initial multivariable regression metabolomic analyses, having

metabolomics at a single timepoint during mid-pregnancy and not being able to reliably assess vertical and horizontal pleiotropy in MR analyses.

In **Chapter 6,** I undertook a similar approach to **Chapter 5** by employing multivariable regression and MR analyses. The work in this chapter examined the relationship of maternal gestational nuclear magnetic resonance (NMR)-derived metabolic traits with offspring CHD. There was evidence that amino acid metabolism during pregnancy could be an important contributing factor which is consistent to results presented in **Chapter 5**. There was also evidence of potential effects for some fatty acid and very low-density lipoprotein (VLDL) traits, albumin, and citrate. This work complements the work in **Chapter 5** by having metabolomic data in larger numbers (~11,000 women versus ~3,000 women) and by constructing genetic instruments for more traits using data from a larger and more powered genome-wide association study (GWAS). Strengths of this work include being able to assess associations of ~150 metabolic traits in both multivariable regression and MR analyses to explore replication and being able to explore potential ethnicity-specific associations. I have plans in place to perform external replication analyses for this work in MoBa soon which will further strengthen the study. Limitations of this work are similar to those presented above for **Chapter 5**. These include only being able to assess associations of metabolic traits in a single cohort (BiB), having metabolomics at a single timepoint and not being able to reliable assess vertical and horizontal pleiotropy in MR analyses.

Bringing this together, in this thesis I have explored how the pregnancy environment may contribute to offspring CHD. Firstly, I examined associations of conventional pregnancy exposures (BMI, smoking, alcohol) using multiple datasets and study designs. I found evidence of a possible intrauterine effect for maternal pregnancy smoking on offspring CHD and found that higher maternal alcohol intake may be causally related to offspring CHD. It should be noted that the results for smoking analyses were not concordant between the two methods used to approach the research question (negative control and MR analyses). As discussed, the MR work is relatively underpowered and does not definitively rule out an effect (confidence intervals spanning the null). On the other hand, the negative control study was well powered, included more cohorts and included sub-categories of CHD. Therefore, in this thesis, more weight is given to the negative control study when drawing conclusions. As an extension to the work exploring conventional exposures and recognising for the potential to elucidate modifiable upstream risk factors, I then explored associations of the pregnancy metabolome with offspring CHD. To my knowledge, the metabolomic studies presented within this thesis are the first to attempt to explore the relationship between pregnancy metabolites and offspring CHD within a causal framework. The results from this work have uncovered some promising avenues for future research.

7.2. Strengths and limitations

Here, I briefly discuss some of the overall strengths and limitations of the work presented in this thesis. A key strength of the work is that I have included multiple studies to explore replication and triangulated findings using different approaches. This is important, as if results are consistent between different populations and study designs, this provides greater confidence in findings. Next, because I used data from birth cohorts which follow participants throughout the life course, in some instances I was able to obtain CHD cases from stillbirths, miscarriages and cases diagnosed later in life which often has not been possible in previous studies. I have used pre-defined analysis plans for all of my studies and have openly documented these online via the Open Science Framework. Thinking back to the Introduction where several systematic reviews found evidence of publication bias, this is one way to help mitigate this and to promote honest, robust science. Throughout my PhD, I have convinced colleagues to adopt this practice (pre-specifying analyses and registering them online) and I hope this approach continues to grow and becomes the norm.

An overall limitation of this thesis is not being able to explore results for CHD subtypes. In negative control analyses, I was able to explore findings for CHD severity and showed that the effect I saw for smoking could be driven by non-severe cases. However, I was not able to explore further what specific subtypes of non-severe CHDs might have been driving this association due to the numbers required for meaningful analyses. For most analyses, I only explored effects of any CHD, which, as stated in the Introduction is important to know for policy makers and prospective parents, but I acknowledge that future work should explore subtypes where possible provided the work is powered to do so. For example, previous work has shown that maternal overweight and obesity status (aortic arch defects and ASDs) [65], smoking (ASDs)[55], and alcohol consumption (ToF) [41] have been associated with CHD subtypes. Although, these previous studies did not use causal methods (as was done in this thesis), a natural progression of these studies and the work presented throughout this thesis would be to perform causal analyses (negative control and MR) with CHD subtypes. However, finding datasets large enough to do this may prove to be difficult. In relation to this, it would also be useful to categorise CHD into isolated (CHD occurring without other anomalies) and CHD occurring with other CAs as well as taking into account previous history of CHD in the mother (familial recurrence), however, the datasets that were used did not provide an opportunity to do this. Another limitation is that the metabolomics work was heavily reliant on BiB data which has a unique ethnic structure. As noted in the COVID-19 statement, I had planned to perform external replication analyses in MoBa for **Chapter 6**, but COVID-19 disruption prevented this. The

MR analyses did include data from other cohorts and the results in which MR shows replication add another layer of evidence to provide robustness to the findings. Levels of missing data differed depending on the analyses and cohort used. Where possible, I assessed whether missing data was influencing my results by performing complete case analyses and didn't find any evidence to suggest that missing data were influencing my findings. It is possible that findings could have been influenced by selection bias via selection into a cohort study or by attrition over time [181,182]. Given that I mostly used pregnancy data (i.e., around the time or soon after recruitment), the latter is unlikely to be an issue.

7.3. Public health and clinical implications of findings

Findings from negative control and MR analyses suggest that lowering maternal pre-/early-pregnancy BMI is unlikely to be an effective prevention target for CHD. However, an effect of maternal underweight status and offspring CHD cannot be ruled out and should be explored in future studies. Given that maternal underweight status during pregnancy is associated with multiple adverse outcomes, such as low birthweight and preterm birth [237], it is plausible that maternal underweight status could impair fetal cardiac development. This could have important implications for public health policy such as additional screening for underweight pregnant women or underweight women of child-bearing age. As noted above, I was unable to explore effects for CHD subtypes. A large Swedish registry study found associations for maternal overweight and obesity status for several CHD subtypes [65]. They also reported associations for maternal underweight status and CHD subtypes, but results were imprecise due to the few numbers of underweight women. Due to the observational study design, the causal nature of associations with CHD subtypes is unclear. Therefore, it remains uncertain as to whether any maternal BMI-related prevention strategies could reduce CHD incidence.

There was evidence of a causal intrauterine effect of maternal pregnancy smoking on offspring CHD using parental negative exposure control analyses. These findings suggest that reducing smoking during pregnancy could help prevent some CHD cases. Given that all clinical guidelines advocate not starting smoking, and if women do smoke, to quit before becoming pregnant, more research is needed to understand how best to reduce smoking during pregnancy. Smoking prevalence worldwide continues to fall, but the absolute numbers of smoking have increased, particularly in developing countries [238]. Robust evidence-based policies are required to reduce these numbers, which in turn could help decrease global CHD incidence and disease burden. Furthermore, understanding the specific mechanisms that link maternal smoking to increased offspring CHD risk could identify targets for interventions for its

prevention. Findings from MR analyses did not support those from negative control analyses for smoking. However, this was the first MR study of its kind and was performed in small numbers and so cannot definitively rule out the absence of a causal effect. Of note, in presence/absence adjusted analyses in **Chapter 5**, the presence of any level of cotinine was positively associated with CHDs (OR: 1.95 (0.95, 3.85)) although confidence intervals included the null which is why it is not presented in the results within the main text. This demonstrates how metabolomics studies have the potential to compliment studies that measure a pregnancy exposure.

In this thesis, there was some evidence of a positive association of alcohol consumption and offspring CHD in observational and MR analyses. It is possible that policies that aim to reduce alcohol consumption during pregnancy could decrease CHD disease burden. Larger sample sizes and stronger instruments (for MR) in future analyses may help to clarify this further.

Metabolomics is still a relatively novel technology in the field of pregnancy and offspring outcomes. It would be overambitious to expect immediate clinical translation from metabolomics studies in relation to CHD. Potential clinical and public health impacts from metabolomics could arise from two broad areas: (i) identifying novel causal pathways/biomarkers for CHD and (ii) improving prediction for CHD. In this thesis, I have focused on identifying causal maternal gestational metabolites/traits for offspring CHD. The idea behind this is to enhance our understanding of how the pregnancy environment may relate to offspring CHD in a real-world population health setting. The work in this thesis has shown that levels of amino acids, androgenic steroid metabolites, succinylcarnitine, fatty acids, VLDL traits, albumin and citrate during pregnancy may contribute to CHD. All of these had at least some evidence of replication in MR analyses. However, I acknowledge that external replication is necessary for drawing robust conclusions. Although, these results do contribute to our overall understanding of CHDs and will hopefully encourage others to invest in similar types of data. If we can identify robust causal associations for certain metabolic pathways, this could help identify preventive therapeutic interventions.

Overall, this thesis has provided evidence that maternal exposures can contribute to offspring CHD status. Policy makers will want to know which exposures would be best to target as the money for preventive interventions is limited. The work in this thesis contributes to the body of evidence for key exposures (BMI, smoking and alcohol) and serves as a strong starting point for the use of metabolomics during pregnancy to disentangle potentially important biological pathways. Clinicians will also be interested in these results. Although the first antenatal clinic (~12 weeks' gestation) is already too late for prevention given that CHDs manifest earlier in pregnancy, clinicians will still be interested in these results as many of the women they see will go onto have subsequent pregnancies. Therefore, clinicians will be

able to communicate the risks of certain exposures of fetal cardiac development which in turn could elicit behaviour change for future pregnancies and thus help reduce CHD burden.

7.4. Future work

In this thesis, I have attempted to use methods that improve causal inference in the context of identifying maternal gestational risk factors for offspring CHD. A natural extension to this work would be to use similar/additional methods to interrogate maternal exposures for offspring CHD subtypes. This would require considerable sample sizes for meaningful analyses. Electronic medical records data are now becoming more accessible for scientific research but may not have the depth of data that birth cohorts possess. For example, paternal exposures (for negative control analyses) around the time of pregnancy may not be routinely collected. Other methods that could be used in aetiological CHD research as part of a triangulation framework to improve causal inference include: (i) natural experiments; e.g., explore policy changes that affect smoking and alcohol in relation to CHDs (done previously for smoking and pregnancy complications using Scottish national administrative data) [239]. (ii) Within sibling comparisons; assesses associations within sibships (comparing outcomes between siblings who are discordant for the exposure). This would require maternal and offspring CHD data across two pregnancies meaning statistical power may be difficult to obtain. (iii) Cross-context comparisons; compares results between two or more populations in different contexts that result in confounding structures being different. E.g., a recent study confirmed the strong association between maternal smoking and birth weight by using 2 birth cohorts born 40 years apart [240]. However, finding relevant datasets for CHD could be difficult. These three methods along with multivariable regression, negative control analyses and MR analyses should be used where possible and triangulated to improve causal inference [96].

To date, MR has been underutilised in aetiological CHD research. A key reason for this is that not many studies have genome-wide data in mothers and offspring as well as offspring CHD data, which would be required for one-sample MR as demonstrated throughout this thesis. However, there are now methods that can explore whether maternal exposures are causally related to offspring outcomes using two-sample MR [241,242]. This requires largescale GWAS data on CHD. To the best of my knowledge, there are currently no publicly available GWAS summary statistics for CHD. To date, the largest GWAS for CHD in a European population included ~4,000 cases and found one genetic risk factor that was significantly associated with all CHD phenotypes, as well as uncovering associations for specific subtypes [243]. As these

GWAS continue to grow, significant data sharing and collaboration will be required, which could then pave way for large-scale two-sample MR studies to explore maternal risk factors for CHDs.

As mentioned previously, there are still relatively few studies that have used metabolomics measurements during pregnancy in relation to offspring CHD. Firstly, I propose for confirmatory studies to seek replications of previous findings to target promising future avenues of investigation. Replication studies and reporting of null results is crucial for improving our scientific understanding of CHD aetiology. Next, if the cost of metabolomics decreases, larger studies with repeat measurements will be required to uncover potential effects and where possible methods that improve causal inference (described above) should be used. Although not used within this thesis, other omics' technologies should also be considered and combined to provide a deeper understanding of CHD aetiology [244].

Finally, there is a pressing need for CHD-related research in developing nations. Throughout this thesis, I have largely used data from European birth cohorts, meaning that results are not necessarily generalisable to other populations. There is also a significant amount of CHD-related research in Chinese populations which was evident in the umbrella review presented in **Chapter 1**. However, research in places such as Africa is extremely limited. For example, highest rates of CHD have been observed in western, central, and eastern sub-Saharan Africa, central and southeast Asia, with lowest rates in Europe and the Americas [9]. More research is needed to understand why this is happening, what the causes are and how to prevent it.

7.5. Concluding remarks

The findings presented in this thesis have contributed to further our understanding of modifiable factors that influence CHD risk. They have shown that using different and complimentary statistical methods and data sources can augment epidemiological understanding of exposure-disease relationships. This thesis provides evidence using a causal framework that maternal lifestyle factors during pregnancy can increase CHD risk as well as providing novel insights into maternal metabolism and its relevance to CHD. Key next steps will be to continue integrating findings within a triangulation framework to explore effects of CHD and CHD subtypes as well as expanding research into developing nations where CHDs are a considerable burden.

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Appendices

Please note that for some chapters, the methodological details are identical. For example, the definitions of CHD and the genetic data across different cohorts have been used in multiple chapters throughout this thesis. To avoid duplication, I have inserted this supplementary information in Appendices where it is first used. I first use the genetic data in Chapter 4 and so have included the supplementary information in relation to that under the Chapter 4 section. I then use these data again in Chapters 5 and 6 and use hyperlinks to link back to the supplementary information in Chapter 4.

Chapter 2

Table S2.1. GP Read codes used to extract primary care data.

GP read code	Name	Description (Domain)
P....	Congenital anomalies	All congenital anomalies code beginning with "P"
Q....	Perinatal conditions	All perinatal conditions code beginning with "Q"
A90..	Congenital syphilis	Infectious/parasitic diseases
J0002	Congenital anodontia	Digestive system diseases
F4K51	Congenital nystagmus	Nervous system/sense organ dis
C3710	Congenital porphyria	Endo/nutr/metab/immun.diseas
B7J1.	Congenital lymphangioma	Neoplasms
14H..	H/O: congenital anomaly	History/Symptoms
C03..	Congenital hypothyroidism	Endo/nutr/metab/immun.diseas
D4010	Congenital dysphagocytosis	Blood/blood forming organs dis
BBG8.	[M]Congenital fibrosarcoma	Neoplasms
J6900	Congenital coeliac disease	Digestive system diseases
D4170	Congenital methaemoglobinaemia	Blood/blood forming organs dis
D2003	Congenital red cell hypoplasia	Blood/blood forming organs dis
D2000	Congenital hypoplastic anaemia	Blood/blood forming organs dis
F145.	Congenital nonprogressive ataxia	Nervous system/sense organ dis
G8610	Primary (congenital) lymphoedema	Circulatory system diseases
F5914	Congenital sensorineural deafness	Nervous system/sense organ dis
C1520	Congenital adrenogenital syndrome	Endo/nutr/metab/immun.diseas
C3905	Congenital hypogammaglobulinaemia	Endo/nutr/metab/immun.diseas
Eu802	[X]Congenital auditory imperception	Mental disorders
C0A..	Congenital iodine deficiency syndrome	Endo/nutr/metab/immun.diseas
66g..	Congenital heart condition monitoring	Prentive procedures
F383.	Congenital and developmental myasthenia	Nervous system/sense organ dis
ZV136	[V]Personal history of congenital malformations	Unspecified conditions
F591A	Bilateral congenital sensorineural hearing loss	Nervous system/sense organ dis
D303z	Congenital deficiency of other clotting factor NOS	Blood/blood forming organs dis
L185.	Congenital cardiovascular disorders during pregnancy, childbirth and the puerperium	Pregnancy/childbirth/puerperium
L240.	Congenital abnormality of uterus in pregnancy, childbirth and the puerperium	Pregnancy/childbirth/puerperium
79...	Heart procedure	Operations, procedures, sites

7L0G.	Correction of congenital deformity of hip	Operations, procedures, sites
7L0H.	Correction of congenital deformity of leg	Operations, procedures, sites
7L0F.	Correction of congenital deformity of hand	Operations, procedures, sites
7L0E.	Correction of congenital deformity of forearm	Operations, procedures, sites
7L0L.	Correction of minor congenital deformity of foot	Operations, procedures, sites
7L0K.	Other correction of congenital deformity of foot	Operations, procedures, sites
7L0J.	Primary correction of congenital deformity of foot	Operations, procedures, sites
7G03L	Excision of congenital pigmented naevus of head or neck	Operations, procedures, sites
7L0K4	Triple arthrodesis for correction of congenital deformity	Operations, procedures, sites
7L0D.	Correction of congenital deformity of shoulder or upper arm	Operations, procedures, sites
x01O4	Warfarin	Drug
b31..	Frusemide	Drug
x01Qk	Oral digoxin	Drug
b43..	Spironolactone	Drug
x005J	Heparin	Drug
x01Qe	Enalapril	Drug
bi...	Angiotensin-converting enzyme inhibitor	Drug
x000Z	Antiarrhythmic drug	Drug
x01Nx	Anticoagulant	Drug
bu...	Antiplatelet drug	Drug
x01Ch	Selective beta-1 adrenoceptor stimulants	Drug
bkA..	Bosentan	Drug
b9...	Diuretics with potassium	Drug
gh5..	Sildenafil	Drug
be...	Vasodilator antihypertensive drugs	Drug

Table S2.2. ALSPAC questionnaires and questions used for the child-based questionnaire category. All questionnaires can be downloaded from: http://www.bristol.ac.uk/alspac/researchers/access/

File (questionnaire)	Timepoint	Question number	Questionnaire response rate (based on total live births)
D3A		KE	
KA	4 weeks	A4A	88%
KA	4 weeks	F2B.2	88%
KA	4 weeks	A5B	88%
KA	4 weeks	F2A.2	88%
KA	4 weeks	A5F	88%
KA	4 weeks	F2B	88%
KB	6 months	G6D	81.3%
KB	6 months	G6C	81.3%
KB	6 months	G5C	81.3%
KB	6 months	G6E	81.3%
KB	6 months	G5E	81.3%
KB	6 months	A8C	81.3%
KB	6 months	A7C	81.3%
KC	1y 3m	B6C	78%
KC	1y 3m	B5F	78%
KC	1y 3m	B4E	78%
KC	1y 3m	C1C	78%
KC	1y 3m	B5C	78%
KC	1y 3m	B3A	78%
KD	1y 6m	A19K.6	76%
KD	1y 6m	F5	76%
KE	2y 0m	D3A	74%
KE	2y 0m	D3C	74%
KE	2y 0m	D3D	74%
KF	2y 6m	A7D	74%
KF	2y 6m	A6C	74%
KF	2y 6m	A18B	74%
KF	2y 6m	A7C	74%
KG	3y 2m	A6C	71.7%
KG	3y 2m	A5C	71.7%
KG	3y 2m	A5F	71.7%
KG	3y 2m	I11F	71.7%
KJ	3y 6m	A4D	71.3%
KK	4y 6m	B5B	
KK	4y 6m	B10C	
KK	4y 6m	B10F	
KK	4y 6m	B5E	
KK	4y 6m	B9J	
KL	4y 9m	A6H	68%
KL	4y 9m	A5C	68%

KL	4y 9m	E13	68%
KL	4y 9m	A6H	68%
KM	5y 5m	B10F	64%
KM	5y 5m	B10C	64%
KM	5y 5m	B5A	64%
KM	5y 5m	B10G	64%
KM	5y 5m	B4D	64%
KN	5y 9m	A5H	62%
KN	5y 9m	E13	62%
KP	6y 5m	C9D	61%
KP	6y 5m	C12C	61%
KP	6y 5m	C12F	61%
KP	6y 5m	C6B	61%
KR	7y 7m	N2B(V)	58.8%
KS	8y 7m	A4P	56.8%
KS	8y 7m	A6H	56.8%
KS	8y 7m	D8G	56.8%
KS	8y 7m	D4E	56.8%
KS	8y 7m	D5D	56.8%
KV	10y 8m	O2A	53%
KV	10y 8m	A10B	53%
KW	11y 8m	B5D	51%
KW	11y 8m	B8C	51%
KW	11y 8m	B8F	51%
KW	11y 8m	H8F	51%
TA	13y 1m	A4C	49%
TB	13y 10m	A4W	48%

Table S2.3. Search strategy for child-based questionnaires.

Search Term(s)
“congenital”
“heart”
“cardiac”
“hart”
“conjenital”
“defect”
“ventric”
“fallot”
“septal”
“VSD”
“abnormal”
“testes”
“testic”
“testis”
“renal”
“kidney”
“lung”
“respir”
“web”
“malform”
“chrom”
“genet”
“gene”
“anencephaly”
“neural”
“hypoplastic”
“bladder”
“born with”
“hydrocephalus”
“spina”
“bif”
“syndrom”
“ephaly”
“phalus”
“spad”
“feet”
“talipes”
“spine”
“hip”
“deform”
“toe”
“finger”
“ulna”

“radius”
“operat”
“surgery”
“anomal”
“procedure”
“hernia”
“splint”
“tongue”
“cleft”
“palat”
“palet”
“pylo”
“stenosis”
“club”

Table S2.4. Subcategories of congenital anomalies defined using EUROCAT guide: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/Section%203.3-%2027_Oct2016.pdf

Category	ICD-10 Codes	Minor anomalies excluded according to EUROCAT
All congenital anomalies	Q00-Q07, Q10-Q15, Q16-Q18, Q20-Q25, Q260, Q262-Q269*, Q300, Q32- Q34, Q35-Q37, Q38-Q45, Q790, Q792, Q793, Q795, Q60-Q64, Q794, Q50-Q52, Q54-Q56, Q65-Q74, Q750, Q77, Q782-Q788, Q80-Q81, Q820- Q824, Q826-Q829, Q860, Q890, Q893-Q894, Q90-Q92, Q93 , Q96- Q99.	Q0461, Q0782, Q101-Q103, Q105, Q135, Q170-Q175, Q179, Q180- Q182, Q184-Q187, Q1880, Q189, Q320, Q331, Q381, Q382, Q3850, Q400, Q401, Q4021, Q430, Q4320, Q4381, Q4382, Q610, Q627, Q633, Q523, Q525, Q527, Q5520, Q5521, Q653-Q656, Q662-Q669, Q670-Q678, Q680, Q6810, Q6821, Q683- Q685, Q7400, Q936.
CHD	Q20-Q25, Q260, Q262-Q269*	Q2111, Q2111, Q250 if GA <37 weeks, Q2541, Q256 if GA<37 weeks, Q261.
Nervous system	Q00, Q01, Q02, Q03, Q04, Q05, Q06, Q07.	Q0461, Q0782.
Respiratory	Q300, Q32- Q34	Q314, Q315, Q320, Q331, Q3310, Q336, Q315, Q320
Orofacial clefts	Q35-Q37	-
Eye	Q10-Q15	Q101, Q102, Q103, Q105, Q135.
Ear, face, neck	Q16, Q17, Q18	Q189, Q1880, Q170, Q173, Q175, Q174, Q171, Q172, Q181, Q179, Q186, Q184, Q187, Q185, Q182, Q180
Digestive system	Q38-Q45, Q790	Q381, Q382, Q3850, Q400, Q401, Q4021, Q430, Q4320, Q4381, Q4382
ABWD	Q792, Q793, Q795	-
Urinary	Q60-Q64, Q794	Q633, Q610, Q627
Genital	Q50-Q52, Q54-Q56	Q523, Q525, Q527, Q5520, Q5521, Q53
Limb	Q65-Q74	Q653, Q654, Q655, Q656, Q662, Q663, Q664, Q665, Q666, Q667, Q668, Q669, Q662, Q663, Q664, Q665, Q666, Q667, Q668, Q669, Q670, Q671, Q672, Q673, Q674, Q675, Q676, Q677, Q678, Q680, Q6810, Q6821, Q683, Q684, Q685, Q7400.
Other	Q750, Q77, Q782, Q783, Q784, Q785, Q786, Q787, Q788, Q80, Q81, Q820, Q821, Q822, Q823, Q824, Q826, Q827, Q828, Q829, Q860, Q890, Q893, Q894	Q845, Q8280, Q833, Q8252, Q8250, Q825, Q8251, Q899
Chromosomal	Q90-Q92, Q93 , Q96- Q99	Q936

Teratogenic/genetic syndromes, microdeletions and chromosomal abnormalities.	D821, P350-P352, P371, Q619, Q751, Q754, Q771-Q772, Q780, Q796, Q85, Q861-Q869, Q87, Q90-Q92, Q930-Q939, Q95-Q99	-
**Q250 and Q256 not a case if isolated and GA<37weeks Minor anomalies excluded according to the minor anomalies for exclusion EUROCAT guide: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/EUROCAT-Guide-1.4-Section-3.2.pdf		

Table S2.5. ALSPAC cases included in 2 or more sources.

Anomaly subtype[1]	Total N	Neonatal & Delivery (%)	Neonatal & Child-based (%)	Child-based & Delivery (%)	All 3 sources (%)
Any CA	64	8 (12.5)	8 (12.5)	39 (60.9)	9 (14.0)
CHD	15	5 (33.3)	3 (20)	5 (33.3)	1 (6.7)
Nervous system	0	-	-	-	-
Respiratory	0	-	-	-	-
Orofacial clefts	6	2 (33.3)	1 (16.7)	3 (50.0)	0
Eye	0	-	-	-	-
Ear, face, neck	0	-	-	-	-
Digestive system	3	2 (66.6)	1 (33.3)	0	0
ABWD	1	1 (100)	0	0	0
Urinary	5	1 (20.0)	1 (20.0)	3 (60.0)	0
Genital	10	1	0	9	0
Limb	25	3	3	19	0
Other	0	-	-	-	-
Chromosomal	2	1	1	0	0
Probable cause[2]	6	1	2	3	0

Abbreviations: CA, congenital anomaly; CHD, congenital heart disease; ABWD, abdominal wall defects;

[1] ICD codes used to define subtypes can be found in the extended data.

* used when there were less than 5 cases in a given category.

[2] Teratogenic/genetic syndromes, microdeletions and chromosomal abnormalities.

Table S2.6. Remaining ALSPAC cases only included in 1 source. These are not included as a case in the final dataset (unless identified by a separate source such as record linkage). These are deemed 'possible CAs' and will be made available to researchers.

Anomaly subtype[1]	Total N	Neonatal	Delivery	Child-based
Any CA*	608	57	261	324
CHD	119	18	23	78
Nervous system	19	0	11	8
Respiratory	6	0	3	3
Orofacial clefts	18	3	0	15
Eye	11	0	4	7
Ear, face, neck	8	0	2	6
Digestive system	26	4	14	8
ABWD	6	1	3	2
Urinary	53	3	20	30
Genital	89	5	55	29
Limb	207	22	57	128
Other	78	0	74	4
Chromosomal	24	4	7	13
Probable cause[2]	40	6	17	19

Abbreviations: CA, congenital anomaly; CHD, congenital heart disease; ABWD, abdominal wall defects;

[1] ICD codes used to define subtypes can be found in the extended data.

* The reason because this row does not add up is because CA_any is based on being a case for the other sub grouped variables. For example, if the neonatal source had a CHD for one person and the delivery source has a ABWD for the same person, that person would be a CA_any in both sources, however, when defining the cases based on 2 or more sources, this was done on subgroups so this person used as an example would still effectively class as a case included in one ALSPAC source.

[2] Teratogenic/genetic syndromes, microdeletions and chromosomal abnormalities.

Chapter 3

Text S3.1. Cohort descriptions.

The Amsterdam Born Children and their Development Study (ABCD)

The following text was adapted from the ABCD cohort profile where full study details are described (https://doi.org/10.1093/ije/dyq128) [15]:

Between January 2003 and March 2004, all pregnant women living in Amsterdam were asked to participate in the ABCD study during their first prenatal visit to an obstetric care provider (general practitioner, midwife or gynaecologist). Altogether, 12 373 women were approached—by estimate, ≥99% of the target population. According to Dutch law, all pregnant women, including illegal immigrants and asylum-seekers, are entitled to receive prenatal care, which is free of charge if costs are a problem. For all of the women approached, the care provider completed a registration form which included personal data such as name, address and date of birth. Based on this information, a questionnaire covering socio-demographic characteristics, obstetric history, lifestyles and psychosocial conditions was sent to the pregnant women within 2 weeks, to be filled out at home and returned to the Public Health Service by prepaid mail. A reminder was sent 2 weeks later. The questionnaire included an informed consent sheet the women could use to grant permission for follow-up of their infants at the age of 3 months and every 5 years thereafter, and for the perusal of their medical files. Approval for the ABCD study was obtained from the Central Committee on Research involving Human Subjects in the Netherlands, the Medical Ethical Committees of the participating hospitals, and from the Registration Committee of the Municipality of Amsterdam. Written informed consent was obtained from all participating mothers.

Of the 12 373 women approached, 8266 women filled out the pregnancy questionnaire (response rate: 67%). Of this group, 7050 women granted permission for follow-up (85%) and 7043 women granted permission for perusal of her and her child's medical files (85%). To enhance participation among foreign-born women, two supportive measures were taken: (i) a Turkish, Arabic or English translation was provided to women born in Turkey, Morocco or other non-Dutch-speaking countries and (ii) the possibility of completing the questionnaire orally was offered to women who were illiterate or had reading difficulties.

The Avon Longitudinal Study of Parents and Children (ALSPAC)

ALSPAC is a prospective birth cohort study which was devised to investigate the environmental and genetic factors of health and development. Detailed information about the methods and procedures of ALSPAC is available elsewhere [16,17,51]. 14,541 pregnant women with an expected delivery date of April 1991 and December 1992, residing in the former region of Avon, UK were eligible to take part. Additional enrolment provided a baseline sample of 14,901 participants [51]. The study website contains details of all the data that is available through a fully searchable data dictionary. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (http://www.bristol.ac.uk/alspac/researchers/research-ethics/). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

The Cork SCOPE BASELINE Birth Cohort Study (BASELINE)

The following text was adapted from the BASELINE cohort profile where full study details are described: https://doi.org/10.1093/ije/dyu157 [18].

The study is based in Cork, Ireland. The SCOPE Ireland pregnancy cohort formed the basis of recruitment of infants to BASELINE (n = 1537). In 2007, the amalgamation of all three Cork maternity units into one centre, Cork University Maternity Hospital (CUMH), provided a unique opportunity to conduct research in pregnancy in Cork. CUMH, which is co-located with the Cork University Hospital, is the third largest maternity hospital in Ireland, with 8563 deliveries in 2012. As recruitment was regionally based, the generalizability of the data may be limited. In 2008, all primiparous women in Cork were invited to take part in the Screening for Pregnancy Endpoints (SCOPE) pregnancy cohort. The SCOPE cohort is an international collaboration of research groups interested in the study of major adverse outcomes in late pregnancy, particularly but not exclusively, pre-eclampsia, fetal growth restriction and spontaneous preterm birth8 and as a consequence strict exclusion criteria were applied.9 Detailed maternal, fetal and paternal information was obtained antenatally, as well as blood samples at 15 and 20 weeks' gestation, see Table 1. All women who participated in the SCOPE study were informed about the birth cohort, and if consent was obtained infants were registered to the Cork BASELINE birth cohort.

The Born in Bradford Cohort (BiB)

The Born in Bradford study is a population-based prospective birth cohort including 12,453 women who experienced 13,776 pregnancies between 2007 and 2011. The study is unique in that it has almost an equal split between White European and South Asian women, all residing in Bradford, UK. Bradford is a city in the North of England with high levels of socioeconomic deprivation, and the cohort was started due to a high prevalence of poor child health in the city [52]. Full details of the study methodology were reported previously [19]. The study website provides more information, including protocols, questionnaires and information on how researchers can access data and a full list of all available data (https://borninbradford.nhs.uk/research/documents-data/). Mothers, and their partners, recruited into the study provided detailed interview questionnaire data, measurements, and biological samples. They also consented to the linkage of theirs and their child's data.

The Danish National Birth Cohort (DNBC)

The DNBC is a nationwide cohort of pregnant women, recruited from 1996 through 2002 consisting of 100,415 pregnancies [20]. Informed consent was obtained from participants upon enrolment, and the study was approved by the Danish Data Protection Agency through the joint notification of the Faculty of Health and Medical Sciences at the University of Copenhagen (Sund-2017-09), according to Danish regulations. Information on lifestyle and environmental factors potentially associated with offspring health was collected through 4 prenatal and postnatal telephone interviews at target ages gestational weeks 12 and 30 and child ages 6 and 18 months. The parent-child dyads were then invited for follow-up at 7, 11, and 18 years.

The Norwegian Mother, Father and Child Cohort Study (MoBa)

MoBa is a nationwide, pregnancy cohort comprising family triads (mother-father-offspring) who are followed longitudinally. All pregnant women in Norway who were able to read Norwegian were

eligible for participation. The first child was born in October 1999 and the last in July 2009. Invitations were sent to women in 277 702 pregnancies, the participation rate was 41%. The cohort includes more than 114 000 children, 95 000 mothers and 75 000 fathers [21,22]. Extensive longitudinal data were collected using nine questionnaires: three during pregnancy, and then follow-up questionnaires when the children were 6 months, 18 months, 36 months, 5 years, 7 years and 8 years of age. In addition, a single questionnaire was administered to fathers during gestational weeks 15-18. Data collected include general background and health information, including diet and lifestyle, a semi-quantitative food frequency questionnaire, information on birth and pregnancy outcomes, and on several aspects of child nutrition and development, as well as the physical and mental health of both mother and child. MoBa is linked to the Medical Birth Registry of Norway, which provides standardised information about the health of the mother during pregnancy, other essential medical information related to the pregnancy and birth, and standard post-natal measures of the child. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act.

NINFEA study

The NINFEA study is internet-based birth cohort established in 2005 in Italy (http://www.progettoninfea.it) [23,24,53]. The cohort consists of children born to mothers who have access to the internet and enough knowledge of Italian to complete online questionnaires. The recruitment was conducted actively, through obstetrics clinics, and passively, via internet and the media. A baseline questionnaire on general health and exposures before and during pregnancy is completed by mothers at enrolment, which may occur at any time during pregnancy. During the period 2005-2016 around 7500 mothers were recruited. Further follow-up information was obtained with repeated questionnaires completed 6 and 18 months after delivery and when children turn 4, 7, 10 and 13 years. The response rates for each questionnaire are available at https://www.progettoninfea.it/attachments/70. The study was approved by the ethical committee of the San Giovanni Battista Hospital and CTO/CRF/Maria Adelaide Hospital (Turin, Italy) (approval N.0048362 and following amendments).

Table S3.1. Study-specific methods for data collection.

Measurement	***Study-specific details***
	BMI data
Maternal BMI	**ABCD:** Women filled out a questionnaire containing questions on sociodemographic characteristics, medical history, lifestyle and dietary habits (16 weeks of gestation; IQR 12–20 weeks). BMI was based on pre-pregnancy height and weight as reported in the pregnancy questionnaire. **ALSPAC:** In the 2nd pregnancy questionnaire (12 weeks' gestation) women were asked to report their pre-pregnancy weight and height. No definition of pre-pregnancy was provided in the question. Subsequently for the majority of women all weight measurements from any time of pregnancy have been extracted from obstetric records (height was not routinely measured antenatally in the UK when these women were pregnant). First antenatal clinic measurements of weight correlated strongly with the women's self-report (Pearson correlation = 0.93). **Baseline:** At 15 weeks' gestation sociodemographic and anthropometric measurements, including objectively measured weight and height, were collected. **BiB:** Weight and height (unshod and in light clothing and following a standard protocol) were measured at the recruitment assessment. As women were recruited at the oral glucose tolerance test (26-28 weeks of gestation for the majority) this would not provide an accurate measure of pre-/early-pregnancy weight and would include fetal and amniotic weight and pregnancy related weight gain. All measurements of weight from all antenatal clinics were extracted from the obstetric records and pre-/early-pregnancy BMI was calculated using weight from the first antenatal clinic (median 12 weeks' gestation) and height at recruitment (26-28 weeks' gestation). **DNBC:** Self-reported information on pre-pregnancy weight and height from the first pregnancy interview at around 16 weeks' gestation. **MoBa:** Pre-pregnancy weight and height were self-reported during the first interview at week 17 in pregnancy. **NINFEA:** Pre-pregnancy weight and height were self-reported in the baseline questionnaire (completed at any time during pregnancy).
Paternal BMI	**ABCD:** Paternal weight was maternally reported in questionnaire when child was aged 5-6 years (the closest timepoint available to pregnancy). Paternal height was maternally reported in the pregnancy questionnaire at around 16 weeks' gestation. **ALSPAC:** Paternal weight and height were self-reported from the first partner questionnaire completed around 18 weeks' gestation. **Baseline:** Paternal weight and height were measured around the time of pregnancy. **BiB:** Paternal weight and height were self-reported from the first partner questionnaire mostly completed at recruitment (26–28 weeks' gestation).

	DNBC: Paternal weight and height were reported by the mother during the first pregnancy interview conducted at around 16 weeks' gestation. **MoBa:** Paternal weight and height were maternally reported by questionnaire at around 18 weeks' gestation. **NINFEA:** Paternal weight and height were maternally reported in the baseline questionnaire (completed at any time during pregnancy).
Smoking data	
Maternal smoking	**ABCD:** Asked number of cigarettes per day during pregnancy in first questionnaire (16 weeks of gestation; IQR 12–20 weeks). Binary variable used any smoking during pregnancy. **ALSPAC:** Asked number of cigarettes per day during pregnancy in questionnaire at around 18 weeks' gestation. Binary variable used any smoking during the first trimester. **Baseline:** Reported in early pregnancy questionnaire around 14 weeks gestation. Binary variable used any smoking during the first trimester. Baseline smoking data only used to adjust for BMI analyses. **BiB:** Asked number of cigarettes per day during pregnancy in first questionnaire (26-28 weeks' gestation). Binary variable used any smoking during pregnancy. **DNBC:** Maternal smoking in the first trimester was ascertained from a computer-assisted telephone interview conducted at approximately 16 weeks' gestation. Binary variable used any smoking during the first trimester. Smoking heaviness was based on the average number of cigarettes smoked per day reported in interviews 1 and 2. **MoBa:** Smoking habits were assessed from questionnaires sent by mail at 13-17 and 30 weeks. Binary variable used any smoking during pregnancy. **NINFEA:** Smoking habits in the first two trimesters were assessed in the baseline questionnaire (completed any time during pregnancy). Binary variable used any smoking during the first trimester.
Paternal smoking	**ABCD:** NA **ALSPAC:** Asked about smoking habits within the partner questionnaire during pregnancy at around 18 weeks' gestation. **Baseline:** Maternally reported in pregnancy questionnaire around 14 weeks' gestation. **BiB:** Asked about smoking habits within partner questionnaire during pregnancy (26-28 weeks' gestation). **DNBC:** Maternally reported at 16 weeks' gestation. **MoBa:** Self-reported within first partner questionnaire around 15 weeks' gestation. **NINFEA:** NA
Alcohol data	
Maternal alcohol	**ABCD:** Mothers asked how many glasses of alcohol they drunk during first period of pregnancy (16 weeks of gestation; IQR 12–20 weeks). Binary variable used any alcohol intake during pregnancy. **ALSPAC:** Self-reported from pregnancy questionnaire at around 18 weeks' gestation. Binary variable used any alcohol intake during the first trimester.

	Baseline: Reported in early pregnancy questionnaire around 14 weeks gestation. Binary variable used any alcohol intake during the first trimester. Baseline alcohol data only used to adjust for BMI analyses. **BiB:** NA **DNBC:** Self-reported at 16 weeks' gestation. Binary variable used alcohol intake during the first trimester. Drinking heaviness was based on the average number of units drank per week reported in interviews 1 and 2. **MoBa:** Assessed via questionnaire around 17 weeks' gestation. Binary variable used any alcohol intake during the first trimester. **NINFEA:** Drinking habits in the first trimester were assessed in the baseline questionnaire (completed at any time during pregnancy). Binary variable used any alcohol intake during the first trimester.
Paternal alcohol	**ALSPAC:** Self-reported within first partner questionnaire at around 18 weeks' gestation. **MoBa:** Self-reported within first partner questionnaire at around 15 weeks' gestation.

Text S3.2. Paternal alcohol consumption methods.

ALSPAC

We used data from the partners questionnaire which was filled in by partners at around 18 weeks' gestation. I used data from questions B18 and B19 from the PB questionnaire (http://www.bristol.ac.uk/alspac/researchers/our-data/).

B18b. How often have you drunk alcoholic drinks during the last 3 months: 1) Never, 2) less than once a week, 3) at least once a week, 4) 1-2 glasses every day, 5) 3-9 glasses every day, 6) at least 10 glasses every day.

B19b. How many days in the past month did you drink the equivalent of 2 pints of beer, 4 glasses of wine or 4 pub measures of spirit? 1) Every day, 2) more than 10 days, 3) 5-10 days, 4) 3-4 days, 5) 1-2 days, 6) none.

We coded paternal alcohol consumption as follows: non-drinkers = If answered 1 to B18b; light drinkers = answered 5 to B19b; mod/heavy drinkers = answered 1,2,3 or 4 to B19b.

MoBa

Question *FF244. How often do you drink alcohol now that your partner is pregnant? Response options: 1) Approximately 6-7 times per week, 2) Approximately 4-5 times per week, 3) Approximately 2-3 times per week, 4) Approximately once per week, 5) Approximately 1-3 times per month, 6) Less than once per month, 7) Never.*

Using data from FF244***, I*** coded paternal alcohol consumption as follows: non-drinkers = Answered number 7; light drinkers = Answered 4, 5 or 6; mod/heavy drinkers = Answered 1, 2 or 3

Text S3.3. Definition of congenital heart disease (CHD) and other congenital anomalies (CAs).

Here I describe ascertainment of CA cases for each cohort. International Classification of Diseases (ICD; version 10) codes were used to define CA cases when possible (see Table S3.2 above for classifications). However, in some cohorts these data were not available. The following cohorts were used to define CA cases with ICD codes: ALSPAC, BiB, DNBC, NINFEA.

ABCD

The ABCD cohort has previously published research involving CAs [54]. The same methods for data extraction were used for the present study. Data on CAs were obtained from three different sources: the infant questionnaire, which was filled out by the mother at an average infant age of 12.9 weeks (IQR 12.4–13.4 weeks); the questionnaire filled out by the mother at an average infant age of 5.07 years (IQR 5.04–5.13 years), and clinical data of the Youth Health Care Registration (health and development registration of all children in the Netherlands, which is mandatory under the law on medical treatment agreement). The questionnaires were screened by a researcher, and in the case of missing or unclear answers the mothers were contacted. Subsequently, the questionnaires were scanned and transferred to a database by a certified company (Scan serv, Nootdorp, the Netherlands). Missing data in the questionnaires could be supplemented by data from the Youth Health Care Registration, and in the case of any discrepancy the data from the Youth Health Care Registration prevailed. CA data in ABCD was restricted to live-born children.

CAs were categorised as follows: 0 = no defect 1 = congenital malformations of the nervous system 2 = congenital malformations of eye, ear, face, throat 3 = congenital malformations of the cardiovascular system 4 = congenital malformations of the respiratory tract 5 = split lip and/or palate 6 = congenital malformations of the digestive tract 7 = congenital malformations of the kidneys, urinary tract, genitalia 8 = congenital malformations of the musculoskeletal system 9 =neoplasms 10 = other congenital malformations 11 = chromosomal defect 12 = monogenic defect 13 = microdeletions and uniparental disomy 14 = other syndromes 15 = complex cardiovascular defects 16 = multiple defects of the extremities 17 = other multiple defects within an organ system 18 = multiple defects (in multiple organ systems) 21 = minor defect 22 = unclear/uncertain diagnosis 23 = "don't know which defect" 24 = "not applicable" 25 = missing information.

We coded CHD cases if they were “Yes” for category 3. I coded chromosomal/genetic aberrations if “Yes” for any of the following categories: 11, 12, 13, 14.

ALSPAC

Case ascertainment of CAs in the ALSPAC cohort has been described in detail in a recently published data note [29]. Data were combined from multiple sources: NHS records (primary care, paediatric cardiology database, data on fetal deaths and local child health services), midwifery and birth records and maternal self-report via child-based questionnaires. Each source was coded using ICD-10 codes. By combining sources, there would be a greater possibility of capturing all of possible cases within the cohort. The majority of cases of CAs were identified by primary care records (79% for any CA and 68% for any CHD). I included diagnoses made at any age (from birth up until age 25/26). There were no restrictions in cases of CAs in ALSPAC, I included all cases whether live-born or not. However, it is possible that some

cases that were terminated earlier in pregnancy were missed due to them never having an NHS number and thus not being identified through record linkage.

BASELINE

At 2 months, mothers were asked of any medical problems and/or referrals. If a baby had been referred to a specialist, it was checked to see if they had results from an echocardiogram. Echocardiograms were checked by a cardiologist. Exact CHD diagnoses were reported based on the echo. At 6 months, there was one additional baby that had cardiac surgery and added as a case. If a baby had been diagnosed after 6 months, they would have been identified through records on the Echo. Therefore, in BASELINE I obtained all CHDs up until ~age 12.

BiB

In the BiB cohort, there were two separate sources to identify CAs. Both sources were used in this study: (i) CAs up to 5 years of age, identified in GP records by Bishop et al [30] following EUROCAT guidelines. ICD-10 codes were mapped to clinical term (CT)-V3 codes prior to extraction from GP records. (ii) Data extracted from the Yorkshire and Humber CAs register database. Data were ICD-10 coded. All of these were confirmed postnatally. BiB includes data on the birth outcome of each child (live birth, miscarriage, still birth). Therefore, diagnoses were not necessarily restricted to live born children. However, there is the possibility that some women would have terminated the pregnancy after the 12- or 20-week scans which would lead to an under-representation of congenital anomaly cases.

DNBC

In the DNBC, all diagnoses of congenital anomalies (according to EUROCAT guide 1.4 section 3.2 and 3.3) up until the age of 15 years were extracted from the Danish National Patient Register (DNPR) which is linked to the cohort data [31,32]. Diagnoses were ICD-coded. These data were restricted to children born alive.

MoBa

Information on whether a child had a CHD or not was obtained though linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN. The notification form includes the name and personal identity number of the child and parents, as well as information about maternal health before and during pregnancy, and any complications during pregnancy or at birth, including the presence of any heart defects. The MBRN contains information on all births and pregnancies ended after the 12th week of gestation, including stillbirths and abortions after the 12th week, including on heart defects. Heart defects are registered in the MBRN through notifications from clinical staff identifying these defects at delivery or any hospital in patient treatments occurring immediately after birth until the child is discharged. The medical notification is made at discharge, which can be several months after birth. Details of the notified heart defects, such as specific diagnosis or treatment are not provided. Whilst most of the heart defects would have been diagnosed at birth it is possible that some children were admitted to hospital after delivery for non-specific reasons of for diagnoses that at the time were not considered to be related to a heart defect. Therefore, MOBA contribute only to analyses of any CHD and I considered diagnosis to have been made between birth and 6 months (few would remain in hospital after this length).

NINFEA

Congenital anomalies in the NINFEA cohort were reported in the second questionnaire compiled 6 months after birth. Mothers compiled a checklist that included pre-specified anomalies (namely cryptorchidism (also assessed 18 months after birth), congenital hip dysplasia, cleft palate, spina bifida and pyloric stenosis) and anomalies divided by major systems (namely cardiovascular, gastrointestinal, genitourinary, musculoskeletal, respiratory and nervous system, and genetic/chromosomal or metabolic/endocrine disease). If the mother reported an anomaly from a specific system, the exact name of the anomaly was asked. If the child died or had any surgery performed in the first 6 months, the cause of death and type of surgery were also checked to see if any congenital anomaly was reported. All congenital anomalies were coded using ICD-10 codes by an experienced pediatrician and were reassessed by an independent physician. NINFEA included live-born infants only.

Studies with ICD coded data

Table S3.2 shows how cases of CHD were defined in the studies with ICD codes (ALSPAC, BiB, DNBC, NINFEA).

Table S3.2. Subcategories of CHD.

Category	CHDs included/excl	ICD codes
All	Any CHD as defined by EUROCAT* Patent ductus arteriosus (PDA) with gestational age (GA) < 37 weeks not considered a CHD case. Peripheral pulmonary artery stenosis with GA < 37weeks not considered as a CHD case.	Q20-Q25, Q260, Q262-Q269**
Severe	Heterotaxia, Conotruncal defect, Atrioventricular septal defect, Anomalous pulmonary venous return, Left ventricle outflow tract obstruction, Right ventricle outflow tract obstruction, Other complex defects	Q240, Q241, Q206, Q200, Q251, Q252, Q253, Q254, Q203, Q213, Q201, Q214, Q212, Q26, Q262, Q264, Q268, Q269, Q234, Q251, Q230, Q231, Q221, Q224, Q225, Q255, Q204
Non-severe	PDA (in full term infants), valvular pulmonary stenosis, ventricular septal defect (VSD), atrial septum defects (ASD), unspecified septal defects, isolated valve defects, other specified heart defects, unspecified heart defects	Non-severe cases that are All=1 and Severe=0.

* Definitions taken from here: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/EUROCAT-Guide-1.4-Section-3.3.pdf
**Q250 and Q256 not a case if isolated and GA<37weeks

Additional analysis - excluding infants with any known chromosomal/genetic/teratogenic defects

ABCD, ALSPAC, BiB, DNBC, MoBa and NINFEA were able to contribute to this additional analysis. In ALSPAC, BiB, DNBC and NINFEA, I used the ICD codes in Table S3.3 to exclude cases. In ABCD, there were specific categories (described above) which corresponded to chromosomal and genetic anomalies (11 = chromosomal defect 12 = monogenic defect 13 = microdeletions and uniparental disomy 14 = other syndromes). In MoBa, I used questionnaire data which was maternally reported at 6 months after birth: "Is your child suspected of having a syndrome?" and "Is your child suspected of having a chromosomal defect?".

Table S3.3. Subcategories of congenital anomalies with a 'known cause' used in additional analyses.

Category	**ICD-10 Codes**
Teratogenic/genetic syndromes, microdeletions and chromosomal abnormalities (additional analysis).	D821, P350-P352, P371, Q619, Q751, Q754, Q771-Q772, Q780, Q796, Q85, Q861-Q869, Q87, Q90-Q92, Q930-Q939, Q95-Q99

Text S3.4. Confounder data.

By definition a confounder has to cause (or be a plausible cause) of exposure and outcome. The maximum number of confounders used in fully adjusted models are listed below. Confounder and other parent exposure adjusted models are the same as fully adjusted but with additional adjustment for the other parent's exposure and additional adjustment for maternal parity in paternal models.

Exposure = BMI: age, education, parity (maternal), ethnicity, smoking, alcohol, offspring sex.

Exposure = Smoking: age, education, parity (maternal), ethnicity, alcohol, offspring sex.

Exposure = Alcohol: age, education, parity (maternal), ethnicity, smoking, offspring sex.

There is evidence that smoking and alcohol influence BMI [55-58]. I therefore treated those as confounders for the association of maternal/paternal BMI with CHD. Smoking and alcohol are associated with each other in most populations but whether one causes the other is unclear. It is possible that most of their association is due to socioeconomic and cultural factors. Despite being unclear about whether they could be confounders of each other's effect on CHD (e.g. alcohol a confounder for smoking and vice versa) in the final confounder adjusted model I included alcohol as a confounder for smoking and vice versa.

I used maternal/paternal age at birth in complete years. I used educational attainment for both parents' measures of socioeconomic position (SEP). In the harmonised LifeCycle data education has been defined according to the international classification (High: Short cycle tertiary, Bachelor, Masters, Doctoral or equivalent (ISCED-2011: 5-8, ISCED-97: 5-6) Medium: Upper secondary, Post-secondary non-tertiary (ISCED-2011: 3-4, ISCED-97: 3-4) Low: No education; early childhood; pre-primary; primary; lower secondary or second stage of basic education). Mothers' parity was based on previous born children (previous stillbirths included, abortions excluded) (coded as 0, 1, 2, 3, $\geq$ 4). For ethnicity I used the best estimate of the mother's/father's ethnic background based on the cohort's discretion (Western, Non-western, Mixed). Offspring sex was a binary variable (male/female). In additional analyses, I adjusted for folic acid supplementation in fully adjusted maternal models. This was a yes/no variable defined as intake of folic acids (folate, vitamin B9) during the period from conception to early pregnancy (12 weeks).

In NINFEA, due to the smaller sample size, maternal parity and maternal/paternal education were categorised as binary variables (parity: nulliparous and multiparous, education: low and medium combined together).

In ALSPAC, BASELINE, DNBC, MoBa and NINFEA I did not adjust for ethnicity in any analyses. 98% of women were of Western origin in ALSPAC. >98.5% of women in BASELINE were of Western origin. Ethnicity in the DNBC is said to be of >99% White European origin with a recent paper reporting their DNBC population to be 100% of White origin [59]. There were no data available on ethnicity in MoBa, however, it is believed that 99-100% are of Western origin. Ethnicity data were not available in NINFEA, although, the large majority of mothers (>98%) were born in Europe. Data on paternal country of birth was available for approximately half of the cohort and >98% of them were born in Europe.

In BiB only ~28% of mothers had harmonised data on alcohol intake during pregnancy, therefore this was not included in any models within BiB analyses as an exposure and also as a confounder in BMI and smoking models.

ABCD and BASELINE did not have harmonised LifeCycle data available. I describe methods for data harmonization here:

We used available ABCD data and tried to harmonize it as best as possible to match the LifeCycle data. BMI, sex, age, parity and folic acid supplementation were identical variables to the harmonised LifeCycle ones. Paternal height was self-reported by the mother and paternal weight was from 11 months after pregnancy (the closest timepoint available). I used any pregnancy smoking or drinking (yes/no) for the smoking and alcohol variables as there was no trimester specific exposure data. ABCD did not contribute to paternal alcohol or smoking analyses as there were no data for these exposures around the time of pregnancy. Maternal education was defined as: high (Short cycle tertiary, Bachelor, Masters, Doctoral or equivalent (9 or more years)), medium (Upper secondary, Post-secondary non-tertiary (6-9 years)) or low (No education; early childhood; pre-primary; primary; lower secondary or second stage of basic education (<6 years)). Paternal education was from the 11-year questionnaire and split into 3 groups as this was the only data available. For ethnicity, I defined Western and non-western as appropriate from physiological ethnicity of grandmother's birth country for maternal ethnicity. Paternal ethnicity was reported by the mother and recoded to Western/Non-Western/Mixed.

All women were experiencing their first pregnancy in BASELINE; therefore I did not adjust for parity in any analyses. BMI, sex, age and smoking were coded the same as the harmonised LifeCycle data. Education in BASELINE was binary defined as medium or high. This was left unchanged and used as a measure of SEP as in other analyses.

In the analysis plan, I originally stated that I would treat type-1 diabetes (T1D) as a confounder. The rationale for this was that diabetes is a known teratogen for CHDs and could also influence pregnancy lifestyle factors through changes in behaviours. However, after exploring the data, the prevalence of T1D was low in those cohorts with data (0.2% in ALSPAC, 0.1% in BiB and 0.2% in DNBC for maternal T1D) and the other cohorts did not have data on specific diabetes diagnoses. For cohorts with T1D data, the number of CHD cases in those with a diagnosis was either zero or less than 10, making adjustment not meaningful or impossible through complete separation in the logistic model.

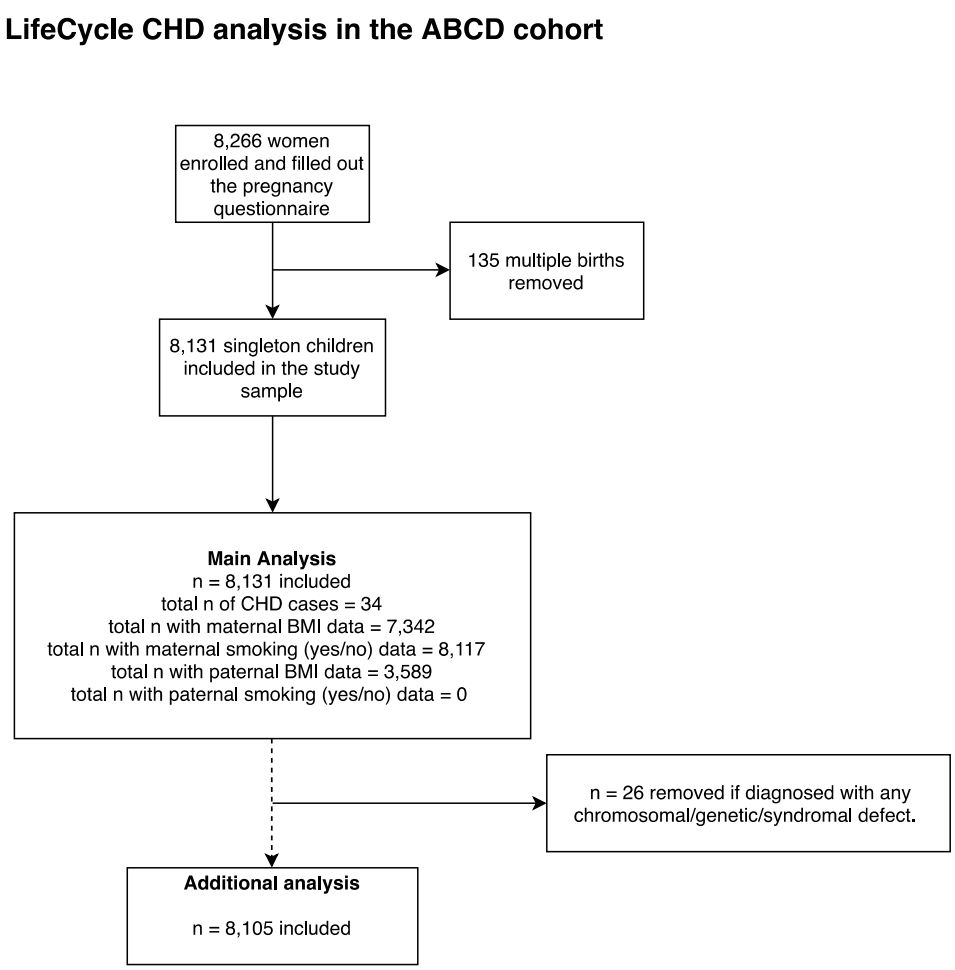


Figure S3.1. Study flow chart illustrating participant selection in the ABCD cohort.

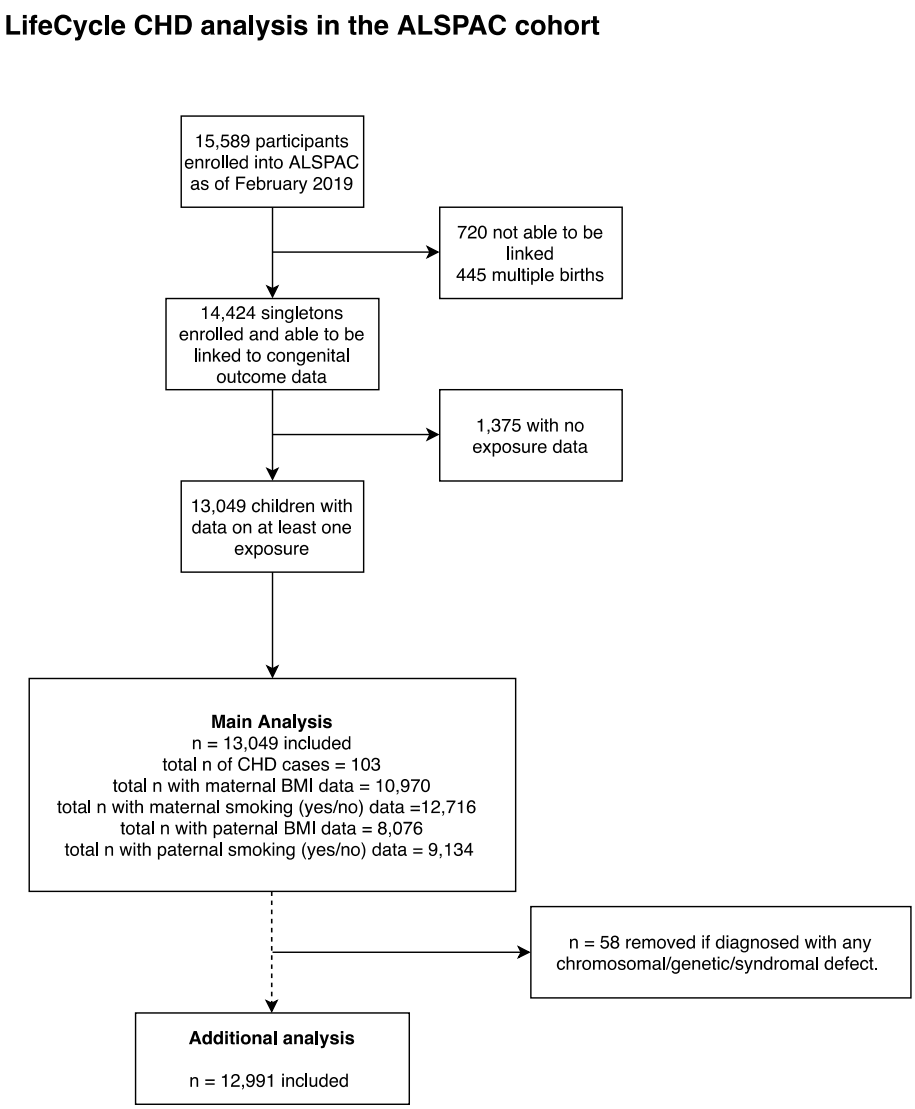


Figure S3.2. Study flow chart illustrating participant selection in the ALSPAC cohort.

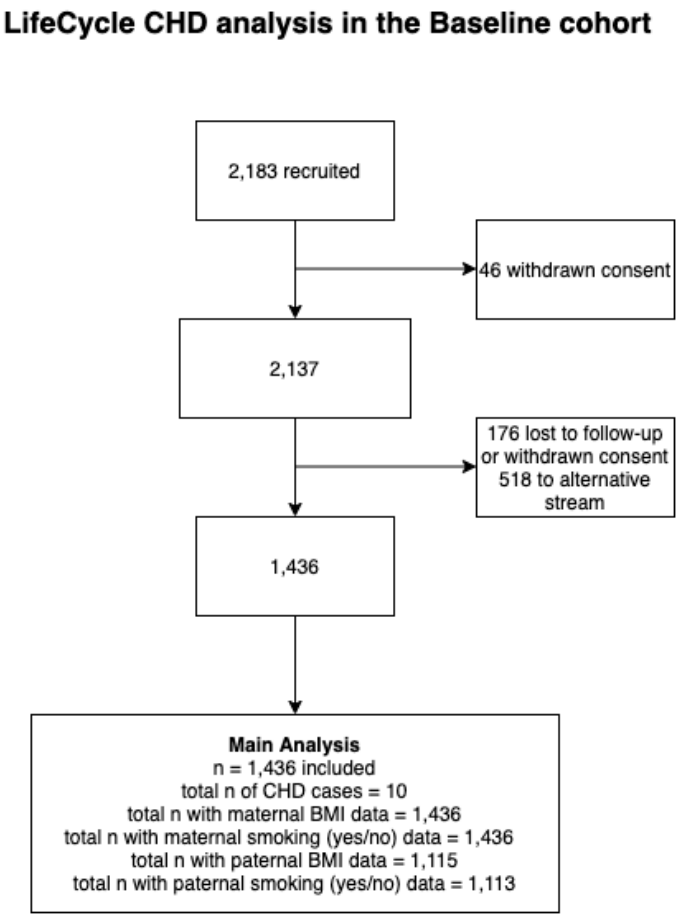


Figure S3.3. Study flow chart illustrating participant selection in the BASELINE cohort. I included 1436 participants in this study (Stream 1). Adapted from: https://doi.org/10.1093/ije/dyu157

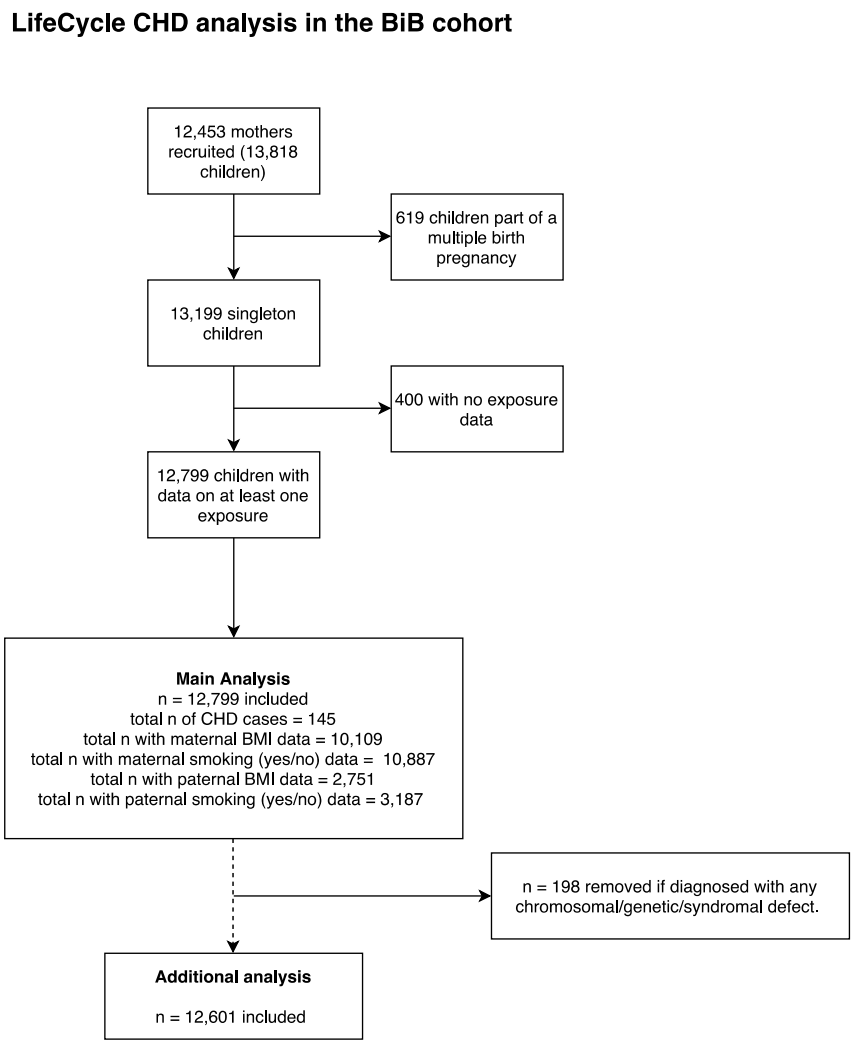


Figure S3.4. Study flow chart illustrating participant selection in the BiB cohort.

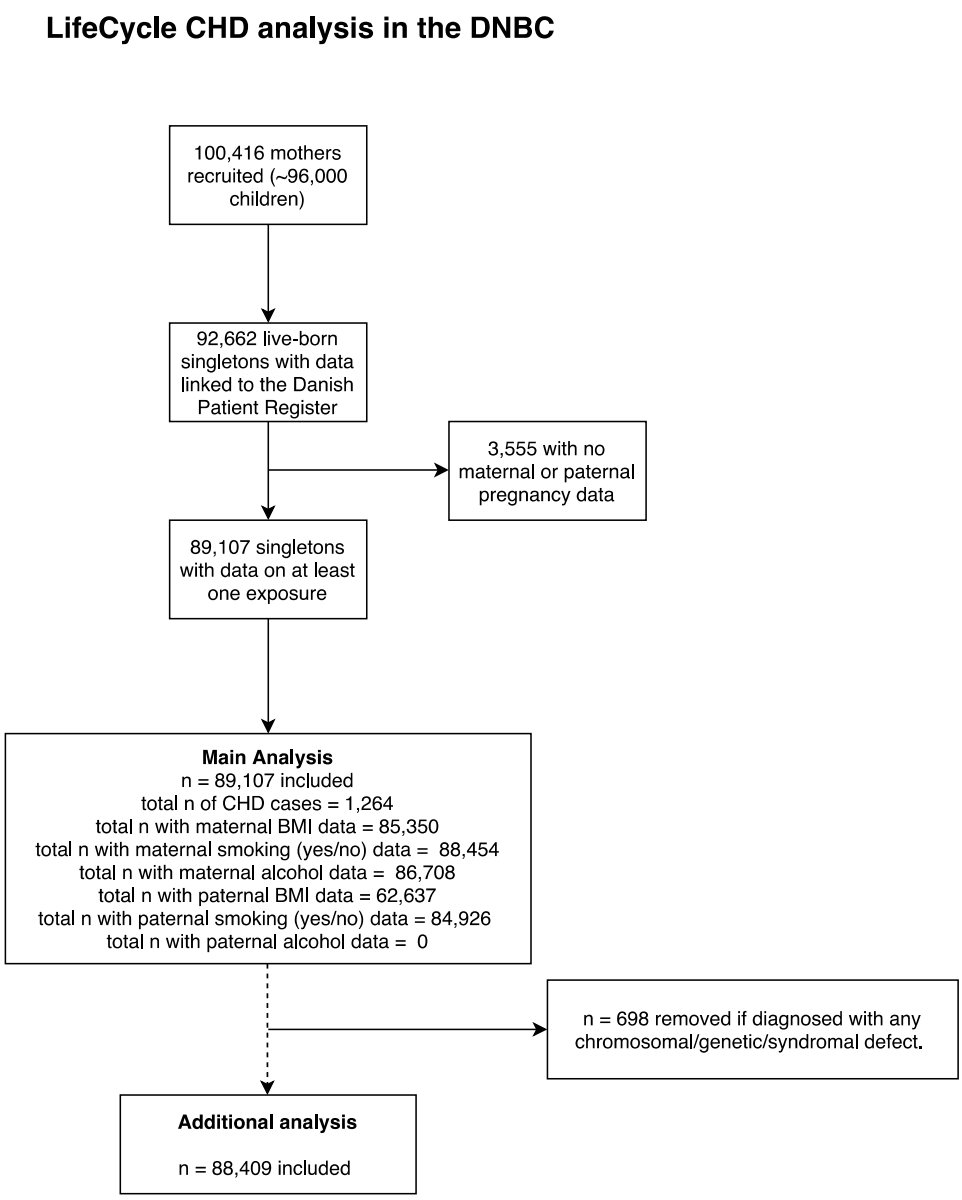


Figure S3.5. Study flow chart illustrating participant selection in the DNBC cohort.

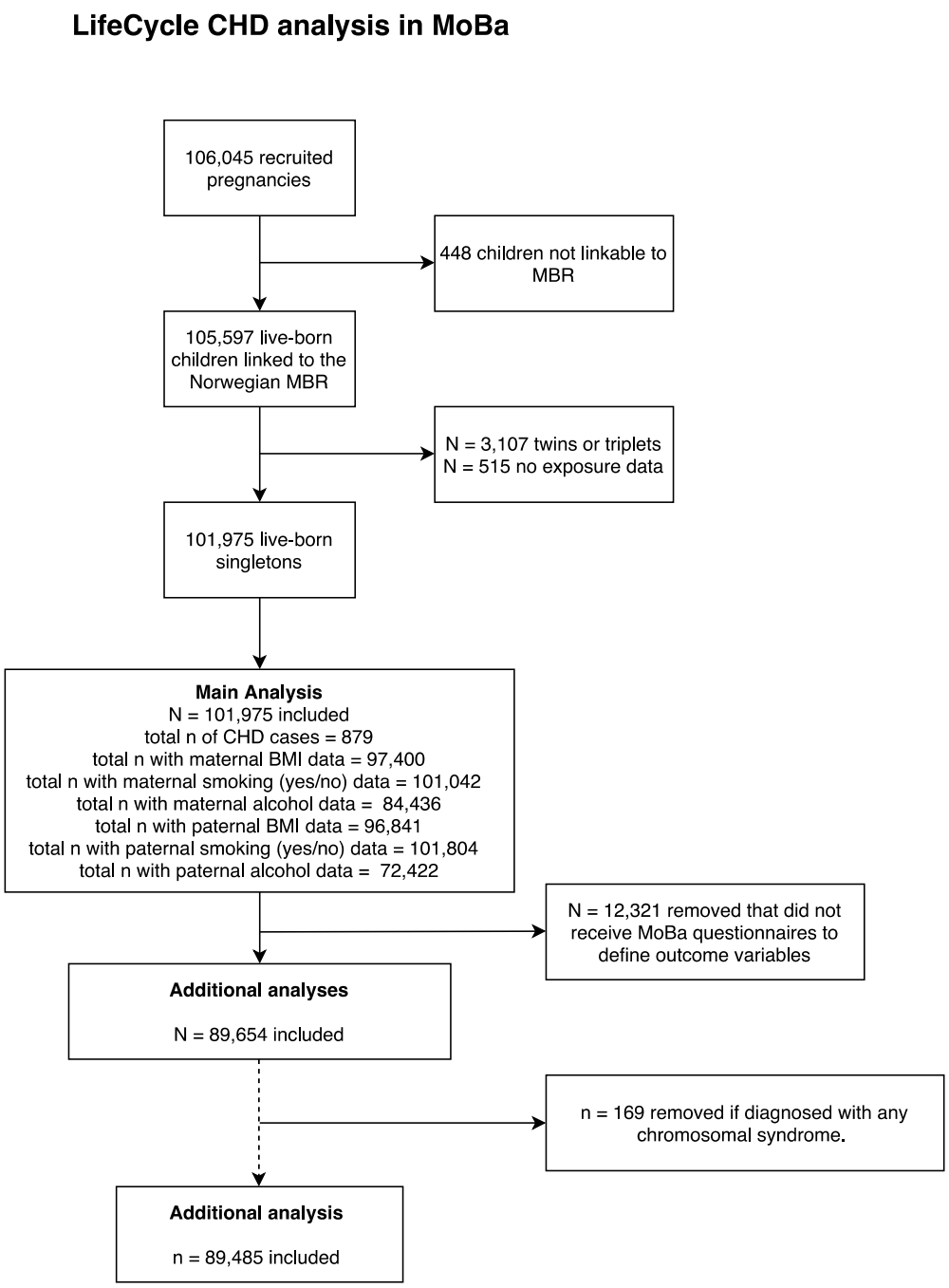


Figure S3.6. Study flow chart illustrating participant selection in the MoBa cohort. MBR = Medical birth registry.

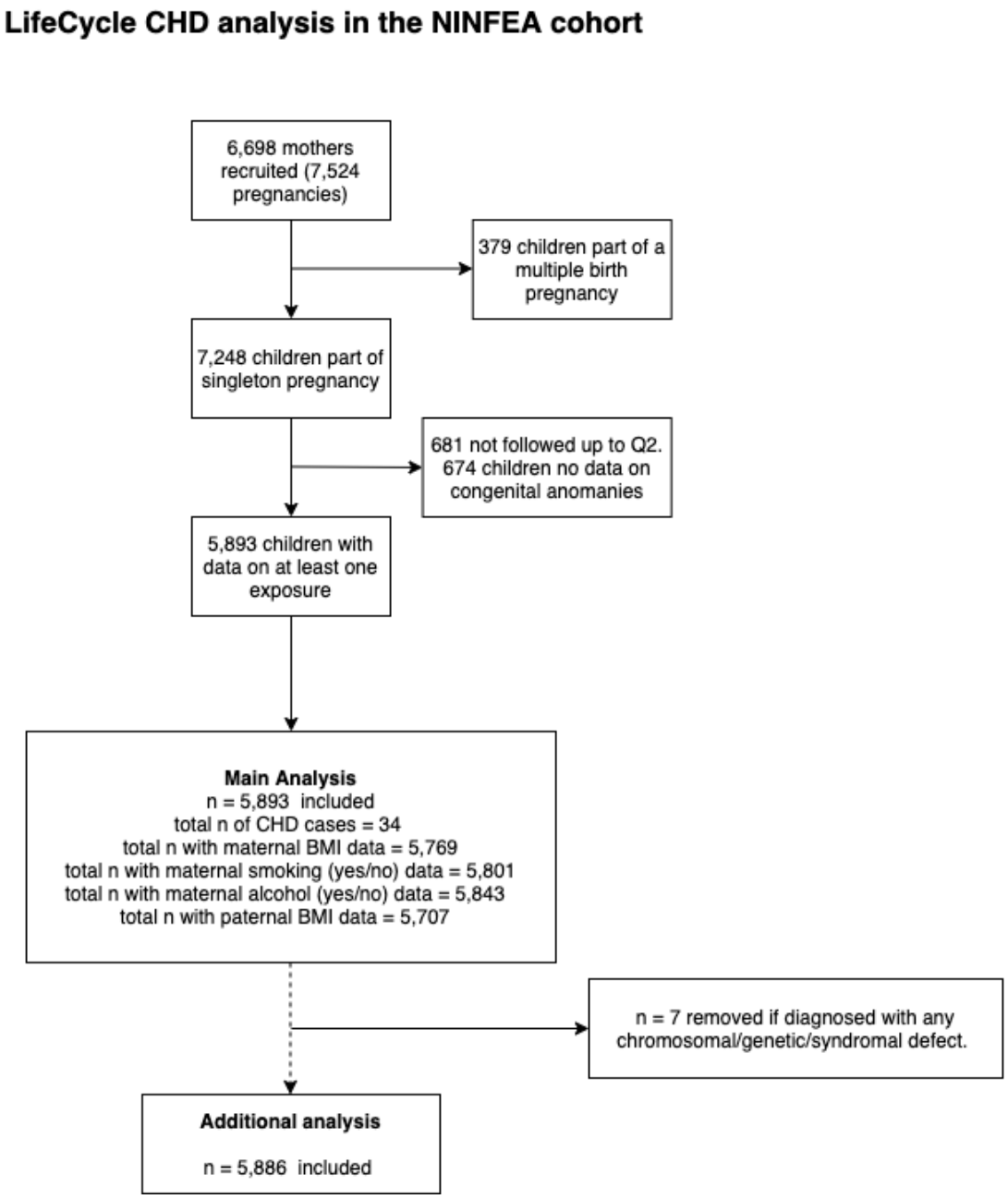


Figure S3.7. Study flow chart illustrating participant selection in the NINFEA cohort

Table S3.4. Summary of missing data in each cohort.

	ABCD ***N* = 8,131**	**ALSPAC** ***N* = 13,049**	**BASELINE** ***N* = 1,436**	**BiB** ***N* = 12,799**	**DNBC** ***N* = 89,107**	**MoBa** ***N* = 101,975**	**NINFEA** ***N* = 5,893**
Country	Netherlands	UK	RoI	UK	Denmark	Norway	Italy
Recruitment period	2003-2004	1991-1992	2008-2011	2007-2011	1996-2002	1999-2008	2005-2016
Maternal (n missing (%))							
Age, years	0	2062 (15.8)	0	0	0	181 (0.2)	1 (0.0)
BMI, kg/m^2	789 (9.7)	2079 (15.9)	0	2690 (21.0)	3757 (4.2)	4575 (4.5)	124 (2.1)
Preg smoking yes/no	14 (0.2)	333 (2.6)	0	1912 (14.9)	2367 (2.7)	933 (0.9)	92 (1.6)
Preg smoking heaviness	-	2350 (18.0)	-	1912 (14.9)	1184 (1.3)	390 (0.4)	72 (1.2)
Preg alcohol yes/no	6 (0.1)	427 (3.3)	43 (3.0)	-	2399 (2.7)	19617 (19.2)	50 (0.9)
Preg alcohol heaviness	-	6548 (50.2)	-	-	758 (0.9)	17539 (17.2)	79 (1.3)
Parity	0	502 (3.8)	0	470 (3.7)	0	1805 (1.8)	272 (4.6)
Education	83 (1.0)	1152 (8.8)	9 (0.6)	2750 (21.5)	8451 (9.5)	6963 (6.8)	46 (0.8)
Ethnicity	14 (0.2)	-	0	1906 (14.9)	-	-	-
Folic acid supp	98 (1.2)	424 (3.2)	-	-	6510 (7.3)	1805 (1.8)	148 (2.5)
Paternal (n missing (%))							
Age, years	4378 (53.8)	5488 (42.1)	321 (22.4)	9439 (73.7)	1371 (1.5)	521 (0.5)	2506 (42.5)
BMI, kg/m^2	4542 (55.9)	4973 (38.1)	321 (22.4)	10074 (78.7)	26470 (29.7)	5134 (5.0)	186 (3.2)
Smoking	-	3915 (30.0)	323 (22.5)	9612 (75.1)	4181 (4.7)	171 (0.2)	-
Alcohol	-	4844 (37.1)	-	-	-	29553 (28.9)	-
Education	5873 (72.2)	1620 (12.4)	0	4676 (36.5)	10690 (12.0)	5372 (5.3)	138 (2.3)
Ethnicity	197 (2.4)	-	321 (22.4)	9625 (75.2)	-	-	-
Offspring sex	203 (2.5)	0	0	0	0	196 (0.2)	1 (0.0)

Table S3.5. Comparison between maximal numbers from **main analyses presented in the manuscript (black, top rows)** and **complete case models (red, bottom rows).** Results are odds ratios (95% CIs) of any offspring CHD per unit difference in BMI.

Model	*ABCD*	*ALSPAC*	*BASELINE*	*BiB*	*DNBC*	*MoBa*	*NINFEA*	*Meta-analysis results*
Maternal BMI unadjusted	1.02 (0.94, 1.09) N = 7,342	1.05 (1.00, 1.09) N = 10,970	1.07 (0.92, 1.20) N = 1,436	1.01 (0.97, 1.04) N = 10,109	1.02 (1.01, 1.03) N = 85,350	0.99 (0.98, 1.01) N = 97,400	0.93 (0.83, 1.03) N = 5,769	**1.01 (1.00, 1.02)** **N = 218,376**
	1.07 (0.95, 1.16) N = 3,415	1.01 (0.93, 1.08) N = 6,452	1.06 (0.87, 1.23) N = 1,078	0.99 (0.89, 1.10) N = 1,753	1.02 (1.00, 1.03) N = 55,564	0.99 (0.97, 1.01) N = 73,637	0.93 (0.83, 1.04) N = 5,393	**1.01 (0.99, 1.02)** **N = 147,292**
Maternal BMI confounder adjusted	1.04 (0.95, 1.11) N = 7,103	1.05 (0.99, 1.10) N = 9,179	1.08 (0.93, 1.21) N = 1,386	1.02 (0.98, 1.05) N = 7,279	1.02 (1.00, 1.03) N = 78,180	0.99 (0.97, 1.01) N = 75,448	0.94 (0.84, 1.05) N = 5,476	**1.01 (1.00, 1.02)** **N = 184,051**
	1.05 (0.93, 1.15) N = 3,415	1.01 (0.94, 1.08) N = 6,452	1.06 (0.87, 1.23) N = 1,078	0.98 (0.87, 1.09) N = 1,753	1.01 (1.00, 1.03) N = 55,564	0.99 (0.97, 1.01) N = 73,637	0.95 (0.85, 1.06) N = 5,393	**1.01 (0.99, 1.02)** **N = 147,292**
Maternal BMI confounder and other parent BMI adjusted	1.05 (0.93, 1.15) N = 3,415	1.02 (0.94, 1.10) N = 6,452	1.05 (0.85, 1.23) N = 1,078	0.99 (0.88, 1.09) N = 1,753	1.01 (1.00, 1.03) N = 55,564	0.99 (0.97, 1.01) N = 73,637	0.94 (0.84, 1.06) N = 5,393	**1.00 (0.99, 1.02)** **N = 147,292**
	1.05 (0.93, 1.15) N = 3,415	1.02 (0.94, 1.10) N = 6,452	1.05 (0.85, 1.23) N = 1,078	0.99 (0.88, 1.09) N = 1,753	1.01 (1.00, 1.03) N = 55,564	0.99 (0.97, 1.01) N = 73,637	0.94 (0.84, 1.06) N = 5,393	**1.00 (0.99, 1.02)** **N = 147,292**
Paternal BMI unadjusted	0.99 (0.84, 1.08) N = 3,589	0.99 (0.91, 1.06) N = 8,076	1.07 (0.86, 1.21) N = 1,115	1.03 (0.94, 1.12) N = 2,706	1.02 (1.00, 1.04) N = 62,637	0.99 (0.97, 1.01) N = 96,841	1.02 (0.92, 1.13) N = 5,707	**1.01 (0.99, 1.02)** **N = 180,690**
	1.04 (0.88, 1.11) N = 1,732	0.97 (0.86, 1.07) N = 5,044	1.07 (0.86, 1.21) N = 1,113	1.01 (0.89, 1.13) N = 1,572	1.02 (1.00, 1.04) N = 53,922	0.99 (0.96, 1.01) N = 67,071	0.96 (0.81, 1.13) N = 3,166	**1.01 (0.99, 1.03)** **N = 133,620**
Paternal BMI confounder adjusted	1.03 (0.84, 1.10) N = 1,800	0.96 (0.86, 1.06) N = 5,550	1.06 (0.86, 1.21) N = 1,113	1.04 (0.93, 1.14) N = 2,085	1.02 (1.00, 1.05) N = 54,710	1.00 (0.97, 1.02) N = 68,623	1.03 (0.89, 1.19) N = 3,294	**1.01 (1.00, 1.03)** **N = 137,175**
	1.03 (0.84, 1.10) N = 1,732	0.97 (0.86, 1.08) N = 5,044	1.06 (0.86, 1.21) N = 1,113	1.04 (0.92, 1.16) N = 1,572	1.02 (1.00, 1.04) N = 53,922	1.00 (0.97, 1.02) N = 67,071	0.96 (0.81, 1.14) N = 3,166	**1.01 (1.00, 1.03)** **N = 133,620**
Paternal BMI confounder and other parent BMI adjusted	1.03 (0.85, 1.10) N = 1,732	0.97 (0.86, 1.08) N = 5,044	1.05 (0.84, 1.21) N = 1,113	1.04 (0.92, 1.15) N = 1,572	1.02 (1.00, 1.04) N = 53,922	1.00 (0.97, 1.02) N = 67,071	0.99 (0.83, 1.18) N = 3,166	**1.01 (0.99, 1.03)** **N = 133,620**
	1.03 (0.85, 1.11) N = 1,732	0.97 (0.86, 1.08) N = 5,044	1.05 (0.84, 1.21) N = 1,113	1.04 (0.92, 1.15) N = 1,572	1.02 (1.00, 1.04) N = 53,922	1.00 (0.97, 1.02) N = 67,071	(0.99, 0.83, 1.18) N = 3,166	**1.01 (0.99, 1.03)** **N = 133,620**

Covariates used for each study in fully adjusted models (mutually adjusted models the same as fully adjusted but with additional adjustment for the other parent's BMI and parity in paternal models);
ABCD: Maternal: offspring sex, age, education, parity, ethnicity, smoking, alcohol. Paternal: offspring sex, age, education, ethnicity.
ALSPAC: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking, alcohol.
BASELINE: Maternal: offspring sex, age, education, smoking, alcohol. Paternal: offspring sex, age, smoking.
BiB: Maternal: offspring sex, age, education, parity, ethnicity, smoking. Paternal: offspring sex, age, education, ethnicity, smoking.
DNBC: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking.
MoBa: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking, alcohol.
NINFEA: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education.

Table S3.6. Comparison between **maximal numbers (black, top rows**) and **complete case models (red, bottom rows)**. Results are odds ratios (95% CIs) of any offspring CHD for a BMI category in comparison to normal BMI. Categories: underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5 to <25 kg/m^2), overweight (BMI 25 to <30 kg/m^2) and obese (BMI ≥30 kg/m^2).

Exposure	*ALSPAC*	*BiB*	*DNBC*	*MoBa*	*Meta-analysis results*
Maternal underweight unadjusted	0.69 (0.26, 1.48) N = 10,970	0.67 (0.17, 0.89) N = 10,109	1.36 (1.05, 1.73) N = 85,350	1.03 (0.70, 1.52) N = 97,400	**1.19 (0.97, 1.46)** **N = 203,829**

	0.63 (0.15, 1.78) N = 6,452	NA	1.35 (0.95, 1.86) N = 55,564	1.06 (0.66, 1.71) N = 73,637	**1.21 (0.92, 1.57)** **N = 135,653**
Maternal underweight confounder adjusted	0.63 (0.19, 1.57) N = 9,179	0.64 (0.10, 2.11) N = 7,360	1.33 (1.01, 1.71) N = 79,288	1.06 (0.66, 1.71) N = 75,448	**1.20 (0.96, 1.50)** **N = 171,275**
	0.68 (0.16, 1.93) N = 6,452	NA	1.34 (0.94, 1.84) N = 55,564	1.08 (0.67, 1.74) N = 73,637	**1.21 (0.93, 1.58)** **N = 135,653**
Maternal underweight confounder and other parent BMI adjusted	0.65 (0.15, 1.84) N = 6,452	NA	1.35 (0.95, 1.86) N = 55,564	1.07 (0.67, 1.73) N = 73,637	**1.21 (0.93, 1.58)** **N = 135,653**
	0.65 (0.15, 1.84) N = 6,452	NA	1.35 (0.95, 1.86) N = 55,564	1.07 (0.67, 1.73) N = 73,637	**1.21 (0.93, 1.58)** **N = 135,653**
Maternal overweight unadjusted	1.23 (0.64, 2.20) N = 10,970	1.35 (0.87, 2.08) N = 10,109	1.24 (1.07, 1.42) N = 85,350	1.01 (0.85, 1.20) 1.02 N = 97,400	**1.15 (1.04, 1.28)** **N = 203,829**
	0.71 (0.21, 1.82) N = 6,452	1.46 (0.41, 5.29) N = 1,753	1.28 (1.07, 1.53) N = 55,564	1.04 (0.86, 1.27) N = 73,637	**1.16 (1.02, 1.32)** **N = 137,406**
Maternal overweight confounder adjusted	0.85 (0.35, 1.80) N = 9,179	1.34 (0.80, 2.22) N = 7,360	1.23 (1.06, 1.42) N = 79,288	1.06 (0.87, 1.29) N = 75,448	**1.17 (1.04, 1.31)** **N = 171,275**
	0.72 (0.21, 1.87) N = 6,452	1.45 (0.39, 5.37) N = 1,753	1.26 (1.05, 1.51) N = 55,564	1.04 (0.85, 1.27) N = 73,637	**1.15 (1.01, 1.31)** **N = 137,406**
Maternal overweight confounder and other parent BMI adjusted	0.77 (0.23, 1.99) N = 6,452	1.46 (0.39, 5.42) N = 1,753	1.24 (1.04, 1.49) N = 55,564	1.05 (0.86, 1.29) N = 73,637	**1.15 (1.01, 1.31)** **N = 137,406**
	0.77 (0.23, 1.99) N = 6,452	1.46 (0.39, 5.42) N = 1,753	1.24 (1.04, 1.49) N = 55,564	1.05 (0.86, 1.29) N = 73,637	**1.15 (1.01, 1.31)** **N = 137,406**
Maternal obesity unadjusted	1.99 (0.95, 3.78) N = 10,970	1.05 (0.62, 1.74) N = 10,109	1.30 (1.06, 1.57) N = 85,350	1.07 (0.85, 1.35) N = 97,400	**1.21 (1.05, 1.39)** **N = 203,829**
	1.56 (0.46, 4.00) N = 6,452	0.84 (0.12, 3.93) N = 1,753	1.16 (0.88, 1.51) N = 55,564	1.10 (0.83, 1.44) N = 73,637	**1.14 (0.94, 1.37)** **N = 137,406**
Maternal obesity confounder adjusted	2.16 (0.93, 4.43) N = 9,179	1.20 (0.66, 2.11) N = 7,360	1.21 (0.97, 1.49) N = 79,288	1.09 (0.83, 1.43) N = 75,448	**1.19 (1.02, 1.40)** **N = 171,275**
	1.72 (0.50, 4.49) N = 6,452	0.67 (0.10, 3.33) N = 1,753	1.14 (0.86, 1.48) N = 55,564	1.09 (0.83, 1.44) N = 73,637	**1.12 (0.93, 1.36)** **N = 137,406**
Maternal obesity confounder and other parent BMI adjusted	1.88 (0.55, 4.93) N = 6,452	0.70 (0.09, 3.44) N = 1,753	1.10 (0.83, 1.43) N = 55,564	1.12 (0.85, 1.49) N = 73,637	**1.12 (0.93, 1.36)** **N = 137,406**
	1.88 (0.55, 4.93) N = 6,452	0.70 (0.09, 3.44) N = 1,753	1.10 (0.83, 1.43) N = 55,564	1.12 (0.85, 1.49) N = 73,637	**1.12 (0.93, 1.36)** **N = 137,406**
Paternal underweight unadjusted	NA	NA	0.59 (0.10, 1.84) N = 62,637	1.97 (0.73, 5.31) N = 96,841	**1.31 (0.58, 2.95)** **N = 159,478**
	NA	NA	0.38 (0.02, 1.71) N = 53,922	0.81 (0.11, 5.80) N = 67,071	**0.56 (0.14, 2.24)** **N = 120,993**
Paternal underweight confounder adjusted	NA	NA	0.36 (0.02, 1.63) N = 54,710	0.82 (0.11, 5.87) N = 68,623	**0.54 (0.13, 2.19)** **N = 123,333**
	NA	NA	0.37 (0.02, 1.67) N = 53,922	0.85 (0.12, 6.09) N = 67,071	**0.56 (0.14, 2.26)** **N = 120,993**
Paternal underweight confounder and other parent BMI adjusted	NA	NA	0.36 (0.02, 1.65) N = 53,922	0.85 (0.12, 6.08) N = 67,071	**0.55 (0.14, 2.24)** **N = 120,993**
	NA	NA	0.36 (0.02, 1.64)	0.85 (0.12, 6.08)	**0.55 (0.14, 2.24)**

			N = 53,922	N = 67,071	**N = 120,993**
Paternal overweight unadjusted	0.90 (0.53, 1.49) N – 8,076	0.60 (0.18, 1.88) N = 2,725	1.10 (0.95, 1.27) N = 62,637	1.02 (0.88, 1.18) N = 96,841	**1.05 (0.95, 1.16)** **N = 159,478**
	0.73 (0.32, 1.54) N = 5,044	0.53 (0.11, 2.17) N = 1,572	1.18 (1.01, 1.38) N = 53,922	1.03 (0.86, 1.23) N = 67,071	**1.10 (0.98, 1.23)** **N = 127,609**
Paternal overweight confounder adjusted	1.07 (0.37, 3.20) N = 5,550	0.67 (0.17, 2.39) N = 2,085	1.20 (0.95, 1.53) N = 54,710	1.08 (0.90, 1.28) N = 68,623	**1.11 (0.97, 1.28)** **N = 130,968**
	1.11 (0.33, 3.78) N = 5,044	0.66 (0.13, 2.76) N = 1,572	1.22 (0.97, 1.56) N = 53,922	1.05 (0.88, 1.25) N = 67,071	**1.10 (0.96, 1.27)** **N = 127,609**
Paternal overweight confounder and other parent BMI adjusted	1.10 (0.33, 3.73) N = 5,044	0.67 (0.13, 2.82) N = 1,572	1.22 (0.96, 1.56) N = 53,922	1.05 (0.88, 1.26) N = 67,071	**1.10 (0.96, 1.27)** **N = 127,609**
	1.10 (0.33, 3.73) N = 5,044	0.67 (0.13, 2.82) N = 1,572	1.22 (0.96, 1.56) N = 53,922	1.05 (0.88, 1.26) N = 67,071	**1.10 (0.96, 1.27)** **N = 127,609**
Paternal obesity unadjusted	1.33 (0.54, 2.81) N – 8,076	1.65 (0.56, 4.83) N = 2,725	1.31 (1.00, 1.67) N = 62,637	1.00 (0.79, 1.37) N = 96,841	**1.15 (0.97, 1.37)** **N = 159,478**
	1.12 (0.26, 3.31) N = 5,044	1.40 (0.34, 5.31) N = 1,572	1.35 (1.01, 1.76) N = 53,922	0.95 (0.71, 1.25) N = 67,071	**1.15 (0.95, 1.40)** **N = 127,609**
Paternal obesity confounder adjusted	2.03 (0.19, 18.64) N = 5,550	1.79 (0.50, 6.16) N = 2,085	1.48 (0.89, 2.48) N = 54,710	1.02 (0.76, 1.37) N = 68,623	**1.15 (0.90, 1.47)** **N = 130,968**
	2.96 (0.24, 33.50) N = 5,044	1.93 (0.46, 7.70) N = 1,572	1.47 (0.88, 2.49) N = 53,922	1.02 (0.76, 1.37) N = 67,071	**1.15 (0.89, 1.48)** **N = 127,609**
Paternal obesity confounder and other parent BMI adjusted	2.99 (0.25, 33.86) N = 5,044	1.96 (0.47, 7.78) N = 1,572	1.46 (0.87, 2.46) N = 53,922	1.03 (0.76, 1.39) N = 67,071	**1.16 (0.90, 1.50)** **N = 127,609**
	2.99 (0.25, 33.86) N = 5,044	1.96 (0.47, 7.78) N = 1,572	1.46 (0.87, 2.46) N = 53,922	1.03 (0.76, 1.39) N = 67,071	**1.16 (0.90, 1.50)** **N = 127,609**

Covariates used for each study in fully adjusted models (mutually adjusted models the same as fully adjusted but with additional adjustment for the other parent's BMI and parity in paternal models);
ABCD: Maternal: offspring sex, age, education, parity, ethnicity, smoking, alcohol. Paternal: offspring sex, age, education, ethnicity.
ALSPAC: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking, alcohol.
BASELINE: Maternal: offspring sex, age, education, smoking, alcohol. Paternal: offspring sex, age, smoking.
BiB: Maternal: offspring sex, age, education, parity, ethnicity, smoking. Paternal: offspring sex, age, education, ethnicity, smoking.
DNBC: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking.
MoBa: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education, smoking, alcohol.
NINFEA: Maternal: offspring sex, age, education, parity, smoking, alcohol. Paternal: offspring sex, age, education.

Table S3.7. Comparison **between maximal numbers (black, top rows)** and **complete case models (red, bottom rows)**. Results are odds ratios (95% CIs) of any offspring CHD for smoking during pregnancy.

Model	*ABCD*	*ALSPAC*	*BiB*	*DNBC*	*MoBa*	*NINFEA*	*Meta-analysis results*
Maternal smoking unadjusted	2.06 (0.77, 4.65) N = 8,117	1.23 (0.78, 1.87) N = 12,716	0.89 (0.53, 1.42) N = 10,887	1.11 (0.98, 1.26) N = 86,740	1.07 (0.86, 1.34) N = 101,042	0.77 (0.18, 3.21) N = 5,801	**1.11 (1.00, 1.23)** **N = 225,303**
	2.04 (0.76, 4.62) N = 7,824	1.40 (0.71, 2.56) N = 7,626	1.62 (0.52, 4.20) N = 2,624	1.10 (0.96, 1.26) N = 78,229	1.03 (0.78, 1.37) N = 77,266	0.79 (0.19, 3.29) N = 5,527	**1.11 (0.99, 1.25)** **N = 179,096**
Maternal smoking confounder adjusted	2.02 (0.73, (4.77) N = 7,824	1.22 (0.69, 2.06) N = 10,217	0.93 (0.50, 1.60) N = 9,646	1.05 (0.91, 1.20) N = 80,571	1.02 (0.77, 1.36) N = 77,311	0.92 (0.22, 3.96) N = 5,527	**1.06 (0.94, 1.18)** **N = 191,096**
	2.02 (0.73, (4.77)	1.31 (0.65, 2.46)	2.09 (0.64, 5.84)	1.07 (0.93, 1.23)	1.02 (0.77, 1.37)	0.92 (0.22, 3.96)	**1.09 (0.97, 1.23)**

	N = 7,824	N = 7,626	N = 2,624	N = 78,229	N = 77,266	N = 5,527	**N = 179,096**
Maternal smoking confounder and other parent smoking adjusted	-	1.27 (0.61, 2.50) N = 7,626	1.77 (0.51, 5.36) N = 2,624	1.11 (0.96, 1.28) N = 79,000	1.05 (0.78, 1.41) N = 77,266	-	**1.11 (0.97, 1.25)** **N = 166,516**
	-	1.27 (0.61, 2.50) N = 7,626	1.77 (0.51, 5.36) N = 2,624	1.13 (0.98, 1.30) N = 78,229	1.05 (0.78, 1.41) N = 77,266	-	**1.12 (0.99, 1.28)** **N = 165,745**
Paternal smoking unadjusted	-	1.29 (0.79, 2.10) N = 9,134	1.20 (0.50, 2.66) N = 3,187	0.95 (0.84, 1.08) N = 84,926	0.96 (0.82, 1.11) N = 101,804	-	**0.97 (0.88, 1.06)** **N = 198,421**
	-	1.28 (0.68, 2.35) N = 6,182	1.46 (0.54, 3.73) N = 2,373	0.96 (0.84, 1.10) N = 77,477	1.00 (0.83, 1.20) N = 70,018	-	**0.99 (0.89, 1.10)** **N = 156,050**
Paternal smoking confounder adjusted	-	1.17 (0.61, 2.19) N = 6,308	1.43 (0.51, 3.76) N = 2,424	0.95 (0.83, 1.08) N = 77,526	1.05 (0.87, 1.26) N = 70,766	-	**0.99 (0.89, 1.10)** **N = 157,024**
	-	1.23 (0.64, 2.30) N = 6,182	1.51 (0.53, 4.06) N = 2,373	0.95 (0.83, 1.08) N = 77,477	1.05 (0.87, 1.27) N = 70,018	-	**0.99 (0.89, 1.10)** **N = 156,050**
Paternal smoking confounder and other parent BMI adjusted	-	1.14 (0.56, 2.23) N = 6,182	1.18 (0.38, 3.41) N = 2,373	0.90 (0.79, 1.04) N = 77,499	1.04 (0.85, 1.26) N = 70,018	-	**0.96 (0.85, 1.07)** **N = 156,072**
	-	1.14 (0.64, 2.30) N = 6,182	1.18 (0.38, 3.41) N = 2,373	0.91 (0.79, 1.04) N = 77,477	1.04 (0.85, 1.26) N = 70,018	-	**0.96 (0.86, 1.07)** **N = 156,050**

Covariates used for each study in fully adjusted models (mutually adjusted models the same as fully adjusted but with additional adjustment for the other parent's smoking);
ABCD: Maternal: offspring sex, age, education, parity, ethnicity, alcohol.
ALSPAC: Maternal: offspring sex, age, education, parity, alcohol. Paternal: offspring sex, age, education, alcohol.
BiB: Maternal: offspring sex, age, education, parity, ethnicity. Paternal: offspring sex, age, education, ethnicity.
DNBC: Maternal: offspring sex, age, education, parity, alcohol. Paternal: offspring sex, age, education.
MoBa: Maternal: offspring sex, age, education, parity, alcohol. Paternal: offspring sex, age, education, alcohol.
NINFEA: Maternal: offspring sex, age, education, parity, alcohol.

Table S3.8. Comparison between **maximal numbers (black, top rows)** and **complete case models (red, bottom rows)**. Results are odds ratios (95% CIs) of any offspring CHD for alcohol intake during pregnancy in comparison to non-drinkers.

Model	*ABCD*	*ALSPAC*	*DNBC*	*MoBa*	*NINFEA*	*Meta-analysis results*
Maternal alcohol (yes/no) unadjusted	1.38 (0.61, 2.85) N = 8,125	1.20 (0.81, 1.80) N = 12,622	1.00 (0.89, 1.12) N = 86,708	1.04 (0.88, 1.23) N = 82,358	1.20 (0.57, 2.51) N = 5,843	**1.03 (0.94, 1.12)** **N = 195,656**
	1.36 (0.60, 2.81) N = 7,824	1.18 (0.56, 2.55) N = 4,585	1.00 (0.89, 1.13) N = 79,648	1.06 (0.86, 1.31) N = 51,006	1.19 (0.57, 2.49) N = 5,527	**1.03 (0.93, 1.14)** **N = 148,590**
Maternal alcohol (yes/no) confounder adjusted	1.17 (0.50, 2.56) N = 7,824	1.24 (0.78, 2.01) N = 10,217	1.01 (0.90, 1.14) N = 80,571	1.03 (0.86, 1.23) N = 77,311	1.18 (0.56, 2.49) N = 5,527	**1.03 (0.94, 1.13)** **N = 181,450**
	1.17 (0.50, 2.56) N = 7,824	1.20 (0.56, 2.63) N = 4,585	1.01 (0.89, 1.14) N = 79,648	1.06 (0.85, 1.31) N = 51,066	1.18 (0.56, 2.49) N = 5,527	**1.03 (0.93, 1.14)** **N = 148,590**
Maternal light drinking unadjusted	-	0.93 (0.52, 1.67) N = 6,501	0.92 (0.82, 1.03) N = 88,349	1.10 (0.88, 1.36) N = 84,436	-	**0.96 (0.87, 1.06)** **N = 179,286**
	-	1.27 (0.58, 2.93) N = 4,585	0.93 (0.82, 1.05) N = 79,648	1.24 (0.94, 1.63) N = 51,006	-	**0.98 (0.88, 1.09)** **N = 135,239**
Maternal light drinking confounder adjusted	-	0.92 (0.48, 1.78) N = 5,797	0.95 (0.85, 1.08) N = 80,214	1.13 (0.90, 1.41) N = 79,695	-	**0.99 (0.89, 1.10)** **N = 165,706**
	-	1.35 (0.61, 3.14) N = 4,585	0.94 (0.83, 1.06) N = 79,648	1.22 (0.92, 1.61) N = 51,006	-	**0.99 (0.88, 1.10)** **N = 135,239**

Maternal light drinking confounder and other parent alcohol adjusted	-	1.40 (0.62, 3.27) N = 4,585	-	1.13 (0.87, 1.47) N = 59,571	-	**1.15 (0.90, 1.48) N = 64,156**
	-	1.40 (0.62, 3.27) N = 4,585	-	1.19 (0.90, 1.57) N = 51,006	-	**1.21 (0.93, 1.57) N = 55,591**
Maternal mod/heavy drinking unadjusted	-	0.67 (0.22, 1.65) N = 6,501	1.14 (0.87, 1.48) N = 88,349	1.85 (0.92, 3.73) N = 84,436	-	**1.17 (0.92, 1.49) N = 179,286**
	-	0.92 (0.21, 3.01) N = 4,585	1.19 (0.89, 1.56) N = 79,648	1.77 (0.66, 4.78) N = 51,006	-	**1.21 (0.93, 1.58) N = 135,239**
Maternal mod/heavy drinking confounder adjusted	-	0.64 (0.18, 1.75) N = 5,797	1.21 (0.90, 1.58) N = 80,214	1.47 (0.65, 3.32) N = 79,695	-	**1.19 (0.92, 1.54) N = 165,706**
	-	0.89 (0.20, 2.98) N = 4,585	1.19 (0.89, 1.57) N = 79,648	1.73 (0.64, 4.69) N = 51,006	-	**1.21 (0.93, 1.58) N = 135,239**
Maternal mod/heavy drinking confounder and other parent alcohol adjusted	-	0.94 (2.06, 3.19) N = 4,585	-	1.31 (0.48, 3.56) N = 59,571	-	**1.16 (0.52, 2.58) N = 64,156**
	-	0.94 (2.06, 3.19) N = 4,585	-	1.57 (0.58, 4.27) N = 51,006	-	**1.30 (0.59, 2.89) N = 55,591**
Paternal light drinking unadjusted	-	0.90 (0.36, 3.02) N = 8,205	-	0.90 (0.61, 1.32) N = 72,422	-	**0.90 (0.63, 1.29) N = 80,627**
	-	1.90 (0.39, 34.09) N = 5,228	-	1.01 (0.62, 1.65) N = 58,847	-	**1.05 (0.65, 1.68) N = 64,075**
Paternal light drinking confounder adjusted	-	2.11 (0.44, 37.99) N = 5,346	-	0.86 (0.58, 1.28) N = 70,766	-	**0.89 (0.60, 1.31) N = 76,112**
	-	2.04 (0.42, 36.80) N = 5,228	-	0.97 (0.60, 1.58) N = 58,847	-	**1.01 (0.63, 1.63) N = 64,075**
Paternal light drinking confounder and other parent alcohol adjusted		1.77 (0.36, 32.20) N = 5,316	-	0.97 (0.63, 1.62) N = 58,847		**1.01 (0.63, 1.62) N = 64,163**
	-	1.74 (0.35, 31.60) N = 5,228	-	0.97 (0.60, 1.59) N = 58,847	-	**1.01 (0.62, 1.62) N = 64,075**
Paternal mod/heavy drinking unadjusted	-	0.86 (0.34, 2.93) N = 8,205	-	1.11 (0.73, 1.70) N = 72,422	-	**1.08 (0.73, 1.59) N = 80,627**
	-	1.83 (0.37, 33.05) N = 5,228	-	1.28 (0.76, 2,17) N = 58,847	-	**1.31 (0.79, 2.18) N = 64,075**
Paternal mod/heavy drinking confounder adjusted	-	2.00 (0.40, 36.05) N = 5,346	-	1.07 (0.69, 1.66) N = 70,766	-	**1.10 (0.72, 1.69) N = 76,112**
	-	1.94 (0.39, 35.05) N = 5,228	-	1.20 (0.71, 2.04) N = 58,847	-	**1.24 (0.74, 2.07) N = 64,075**
Paternal mod/heavy drinking confounder and other parent alcohol adjusted	-	1.72 (0.34, 31.20) N = 5,316	-	1.21 (0.71, 2.05) N = 58,847	-	**1.23 (0.74, 2.06) N = 64,163**
	-	1.70 (0.34, 30.83) N = 5,228	-	1.21 (0.71, 2.05) N = 58,847	-	**1.23 (0.74, 2.06) N = 64,075**

Covariates used for each study in fully adjusted models (mutually adjusted models the same as fully adjusted but with additional adjustment for the other parent's alcohol intake);
ABCD: Maternal: offspring sex, age, education, parity, ethnicity, smoking.
ALSPAC: Maternal: offspring sex, age, education, parity, smoking. Paternal: offspring sex, age, education, smoking.
DNBC: Maternal: offspring sex, age, education, parity, smoking.
MoBa: Maternal: offspring sex, age, education, parity, smoking. Paternal: offspring sex, age, education, smoking.
NINFEA: Maternal: offspring sex, age, education, parity, ethnicity, smoking.

Table S3.9. Meta-analysis results from 4 cohorts (ALSPAC, BiB, DNBC, MoBa) for associations between BMI categories and CHDs with and without removing chromosomal/genetic defects from the study population. Results reported as odds ratios for CHD for parental underweight, overweight or obesity in comparison to parental normal weight.

Model	*Main analysis* *Outcome = CHD*	*Additional analysis* *Outcome = CHD with chromo/gen defects removed from study population*
Confounder adjusted	**M**-Underweight: 1.20 (0.96, 1.50) **P**-Underweight: 0.54 (0.13, 2.19)	**M**- Underweight: 1.16 (0.90, 1.48) **P**-Underweight: 0.67 (0.16, 2.70)
	M-Overweight: 1.17 (1.04, 1.31) **P**-Overweight: 1.11 (0.97, 1.28)	**M**-Overweight: 1.20 (1.06, 1.35) **P**-Overweight: 1.09 (0.94, 1.27)
	M-Obesity: 1.19 (1.02, 1.40) **P**-Obesity: 1.15 (0.90, 1.47)	**M**-Obesity: 1.21 (1.02, 1.44) **P**-Obesity: 1.19 (0.91, 1.58)
Confounder and other parent BMI adjusted	**M**-Underweight: 1.21 (0.93, 1.58) **P**-Underweight: 0.55 (0.14, 2.24)	**M**-Underweight: 1.22 (0.90, 1.63) **P**-Underweight: 0.67 (0.17, 2.72)
	M-Overweight: 1.15 (1.01, 1.31) **P**-Overweight: 1.10 (0.96, 1.27)	**M**-Overweight: 1.20 (1.04, 1.38) **P**-Overweight: 1.08 (0.93, 1.27)
	M-Obesity: 1.12 (0.93, 1.36) **P**-Obesity: 1.16 (0.90, 1.50)	**M**-Obesity: 1.15 (0.93, 1.42) **P**-Obesity: 1.20 (0.90, 1.59)

M = maternal
P = paternal
^ICD codes used to remove these cases from the population can be found in Table S3.3.

Table S3.10. Meta-analysis results from 3 cohorts (ALSPAC, BiB and DNBC) for associations between BMI categories and CHD severity with and without removing chromosomal/genetic defects from the study population. Results reported as odds ratios for CHD for parental underweight, overweight or obesity in comparison to parental normal weight.

Model	*Outcome = Non-severe CHD*	*Outcome = Non-severe CHD (excluding chromo/gen defects)^*	*Outcome = Severe CHD*	*Outcome = Severe CHD (excluding chromo/gen defects)^*
Confounder adjusted	**M**-Underweight: 1.24 (0.91, 1.68) **P**-Underweight: 0.49 (0.07, 3.56)	**M**- Underweight: 1.32 (0.95, 1.83) **P**-Underweight: 0.55 (0.07, 4.10)	**M**-Underweight: 1.25 (0.79, 1.97) **P**-Underweight: *	**M**- Underweight: 1.27 (0.75, 2.16) **P**-Underweight: *
	M-Overweight: 1.24 (1.05, 1.47) **P**-Overweight: 1.16 (0.89, 1.51)	**M**-Overweight: 1.27 (1.06, 1.52) **P**-Overweight: 1.09 (0.82, 1.45)	**M**-Overweight: 1.19 (0.93, 1.53) **P**-Overweight: 1.19 (0.77, 1.84)	**M**-Overweight: 1.29 (0.98, 1.70) **P**-Overweight: 1.09 (0.66, 1.79)
	M-Obesity: 1.36 (1.08, 1.71) **P**-Obesity: 1.49 (0.86, 2.59)	**M**-Obesity: 1.36 (1.06, 1.74) **P**-Obesity: 1.51 (0.83, 2.74)	**M**-Obesity: 1.07 (0.73, 1.56) **P**-Obesity: 1.65 (0.71, 3.87)	**M**-Obesity: 1.12 (0.74, 1.71) **P**-Obesity: 1.58 (0.60, 4.19)
Confounder and other parent BMI adjusted	**M**-Underweight: 1.34 (0.92, 1.93) **P**-Underweight: 0.48 (0.07, 3.54)	**M**-Underweight: 1.41 (0.95, 2.09) **P**-Underweight: 0.56 (0.08, 4.10)	**M**-Underweight: 1.16 (0.61, 2.22) **P**-Underweight: *	**M**-Underweight: 1.42 (0.69, 2.94) **P**-Underweight: *
	M-Overweight: 1.29 (1.05, 1.58) **P**-Overweight: 1.22 (0.93, 1.59)	**M**-Overweight: 1.33 (1.07, 1.66) **P**-Overweight: 1.13 (0.84, 1.51)	**M**-Overweight: 1.11 (0.78, 1.57) **P**-Overweight: 1.13 (0.72, 1.77)	**M**-Overweight: 1.39 (0.95, 2.02) **P**-Overweight: 0.97 (0.58, 1.62)
	M-Obesity: 1.14 (0.84, 1.55) **P**-Obesity: 1.61 (0.91, 2.84)	**M**-Obesity: 1.19 (0.85, 1.66) **P**-Obesity: 1.57 (0.84, 2.91)	**M**-Obesity: 1.17 (0.71, 1.93) **P**-Obesity: 1.36 (0.56, 3.32)	**M**-Obesity: 1.31 (0.74, 2.32) **P**-Obesity: 1.17 (0.42, 3.28)

M = maternal
P = paternal
^ICD codes used to remove these cases from the population can be found in Table S3.3.
*** =** not enough data to compute results.

Table S3.11. Meta-analysis results for associations between alcohol intake and CHDs after removing chromosomal/genetic defects from the study population. Results reported as odds ratios and 95% confidence intervals for CHD in comparison to non-drinkers.

Model	***Main analysis*** ***Outcome = CHD***	***Additional analysis*** ***Outcome = CHD with chromo/gen removed from study population***
Confounder adjusted	**M** – y/n: 1.03 (0.93, 1.13)	**M** – y/n: 1.04 (0.94, 1.15)
	M – light: 0.99 (0.89, 1.10) **P** – light: 0.89 (0.60, 1.31)	**M** – light: 0.95 (0.85, 1.07) **P** – light: 1.13 (0.69, 1.87)
	M – mod/heavy: 1.19 (0.92, 1.54) **P** – mod/heavy: 1.10 (0.72, 1.69)	**M** – mod/heavy: 1.24 (0.94, 1.63) **P** – mod/heavy: 1.36 (0.79, 2.34)
Confounder and other parent BMI adjusted	**M** – light: 1.15 (0.90, 1.48) **P** – light: 1.01 (0.63, 1.62)	**M** – 1.17 (0.88, 1.55) **P** – light: 1.21 (0.68, 2.16)
	M – mod/heavy: 1.16 (0.52, 2.58) **P** – mod/heavy: 1.23 (0.74, 2.06)	**M** – mod/heavy: 1.20 (0.52, 3.17) **P** – mod/heavy: 1.52 (0.82, 2.80)

M = maternal
P = paternal
y/n = alcohol as a binary variable, yes or no.
Estimates from yes/no analyses derived from 5 cohorts (ABCD, ALSPAC, DNBC, MoBa, NINFEA).
Estimates from maternal light and mod/heavy drinking analyses derived from ALSPAC, DNBC and MoBa in fully adjusted results, but only ALSPAC and MoBa in paternal and mutually adjusted results.

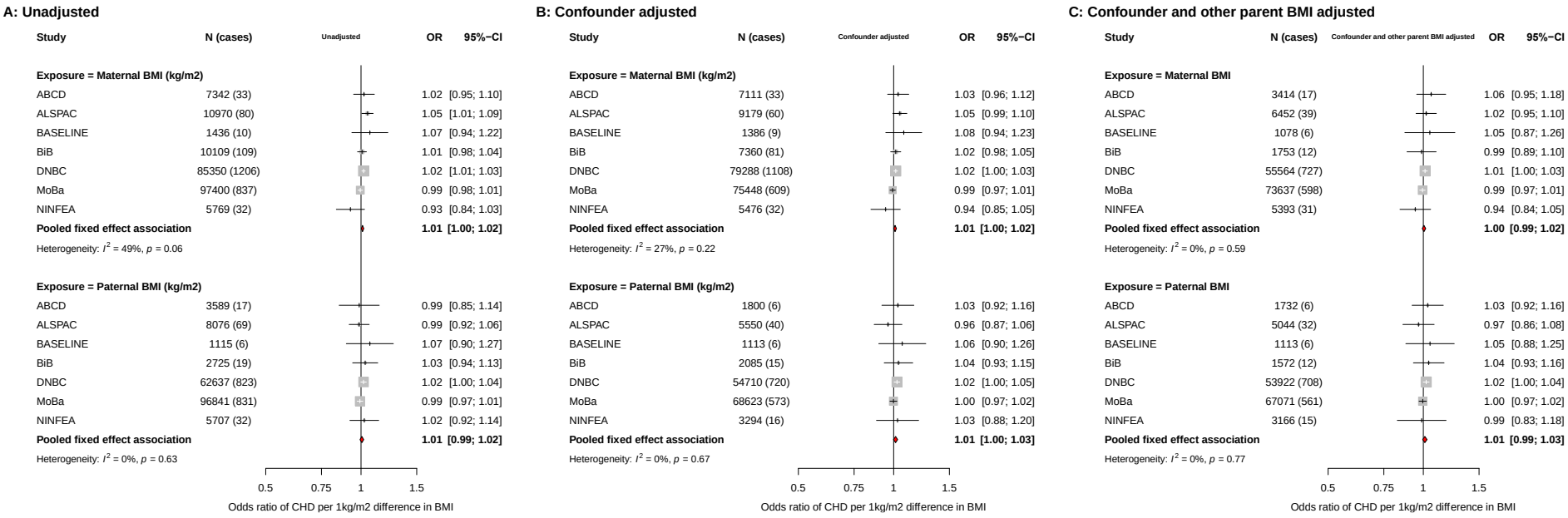


Figure S3.8. Main analysis associations between parental BMI as a continuous measurement in kg/m^2 (maternal top, paternal bottom) and offspring congenital heart disease. Panel A results are unadjusted, panel B results are fully adjusted for all confounders and panel C results are adjusted for all confounders as well as other parent's BMI. Confounders: ABCD: parental age, education, parity, ethnicity, smoking, alcohol, offspring sex; *ALSPAC:* parental age, education, parity, smoking, alcohol, offspring sex; *BASELINE:* parental age, education, smoking, alcohol, offspring sex *BiB*: parental age, education, parity, ethnicity, smoking, offspring sex; *DNBC:*, parental age, education, parity, smoking, alcohol, offspring sex; MoBa: parental age, education, parity, smoking, alcohol, offspring sex; NINFEA: parental age, education, parity, smoking, alcohol, offspring sex.

Figures S3.9 and S3.10 show the odds ratios of CHD by fifths of the BMI distribution for mothers and fathers respectively in DNBC and MoBa. Whilst there was statistical evidence for a linear trend in DNBC mothers (p-value for per fifth increase = 0.05) the graph shows this was driven by increased risk only in the highest fifth, with the 2nd, 3rd and 4th fifth (compared to the first) consistent with the null. In MoBa mothers there was no clear pattern with some evidence that the 4th compared to the 1st fifth was associated with lower risk with the 3 other categories being consistent with the null (p-value for linear trend in MoBa = 0.22). Whilst the p-values for the likelihood ratio comparing the linear model with the category model (0.03 and 0.09, for DNBC and MoBa mothers, respectfully) provide statistical support for the category model in each, this is based on just one of the fifths. Results for the fathers are broadly consistent with those for the mothers, and overall, these results are consistent with no association of maternal or paternal mean BMI with offspring CHD risk.

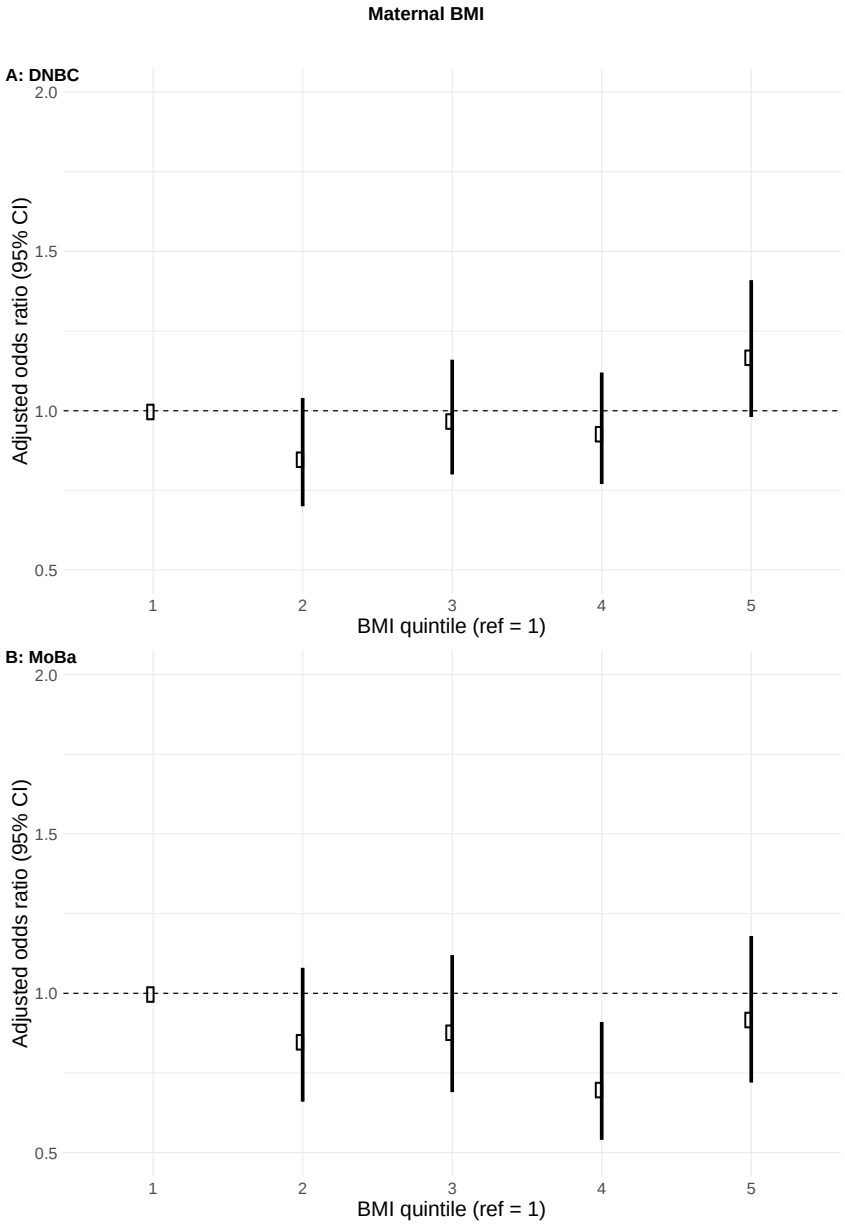


Figure S3.9. Confounder adjusted associations between maternal BMI split into fifths and offspring CHDs in the DNBC (A) and MoBa (B). Results are odds ratios and 95% CIs for maternal BMI quintile and offspring CHD in comparison to BMI quintile 1.

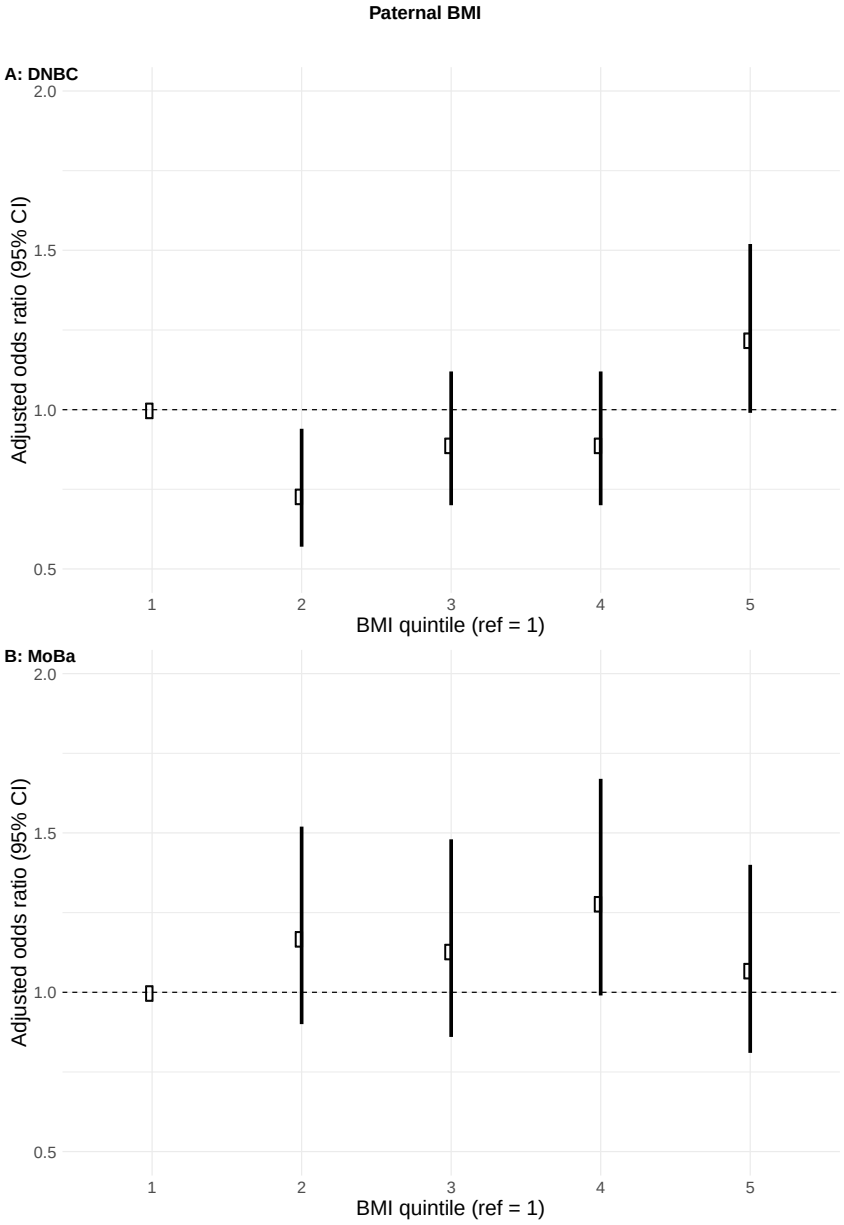
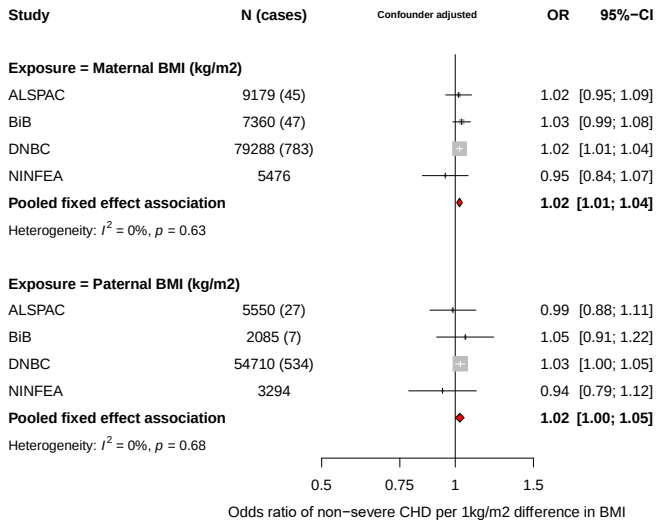
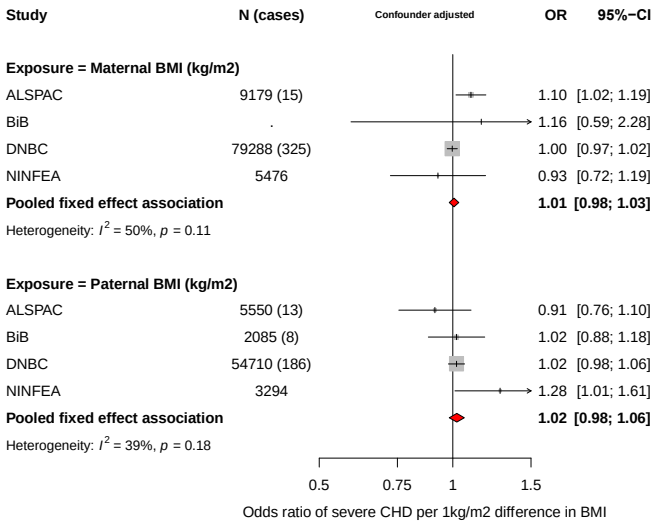


Figure S3.10. Confounder adjusted associations between paternal BMI split into fifths and offspring CHDs in the DNBC (A) and MoBa (B). Results are odds ratios and 95% CIs for paternal BMI quintile and offspring CHD in comparison to BMI quintile 1.

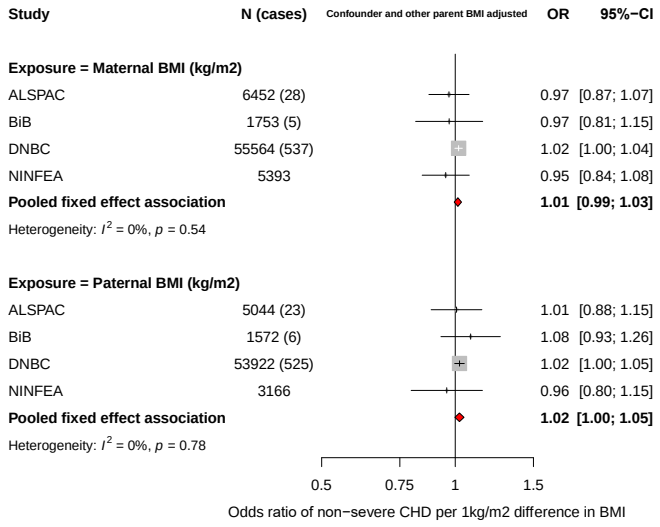
A: Non−severe CHD confounder adjusted



B: Severe CHD confounder adjusted



C: Non−severe CHD confounder and other parent BMI adjusted



D: Severe CHD confounder and other parent BMI adjusted

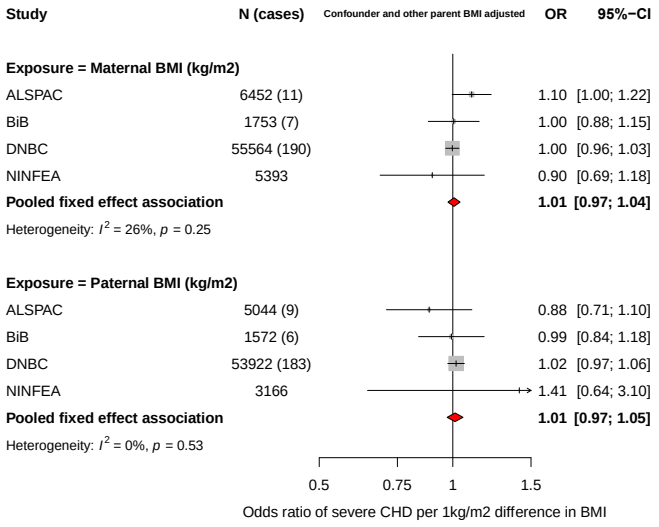


Figure S3.11. Linear associations (top (A&B): confounder adjusted, bottom (C&D): confounder and other parent BMI adjusted) between parental BMI and offspring non-severe congenital heart disease (left) and severe congenital heart disease (right). Definitions for CHD subtypes can be found in Table S3.2.

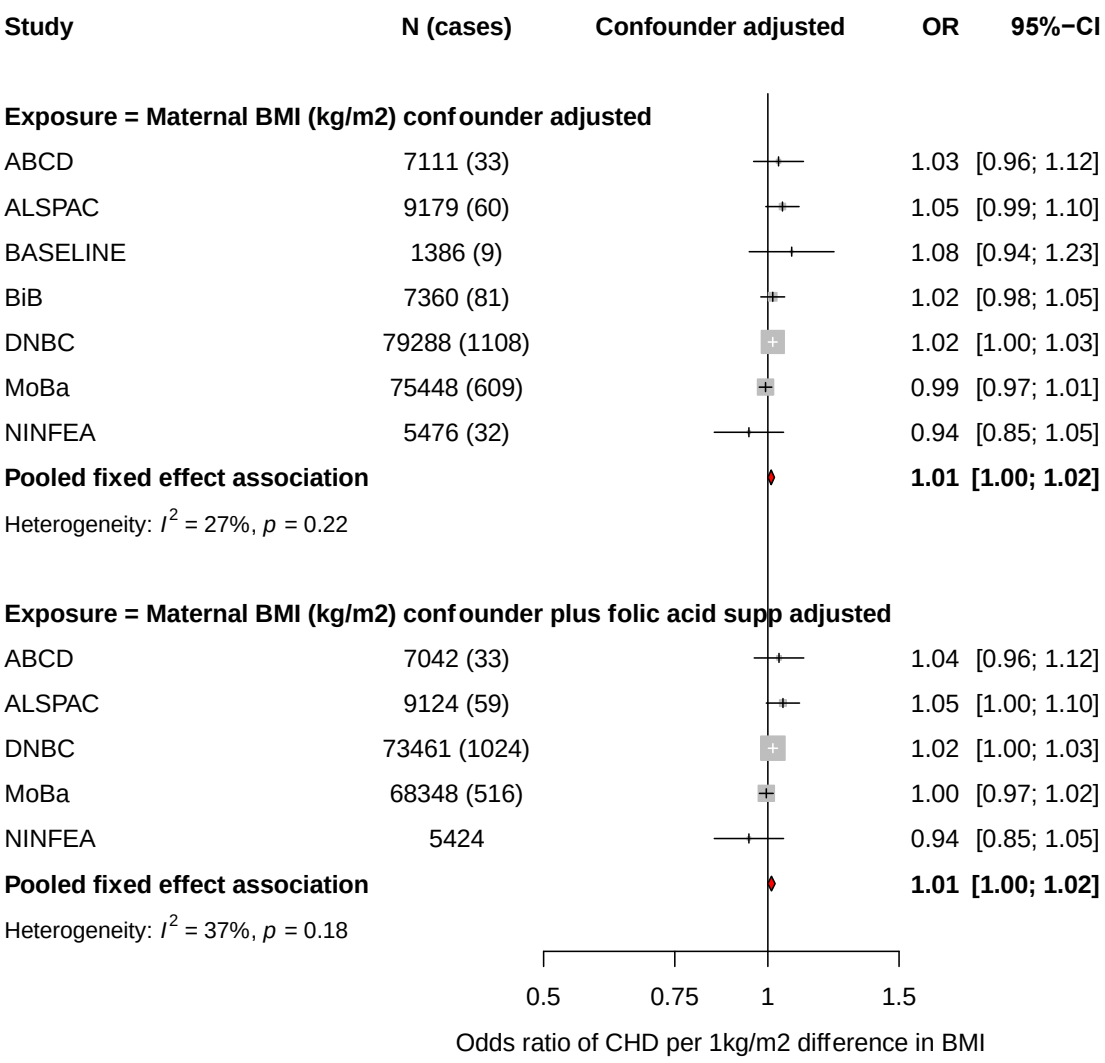


Figure S3.12. Linear associations between maternal BMI and offspring congenital heart disease. Results are fully adjusted for all confounders (top) and all confounders plus additional adjustment for folic acid supplementation during weeks 0-12 of pregnancy (bottom).

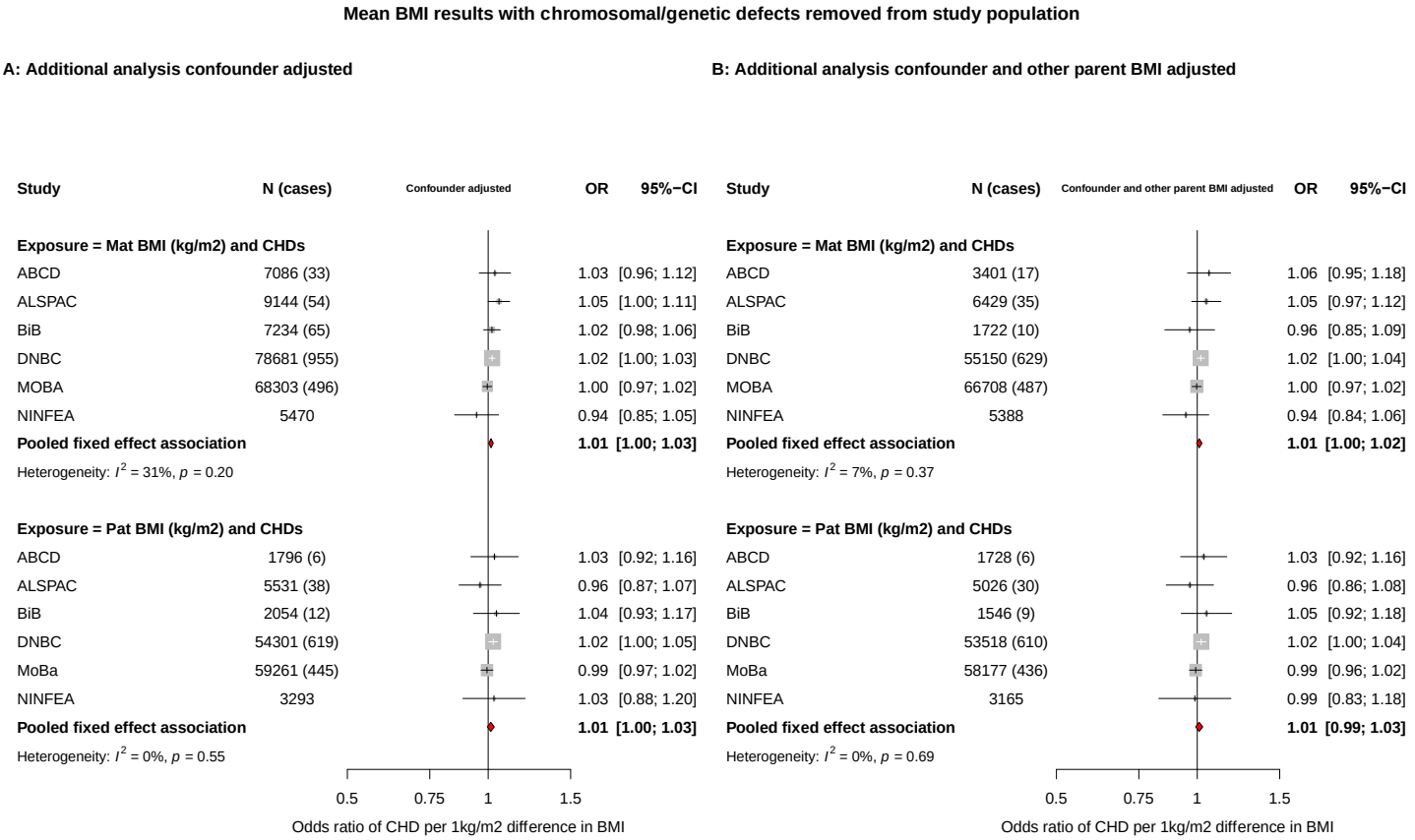


Figure S3.13. Additional analysis: linear associations between parental BMI and offspring congenital heart disease with chromosomal/genetic defects removed from the study population. **A** is adjusted for all confounders, and **B** is adjusted for all confounders and the other parent's BMI. The rationale here is to see if estimates differ when I remove offspring from the population with an anomaly associated with a pre-specified cause such as a genetic, chromosomal or teratogenic aberration. ICD codes used to remove these cases from the population can be found in Table S3.3. For comparison the pooled associations from main analyses (without removal of genetic/chromo disorders) were: 1.01 (1.00, 1.02) & 1.01 (0.99, 1.02) for maternal (top graphs, left and right respectively) and 1.01 (1.00, 1.03) & 1.01 (0.99, 1.03) for paternal (bottom graphs left and right respectively).

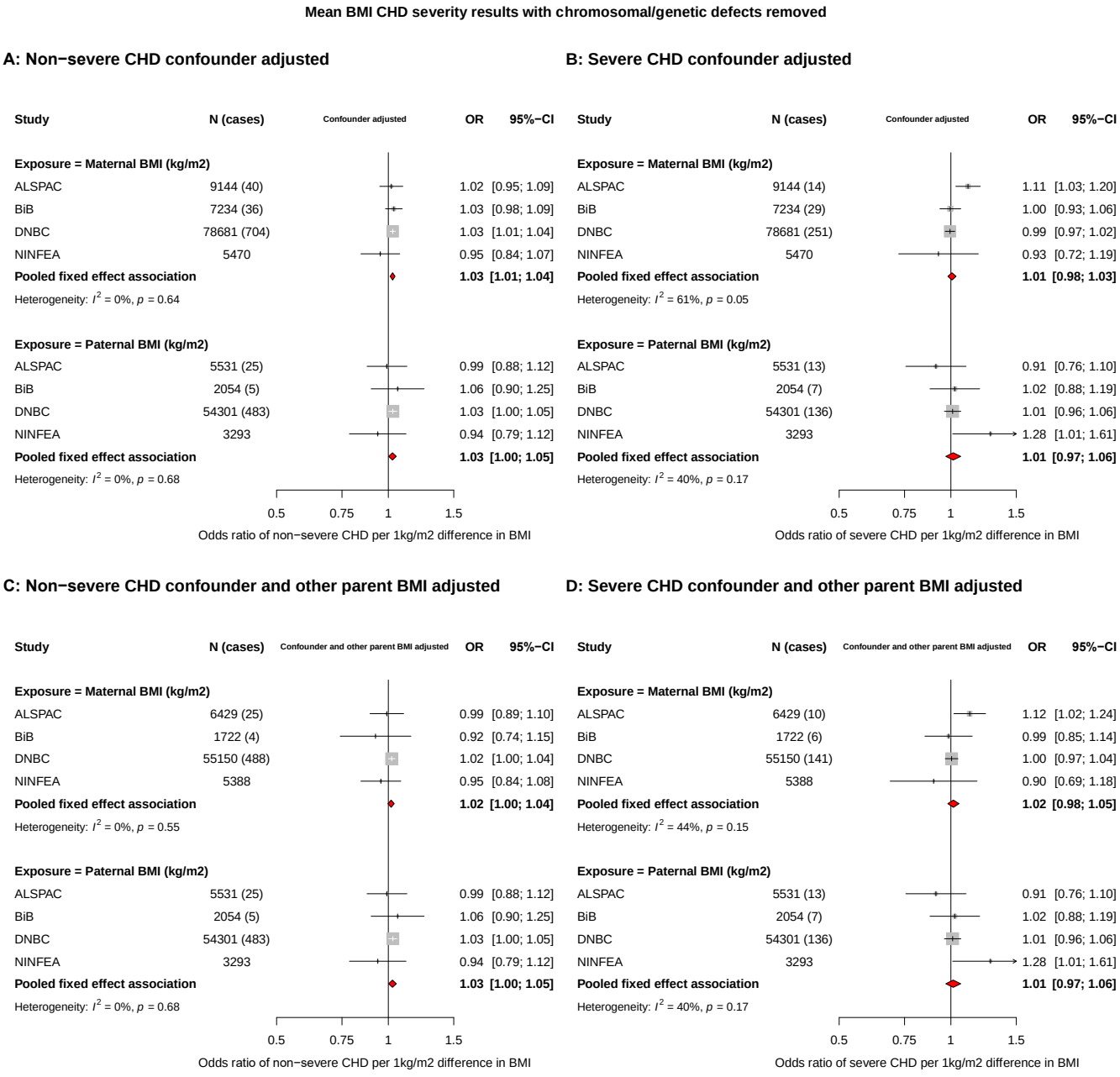
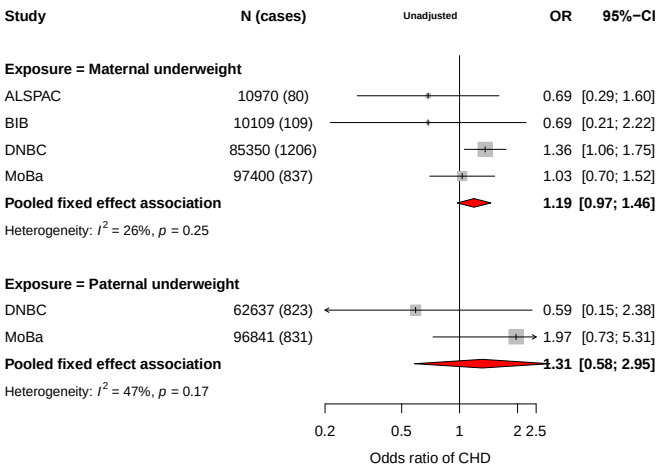
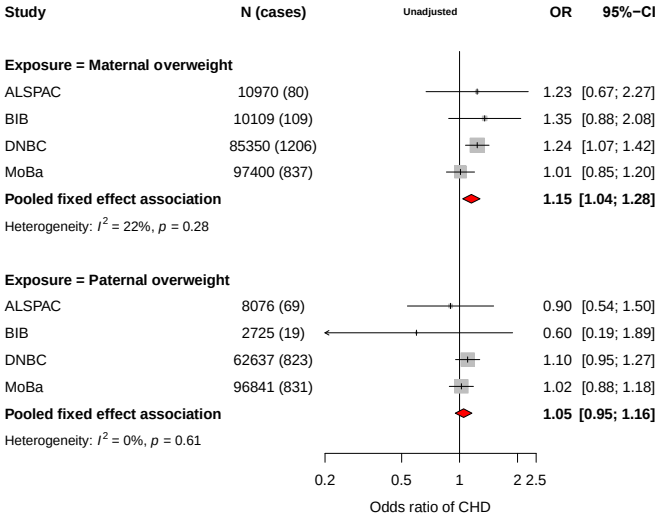


Figure S3.14. Linear associations (top (A&B): confounder adjusted, bottom (C&D): confounder and other parent BMI adjusted) between parental BMI and offspring non-severe congenital heart disease (left) and severe congenital heart disease (right) with cases of chromosomal/genetic defects removed from the study population. Definitions for CHD subtypes can be found in Table S3.2.

A: Underweight



B: Overweight



C: Obesity

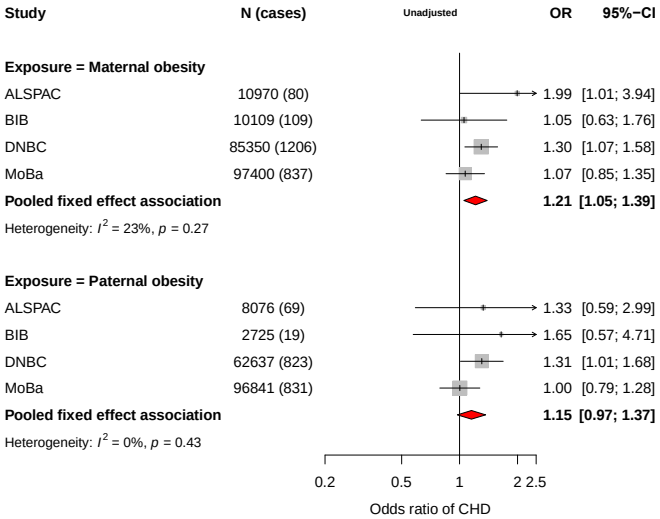
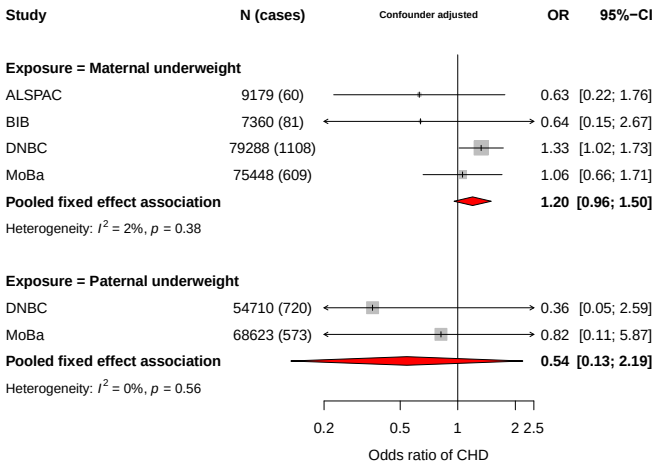
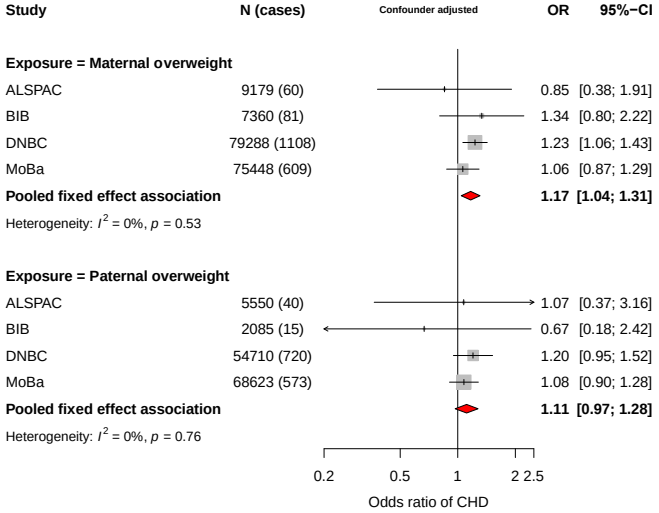


Figure S3.15. Meta-analysis results for unadjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = any CHD in the offspring.

A: Underweight



B: Overweight



C: Obesity

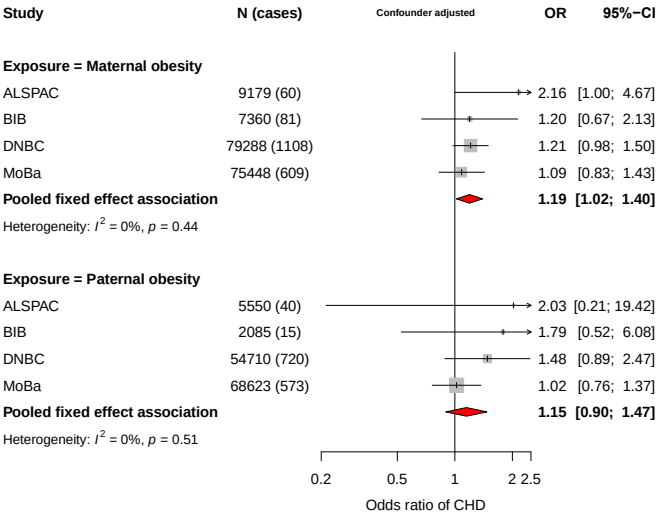
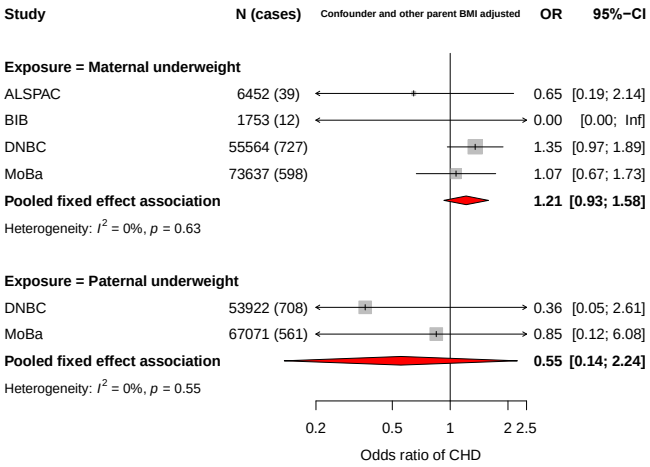
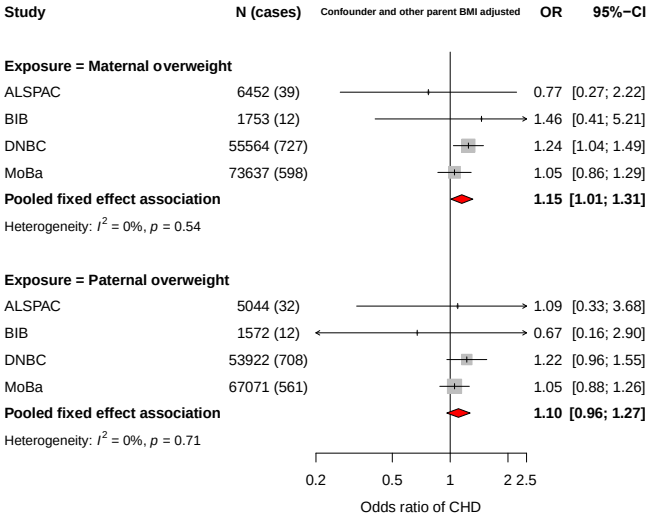


Figure S3.16. Meta-analysis results for confounder adjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = any CHD in the offspring.

A: Underweight



B: Overweight



C: Obesity

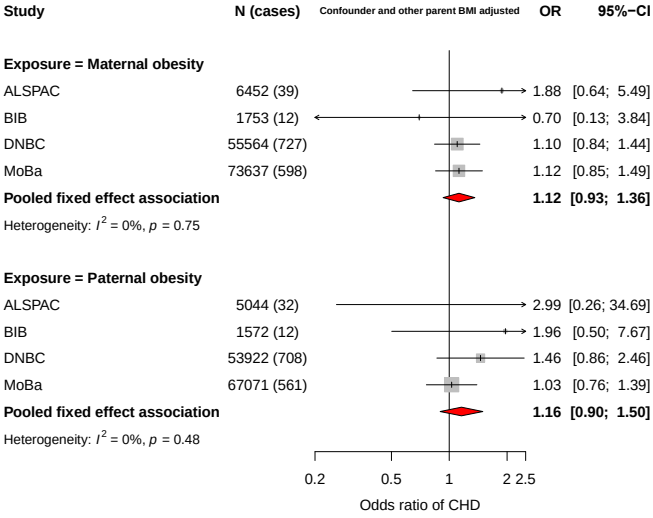


Figure S3.17. Meta-analysis results for confounder and other parent BMI adjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = any CHD in the offspring.

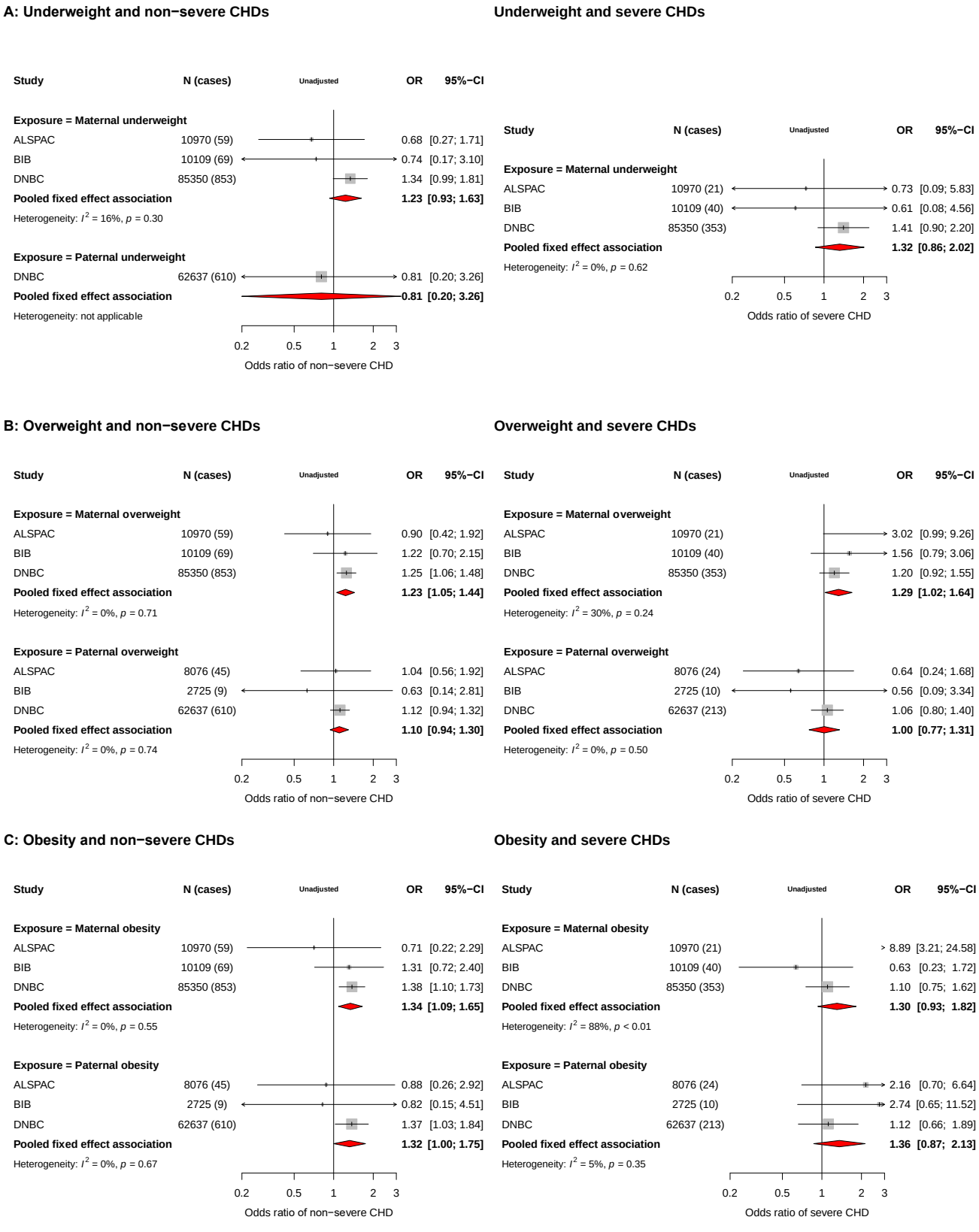


Figure S3.18. Meta-analysis results for unadjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = non-severe CHDs (left) and severe CHDs (right). Ns represent total numbers included in the non-severe/severe analyses presented.

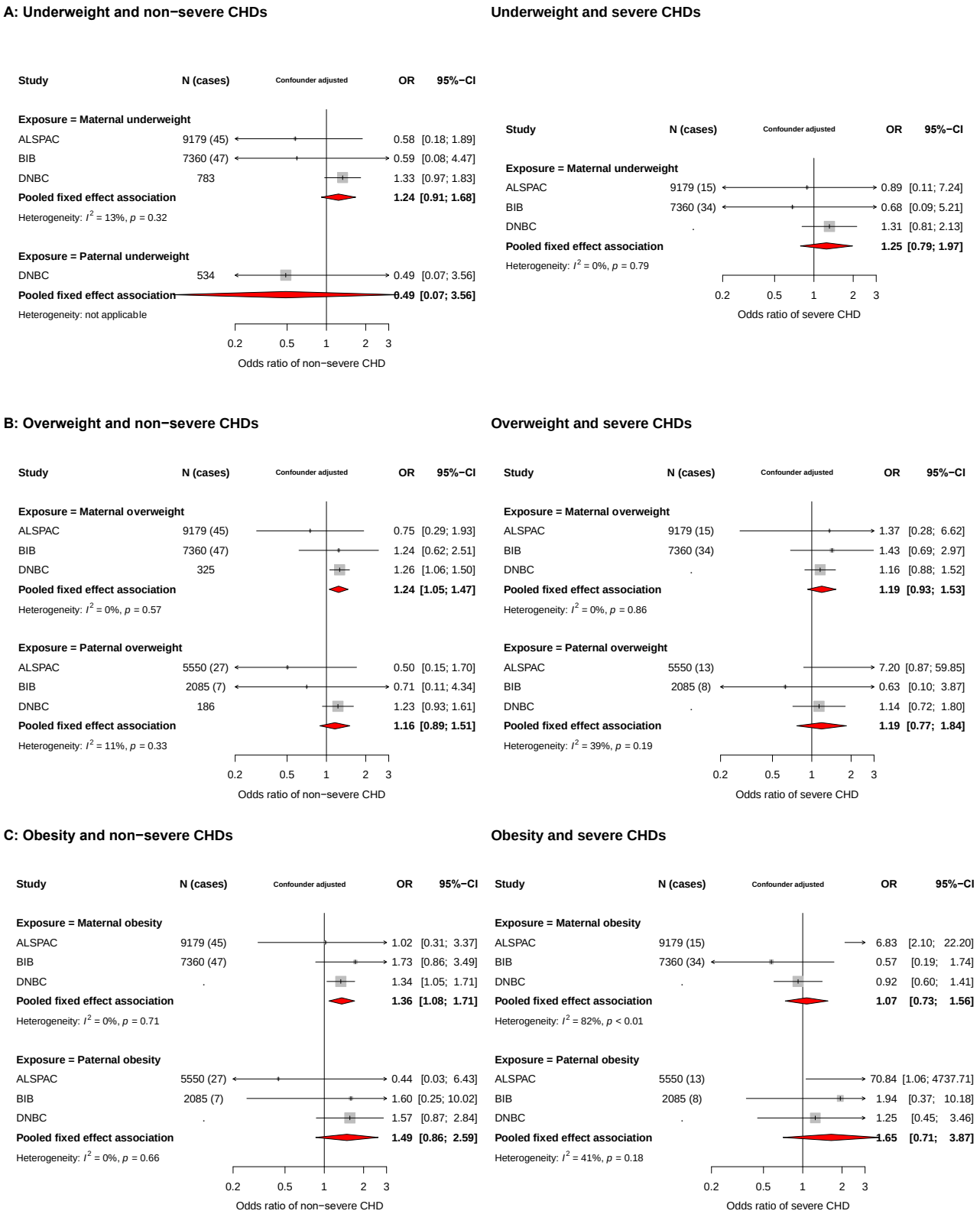


Figure S3.19. Meta-analysis results for confounder adjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = non-severe CHDs (left) and severe CHDs (right). Ns represent total numbers included in the non-severe/severe analyses presented.

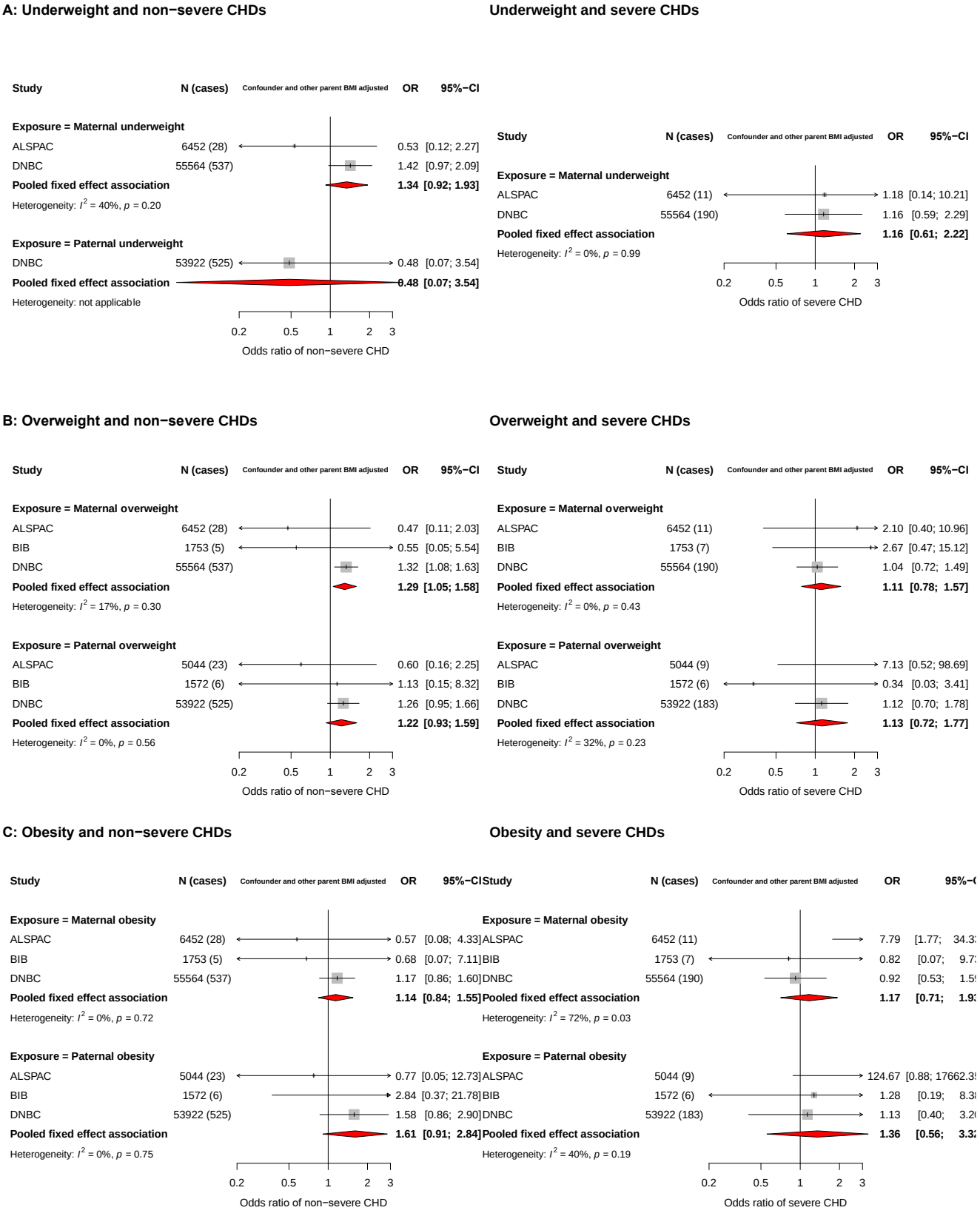


Figure S3.20. Meta-analysis results for confounder and other parent BMI adjusted BMI categories using World Health Organization cut-offs with normal BMI as the reference. Outcome = non-severe CHDs (left) and severe CHDs (right). Ns represent total numbers included in the non-severe/severe analyses presented.

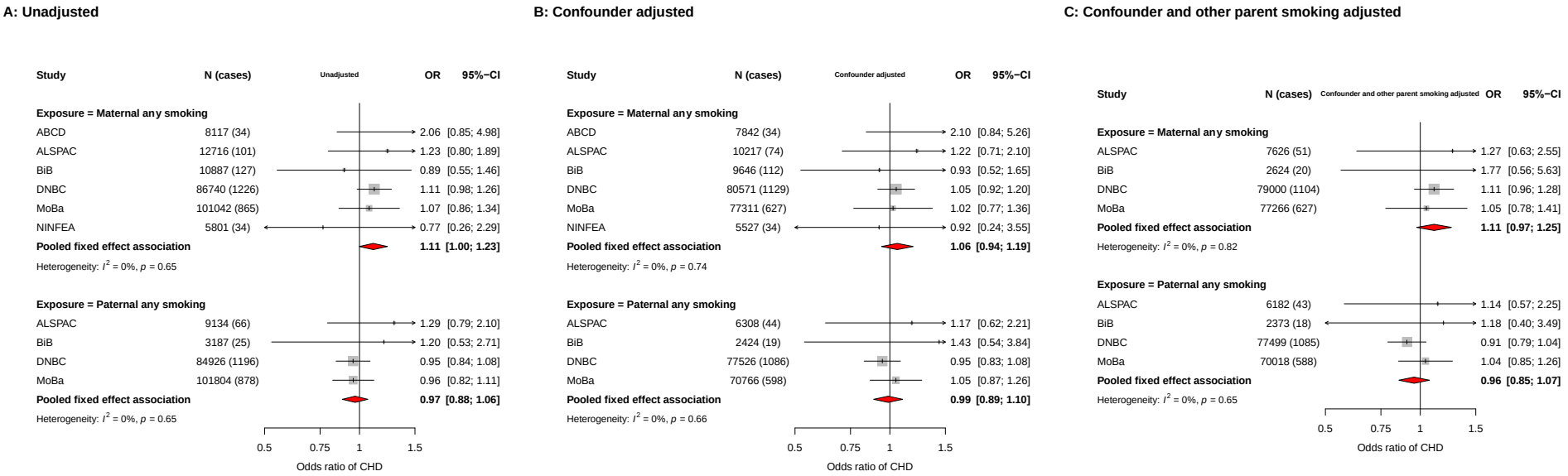


Figure S3.21. Main analysis associations between parental smoking (maternal top, paternal bottom) and offspring congenital heart disease. Panel A results are unadjusted, B results are fully adjusted for all confounders and C results are adjusted for all confounders as well as other parent's smoking. Confounders: ABCD: parental age, education, parity, ethnicity, alcohol, offspring sex; *ALSPAC:* parental age, education, parity, alcohol, offspring sex; *BiB*: parental age, education, parity, ethnicity, offspring sex; *DNBC:*, parental age, education, parity, alcohol, offspring sex; MoBa: parental age, education, parity, alcohol, offspring sex; NINFEA: parental age, education, parity, alcohol, offspring sex.

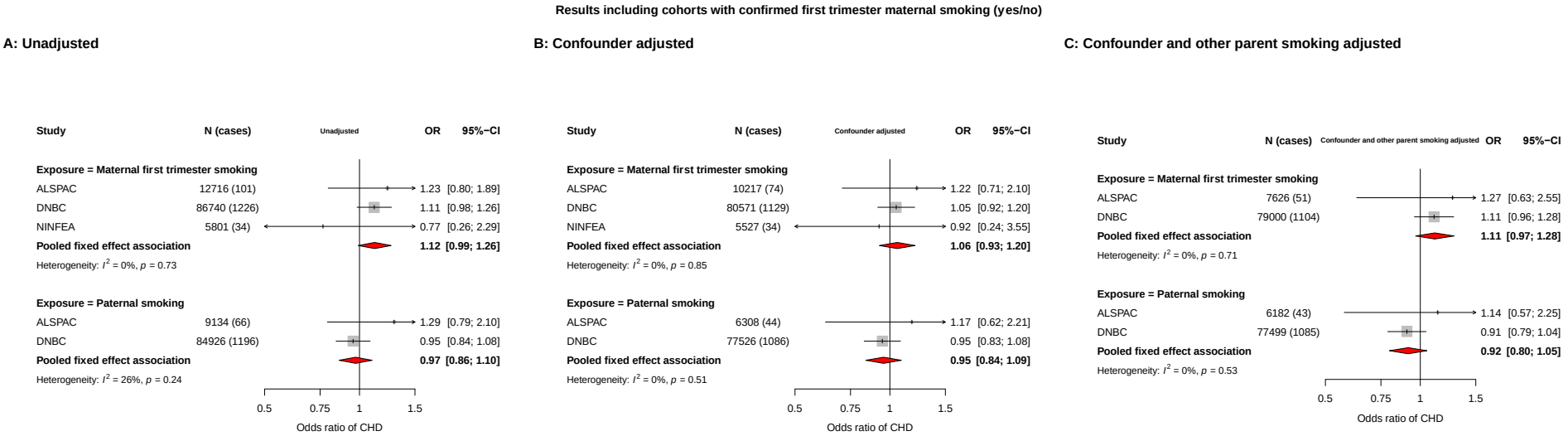


Figure S3.22. Showing the smoking results in those cohorts that had confirmed data on maternal first trimester smoking. Panel A results are unadjusted, B results are fully adjusted for all confounders and C results are adjusted for all confounders as well as other parent's smoking. Confounders: *ALSPAC:* parental age, education, parity, alcohol, offspring sex; *DNBC:*, parental age, education, parity, alcohol, offspring sex; NINFEA: parental age, education, parity, alcohol, offspring sex.

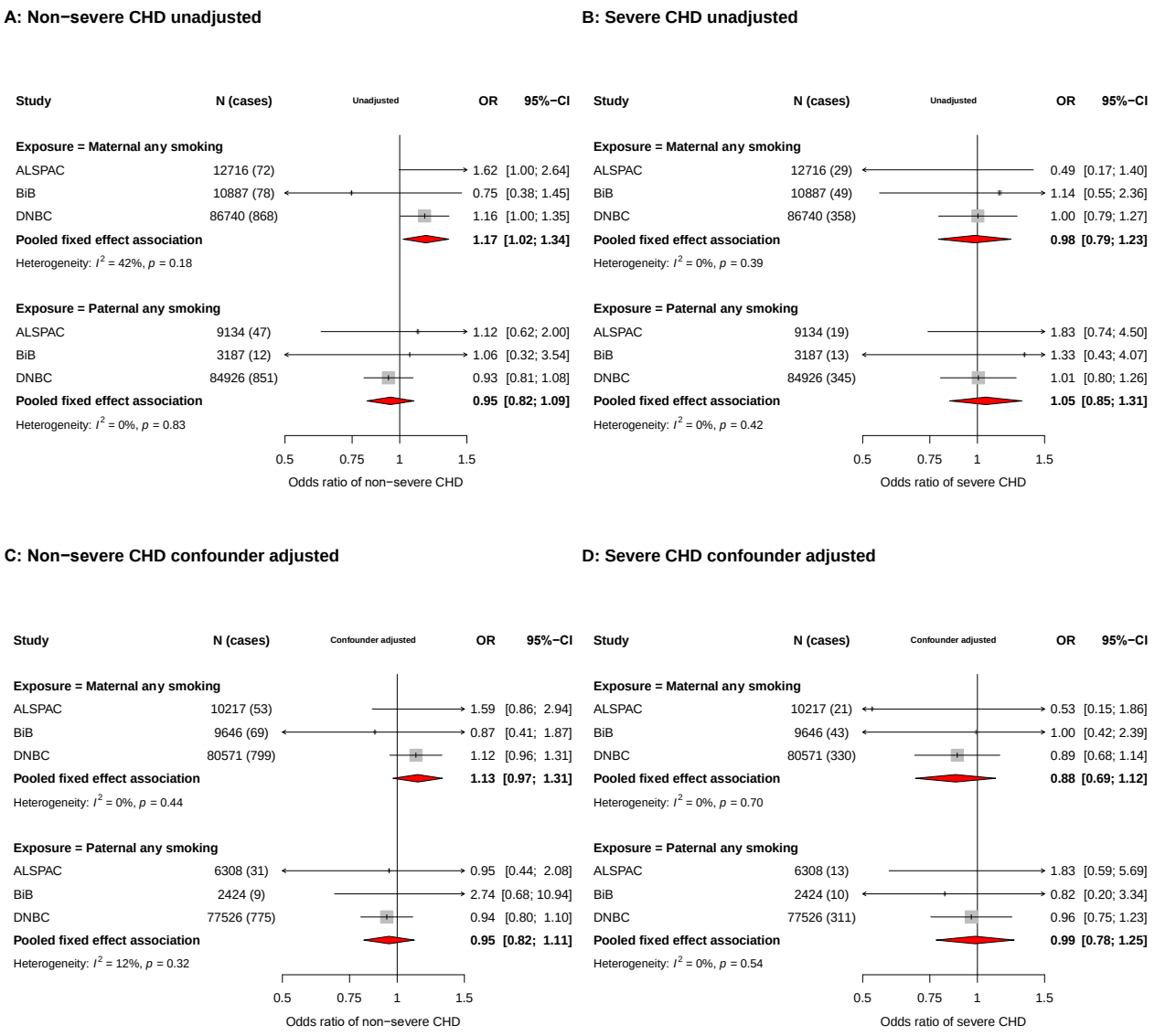


Figure S3.23. Unadjusted (A & B) and confounder adjusted (C &D) results for the smoking and CHD severity analyses presented in the main manuscript Figure 2.

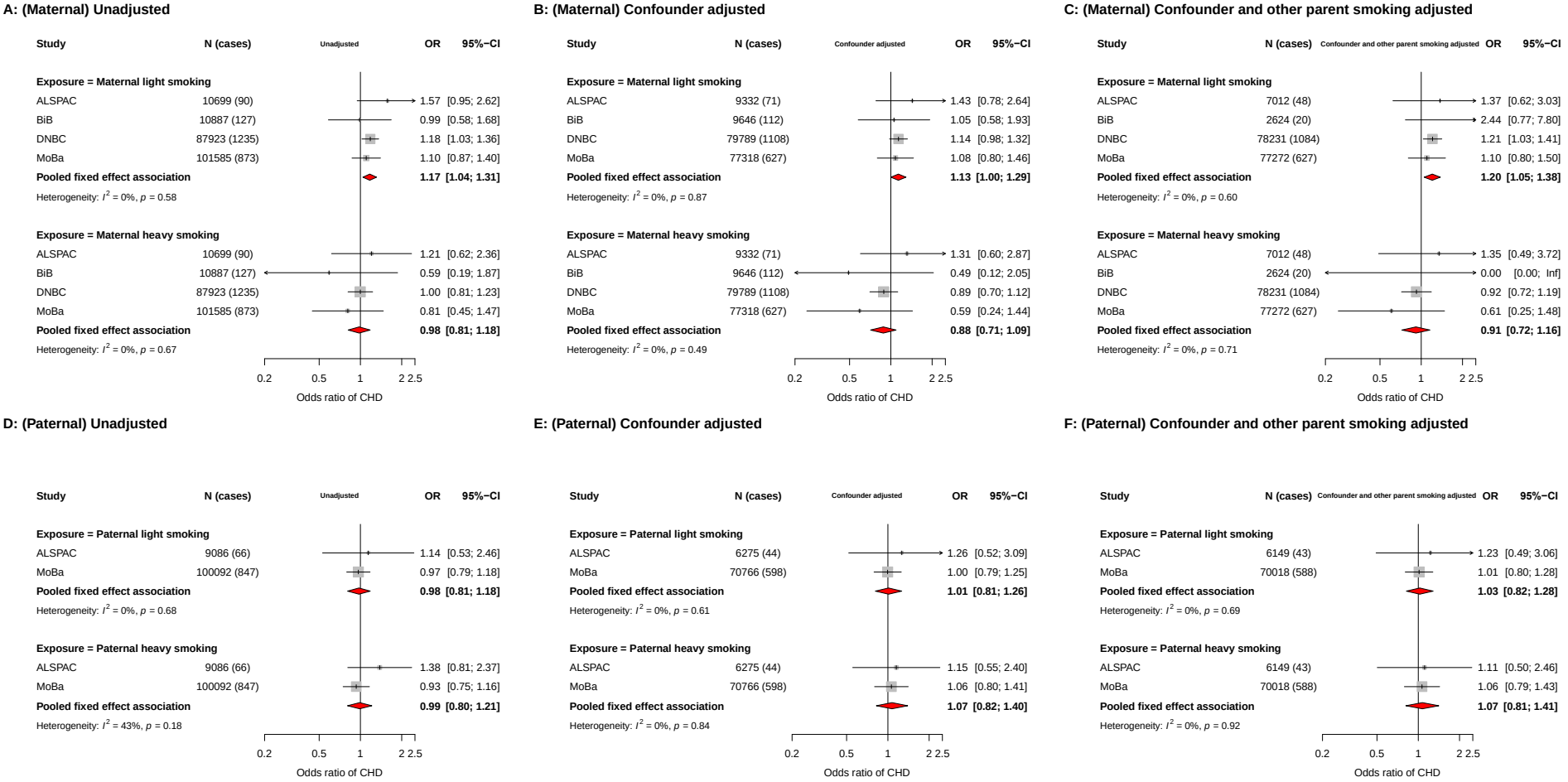


Figure S3.24. Associations between parental smoking heaviness (top (A, B & C): maternal, bottom (D, E & F): paternal) and offspring congenital heart disease. Results are unadjusted (left), adjusted for all confounders (middle) as well as all confounders and other parents smoking (right). Smoking categorised as none (non-smoker), light (< 10 cigarettes smoked per day during pregnancy) and heavy (≥ 10 cigarettes per day). Results presented as odds ratios and 95% confidence intervals for offspring CHD in comparison to non-smokers.

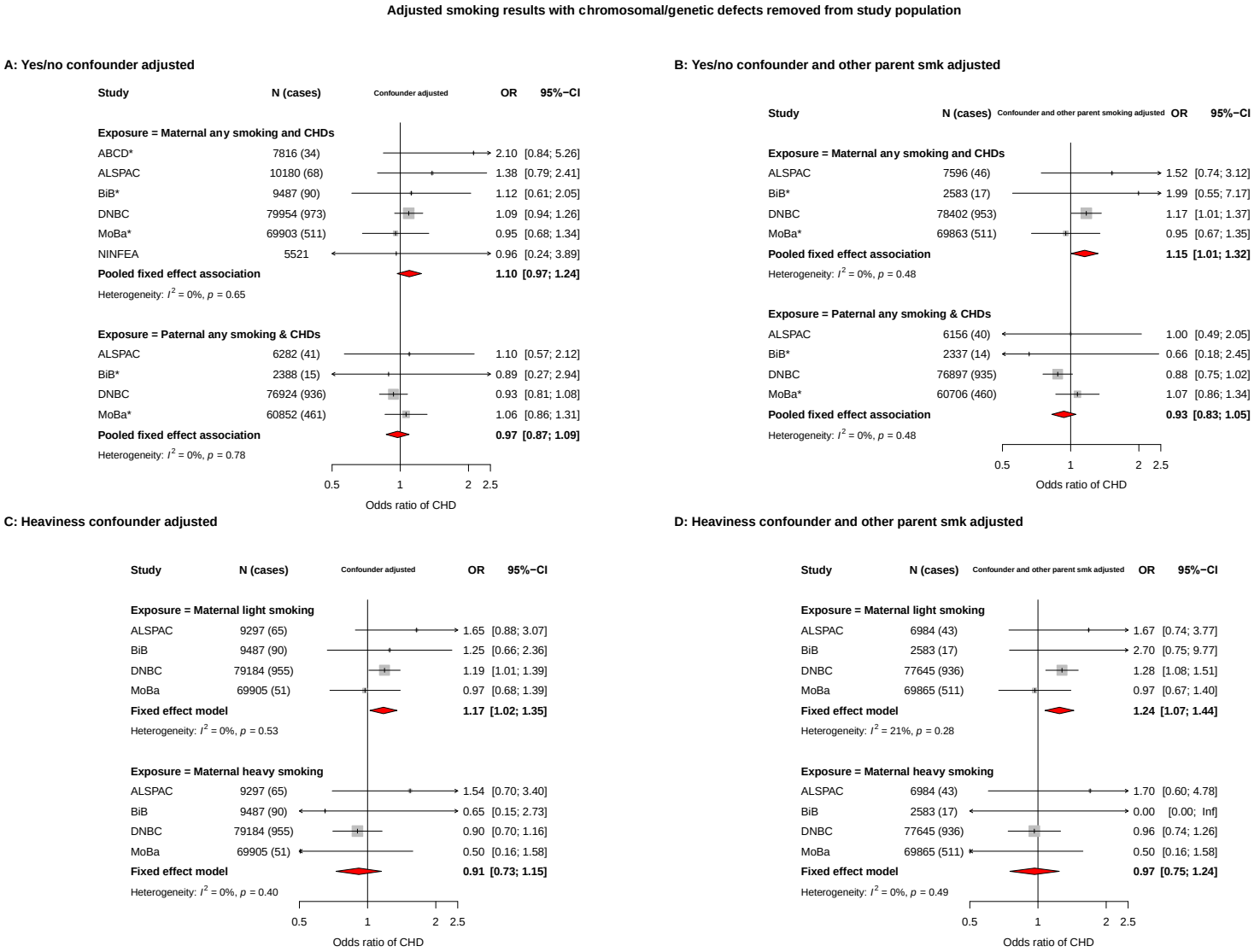


Figure S3.25. Additional analysis: Associations between parental smoking and offspring congenital heart disease with chromosomal/genetic defects removed from the study population. The plots on the top half (A &B) are for smoking yes/no analyses and the plots on the bottom half (C & D) are for smoking heaviness analyses. The rationale here is to see if estimates differ when I remove offspring from the population with an anomaly associated with a pre-specified cause such as a genetic, chromosomal or teratogenic aberration. ICD codes used to remove these cases from the population can be found in Table S3.3.

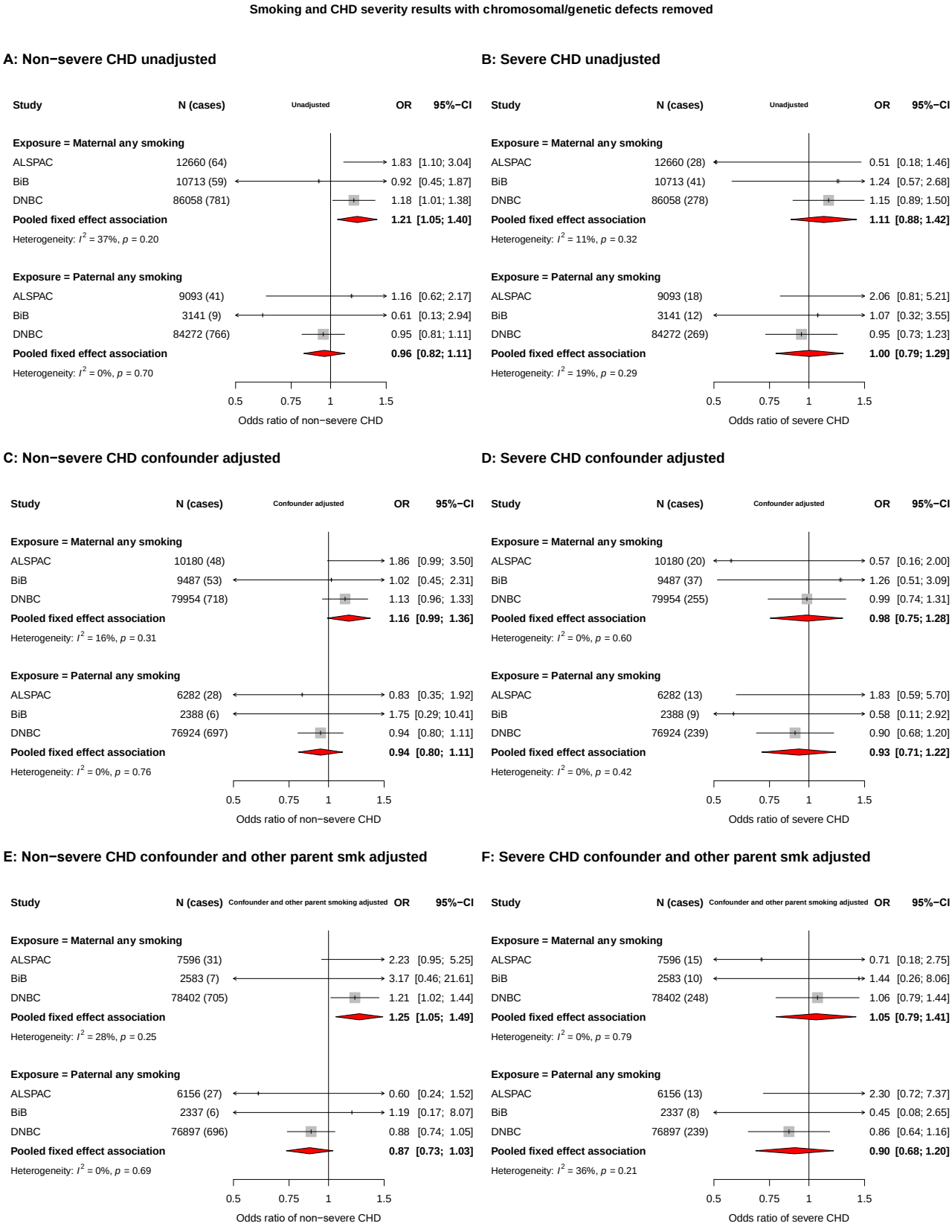


Figure S3.26. Smoking and CHD severity results with chromosomal/genetic defects removed from the study population.

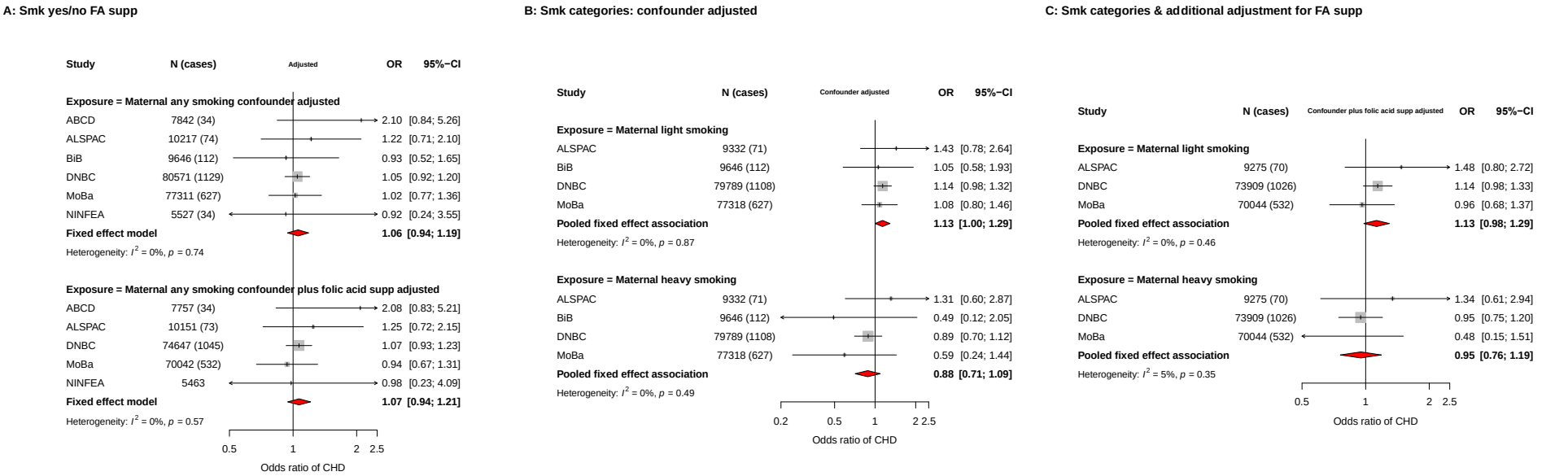
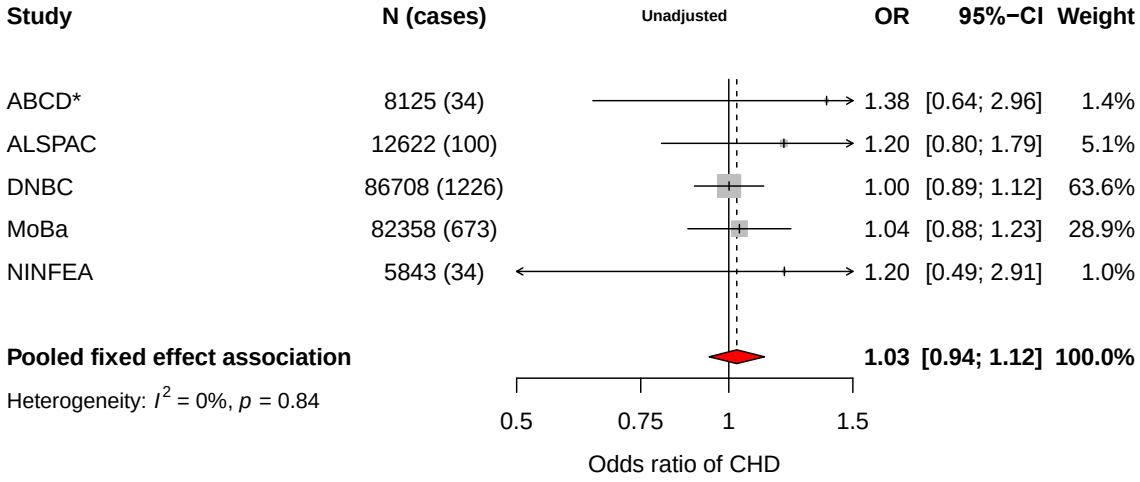


Figure S3.27. Associations between maternal smoking and offspring congenital heart disease. Results are fully adjusted for all confounders and all confounders plus additional adjustment for folic acid supplementation during weeks 0-12 of pregnancy. Panel A shows the results for the yes/no smoking analyses and panels B and C show results for the smoking heaviness analyses.

A: Unadjusted



B: Confounder adjusted

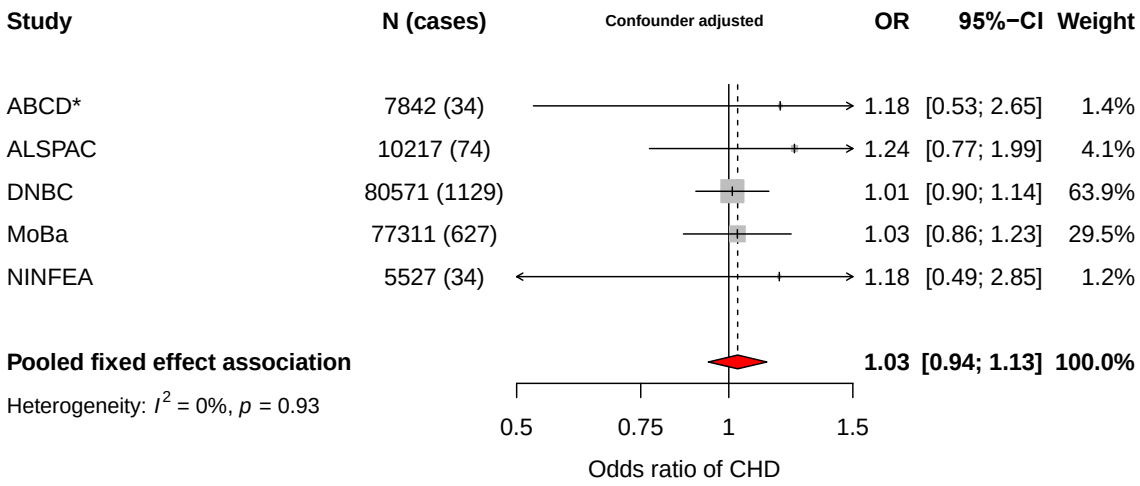
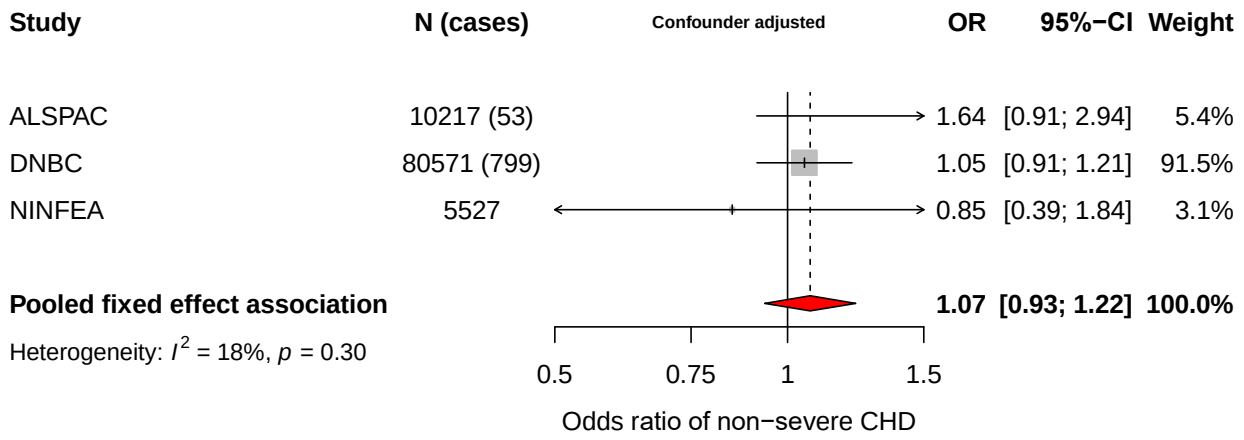


Figure S3.28. Associations between maternal alcohol consumption in the first trimester and offspring congenital heart disease. *ABCD did not have trimester-specific data, therefore analyses presented for ABCD are any alcohol consumption during pregnancy. Results are adjusted for all confounders. Confounders: ABCD: parental age, education, parity, ethnicity, smoking, offspring sex; *ALSPAC:* parental age, education, parity, smoking, offspring sex; *DNBC:* parental age, education, parity, smoking, offspring sex; MoBa: parental age, education, parity, smoking, offspring sex; NINFEA: parental age, education, parity, smoking, offspring sex.

A: Non−severe CHD



B: Severe CHD

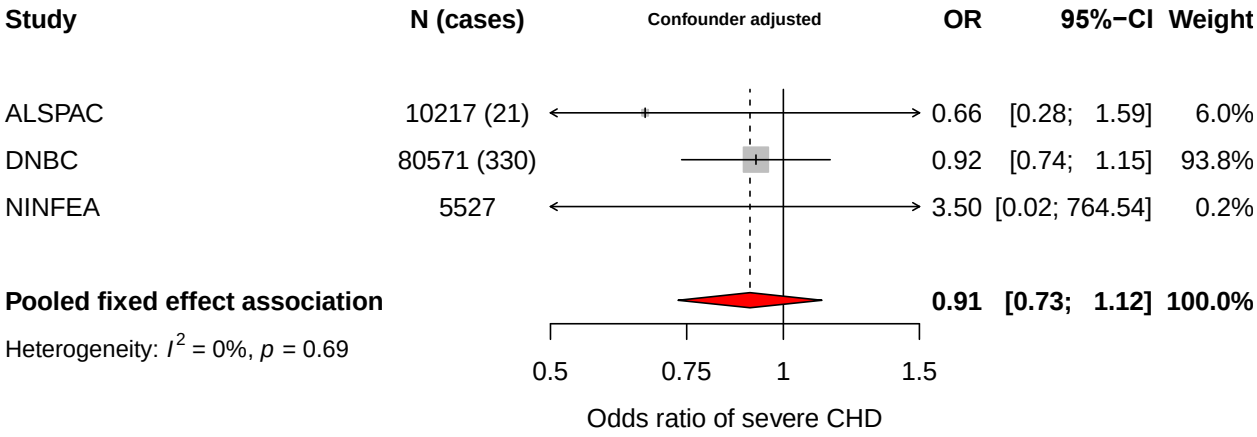


Figure S3.29. Confounder adjusted associations between maternal alcohol consumption during the first trimester and offspring non-severe congenital heart disease (A) and severe congenital heart disease (B). Definitions for CHD subtypes can be found in Table S3.2.

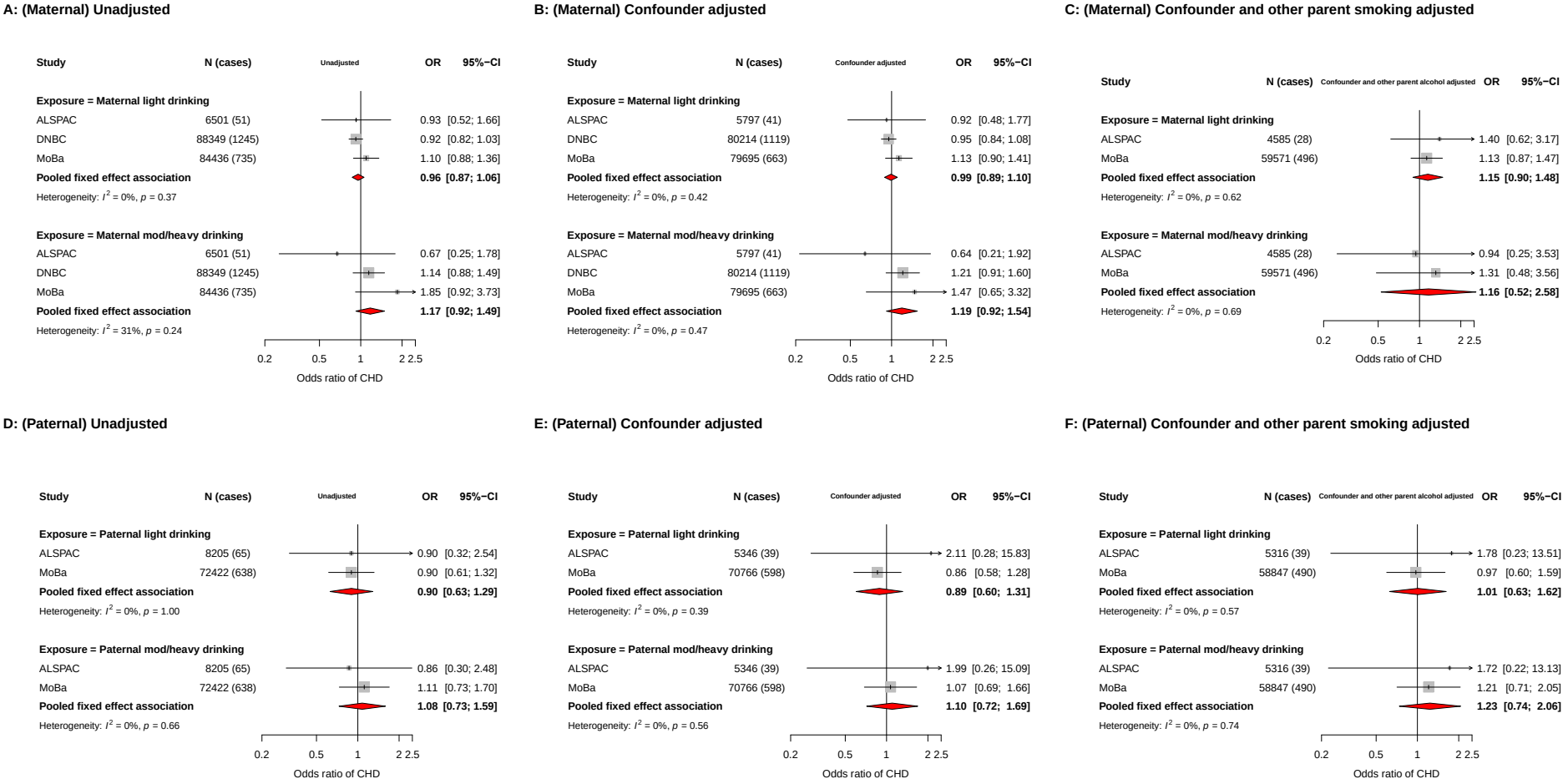
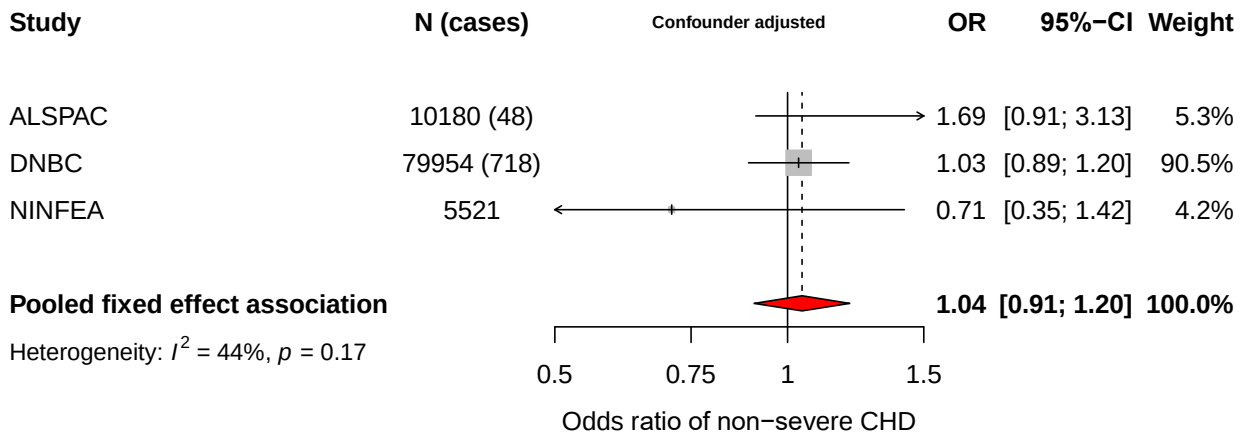


Figure S3.30. Associations (top (A, B & C): maternal, bottom (D, E & F): paternal) between parental alcohol intake and offspring congenital heart disease. Results are unadjusted (left), adjusted for all confounders (middle) as well as all confounders and other parents smoking (right). Maternal alcohol intake categorised as none (non-drinker), light (< 3 units per week during pregnancy) and moderate/heavy (≥ 3 units per week during pregnancy). Paternal alcohol intake categorised as none (non-drinker), light (< 7 units per week during pregnancy) and moderate/heavy (≥ 7 units per week during pregnancy). Results presented as odds ratios and 95% confidence intervals for offspring CHD in comparison to non-drinkers.

A: Non−severe CHD



B: Severe CHD

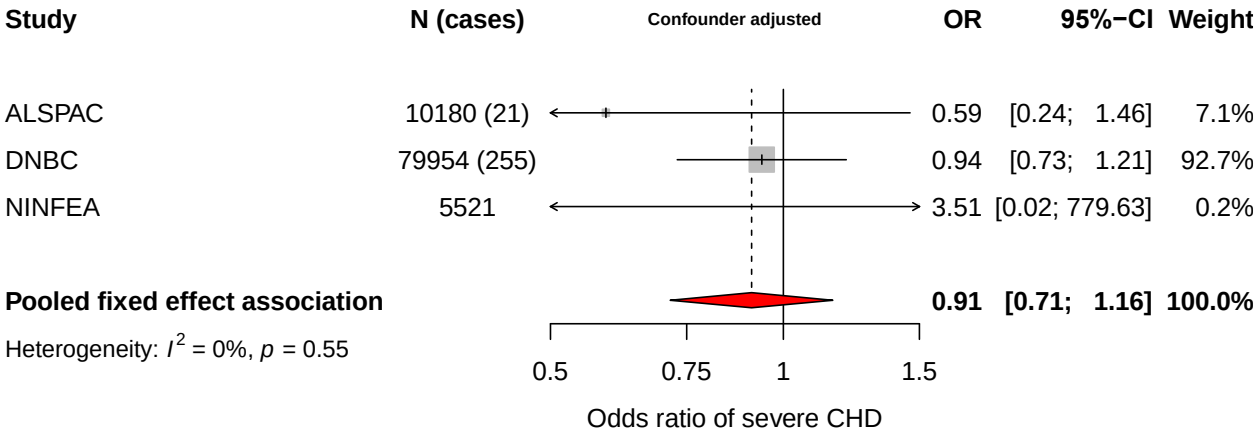


Figure S3.31. Confounder adjusted associations between maternal alcohol consumption during the first trimester and offspring non-severe congenital heart disease (A) and severe congenital heart disease (B) with chromosomal/genetic defects removed from the study population. Definitions for CHD subtypes can be found in Table S3.2.

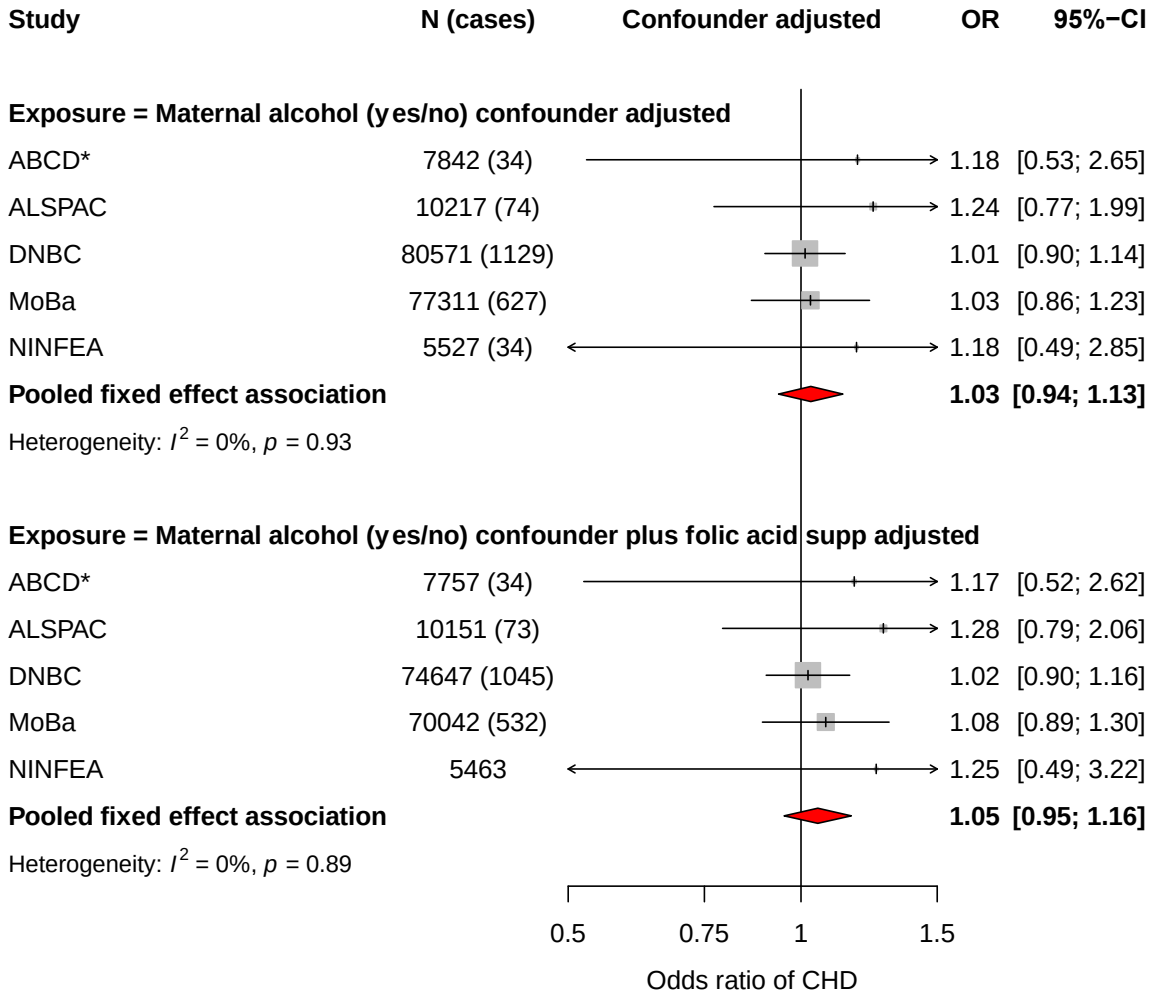


Figure S3.32. Associations between maternal drinking during the first trimester and offspring congenital heart disease. Results are adjusted for all confounders (top) and all confounders plus additional adjustment for folic acid supplementation during weeks 0-12 of pregnancy (bottom). Results are for first trimester drinking or any drinking during pregnancy where trimester data were not available (denoted by *).

Chapter 4

Text S4.1. Genetic data methods.

The Avon Longitudinal Study of Parents and Children (ALSPAC)

Mothers were genotyped on Illumina HumanHap660W quad-chip platform by Centre National de Génotypage (Évry, FR). Offspring were genotyped on Illumina HumanHap550 quad-chip platforms by the Wellcome Trust Sanger Institute (Cambridge, UK) and by the Laboratory Corporation of America (Burlington, USA) using support from 23andMe. Standard quality control was applied to SNPs and individuals. Individuals were excluded based on genotype rate (<5%), sex mismatch, high heterozygosity and cryptic relatedness [defined as identity-by-descent (IBD) >0.125]. In order to remove individuals of non-European descent, principal components (PCs) were derived from linkage disequilibrium-pruned SNPs with MAF >0.01 using plink. Individuals laying 5 standard deviations beyond the 1000 Genomes European population PCs 1 and 2 centroid were excluded. SNPs with a minor allele frequency (MAF) <1%, genotyping rate <5% or with a deviation from Hardy–Weinberg disequilibrium (pP << 1×10−6) were removed from the analysis. Using this QC'd dataset, a list of unrelated mothers was created using an IBD cut-off of 0.05. For imputation, genotypes of ALSPAC mothers and children were combined. Haplotypes were estimated using ShapeIT (v2. r644), which utilises relatedness during phasing. A phased version of the 1000 genomes reference panel (Phase 1, Version 3) was obtained from the Impute2 reference data repository. Imputation was performed using Impute V2.2.2 against the reference panel (all polymorphic SNPs excluding singletons), using all 2186 reference haplotypes (including non-Europeans).

Born in Bradford (BiB)

The samples of the BiB cohort (mothers and offspring) were processed on three different type of Illumina chips: HumanCoreExome12v1.0, HumanCoreExome12v1.1 and HumanCoreExome24v1.0. The pre-processing of samples was done separately for the three chips. Problematic samples which had a Call Rate < 0.95 were removed. Poorly performing SNPs determined by a set of quality matrices were zeroed.

BiB Illumina HumanCoreExome: PLINK and filtering

GenomeStudio output files were converted to PLINK format and subsequently filtered. SNPs where >=20% of individuals were missing genotype were removed. Individuals with >=10% missing genotypes were removed. A further pass over genotype rate was performed, removing SNPs where over

20% are missing genotype. Following inspection of plink *--missing output*, individuals with > 1% missing genotypes were removed. The final pass over genotype rate, removed SNPs where over 0.5% were missing genotype. The final pass over missingness per individual, removed individuals with over 0.5% missing genotypes.

Quality control and imputation

From each of the 3 genotyping sets any individual or SNP missing >3% of their data was dropped and the datasets combined. Genetic duplicates were removed. Reported first degree relatives (mother-child, father-child, child-child siblings) were checked to see if they looked genetically like first degree relatives. If there was no such evidence of this, they were removed. Mother-child discrepancies between phenotype and genotype were removed. People who looked genetically to be clearly South Asian or White British from the principal component analysis (PCA) but had a different ethnicity phenotype were removed. Based on a combination of PCA and reported ethnicity there were two subsets of individuals – white European and south Asian. As a sensitivity analysis, all of the genetic analyses in BiB are repeated after stratifying by these two ethnic groups. SNPs with a minor allele frequency (MAF) <1%, genotyping rate <5% or with a deviation from Hardy–Weinberg disequilibrium (pP << 1×10−6) were removed from the analysis. Imputation was performed for Europeans and South Asians separately, both using the HRC r1.1 as the reference panel. The genotype data was uploaded to the Michigan Imputation Server to perform genotype imputation using Minimac4. Phasing was performed using Eagle v2.4. After imputation, the VCF files were downloaded from the server and BCFtools was used to remove SNPs that were not accurately imputed. Mimimac4 generates a metric (imputation accuracy $R^{-squared}$) for each variant, and variants with estimated imputation accuracy R^2 <0.3 were removed.

The Norwegian Mother, Father and Child Cohort (MoBa)

Compared to other large biobanks like the UK Biobank, where considerable funding was secured upfront allowing for genotyping their entire cohort in a single effort, genotyping in MoBa have had to rely on several projects - each contributing with resources to genotype subsets of MoBa over the last decade. Consequently, genotyping was performed years apart at different labs using different arrays. I used data from MoBaGenetics 1.0. There is an openly available comprehensive GitHub page that documents all quality control for all releases of genetic data in the MoBa cohort (https://github.com/folkehelseinstituttet/mobagen). In this study I used data from the following batches: NORMENT, ROTTERDAM, TED and HARVEST (initial N = ~98,000). 33,047 individuals were genotyped in the NORMENT sample at deCODE genetics, Reykjavik Iceland (Illumina HumanOmniExpress-24v1.0,

Illumina InfiniumOmniExpress-24v1.2, & Illumina Global Screening Array MD v.1.0 + 50k custom OmniExpress overlap content array), 26,680 were genotyped in the ROTTERDAM sample at ERASMUS MC, Rotterdam, Netherlands (Illumina Global Screening Array MD v.1.0 array), 5215 were sampled in the TED samples at deCODE genetics (Illumina InfiniumOmniExpress-24v1.2), and 32,886 were sampled in the HARVEST sample at Genomics Core Facility, Trondheim, Norway (llumina HumanCoreExome12v1.1 & Illumina HumanCoreExome24v1.0). Below, I describe the methods and QC for the merged dataset used in the present study.

Quality control (QC) and imputation was performed to align with current best-practice QC protocols in human genetics and the family-based pipeline Picopili. The primary software used for the QC was PLINK 1.9 and KING 2.2.5. To identify core subpopulations filtering of was performed for minor allele frequency of 1%, SNP and individual call rate of 95%, and Hardy-Weinberg Equilibrium (HWE) p-value of 0.001. Principle component (PC) analysis with 1000 Genomes phase 1 data was used to identify the European, Asian, and African core subpopulations.

Pre-imputation QC was performed for each of the core subpopulations on the SNP and individual level. QC on a SNP level involved filtering for 0.5% MAF, 95% call rate, HWE p-value 0.000001, discordant in duplicate pairs, association with genotype plate and genotype batch at p-value 0.001. Individual level QC was performed by filtering for heterozygosity outliers Fhet ± 0.2, erroneous sex assignment, known relatedness, cryptic relatedness, identity-by-decent (PI_HAT threshold of 0.15), and PC outliers both with and without 1000 Genomes. Mendel errors were assessed for families with a minimum of one PO duo. Families with more than 5% Mendel errors and SNPs with more than 1% of Mendel errors were removed, while other minor Mendel errors were zeroed out. Batches that were genotyped using the same array were merged (keeping only SNPs present in all batches) and the pre-imputation QC was performed on the merged batches.

Phasing and imputation was performed using the publicly available Haplotype Reference Consortium data. Phasing was performed using SHAPEIT2 with the duoHMM algorithm to incorporate the pedigree information into the haplotype estimates. IMPUTE 4 was then used to perform imputation. Dosage data was then converted to best-guess, hard call genotype data with an imputation quality score (INFO) of 0.8 and default PLINK certainty of 0.9. Post-imputation QC was then performed following the steps outlined in the pre-imputation QC. To ensure the across batch relatedness (both known, such as PO and FS relationships, and unknown, such as sibships within the parent generation) was accounted for in all analyses the three imputation batches were merged, and post-imputation QC was performed on the overall merged dataset. I removed related individuals (cryptic relatedness: IBD >0.05).

Table S4.1. Further information on the genome-wide association studies used to generate genetic risk scores.

Primary outcome	Data	Publication year	N	Ancestry	Imputation reference panel	Control for population structure	Model	Covariables	Units	PMID	Data access
Body mass index	UKBB and GIANT	2018	~700,000	European	HRC imputation reference panel	10 PCs	Linear mixed model - BOLT-LMM v2.3	Age, sex, recruitment centre, genotyping batches and 10 PCs	Kg/m^2	30124842	Open access.
Lifetime smoking – heaviness, duration, initiation	UKBB	2019	462,690	European	UK 10K reference panel	10 PCs	Linear mixed model - BOLT-LMM	Genotype chip, sex, 10PCs	Lifetime smoking score (mean = 0.36).	31689377	Open access.
Alcoholic drinks per week	Large consortium – see paper for all studies.	2019	941,280	Mostly European or US.	Most studies used HRC imputation reference panel.	10 PCs.	Linear mixed model with a genetic kinship matrix.	PCs, age, sex, age x sex interaction.	Alcoholic drinks per week	30643251	Open access.

PMID, PubMed ID number; UKBB, UKBioBank; HRC, haplotype reference consortium; PC, principal component.

Text S4.2. Genetic risk score generation.

ALSPAC

Selected SNPs were extracted from the imputed genotype data in dosage format using QCTOOL (v2.0). PLINK (v1.9) was then used to construct the GRS for each exposure coded so that an increased score associated with increased exposure.

BiB

Selected SNPs were extracted from the imputed genotype data in dosage format using VCF tools (v 0.1.12b). PLINK (v1.9) was then used to construct the GRS for each exposure coded so that an increased score associated with increased exposure.

MoBa

In MoBa, I constructed the GRSs from the QC'd data in PLINK format. In MoBa, there were a large proportion of missing SNPs. I subset SNPs included in full GWAS results to SNPs also available in the QC'd MoBa data. From SNPs available in both, independent genome-wide significant associations were identified by clumping in MRBase, specifying r=0.01 and $p<5.0x10^{-8}$ [213]. Subsetting to SNPs available in MoBa and then clumping within these avoids the need for an additional step identifying proxy SNPs. These steps produced a similar number of SNPs for the BMI and smoking GRS in comparison to the GRSs generated in ALSPAC and BiB (941 and 939 in ALSPAC and BiB, respectively vs 868 in MoBa for BMI and 126 in ALSPAC and BiB vs 119 in MoBa for smoking). However, for alcohol, there was significantly less SNPs (98 and 99 in ALSPAC and BiB, respectively vs 37 in MoBa) most likely due to the approach the original GWAS used which was not possible to replicate. Therefore, as an alternative, I used the same summary data as ALSPAC and BiB and used a proxy SNP where available based on $r^2 > 0.8$ using the European reference panel in the LDLink R package [216], which left a total of 73 SNPs.

Text S4.3. Defining congenital heart disease.

ALSPAC

Case ascertainment of CAs in the ALSPAC cohort has been described in detail in a recently published data note [29]. Data were combined from multiple sources: NHS records (primary care, paediatric cardiology database, data on fetal deaths and local child health services), midwifery and birth records and maternal self-report via child-based questionnaires. Each source was coded using ICD-10 codes. By combining sources, there would be a greater possibility of capturing all of possible cases within the cohort. The majority of cases of CAs were identified by primary care records (79% for any CA and 68% for any CHD). I included diagnoses made at any age (from birth up until age 25/26). There were no restrictions in cases of CAs in ALSPAC, I included all cases whether live-born or not. However, it is possible that some cases that were terminated earlier in pregnancy were missed due to them never having an NHS number and thus not being identified through record linkage.

BiB

In the BiB cohort, there were two separate sources to identify CAs. Both sources were used in this study: (i) CAs up to 5 years of age, identified in GP records by Bishop et al [30] following EUROCAT guidelines. ICD-10 codes were mapped to clinical term (CT)-V3 codes prior to extraction from GP records. (ii) Data extracted from the Yorkshire and Humber CAs register database. Data were ICD-10 coded. All of these were confirmed postnatally. BiB includes data on the birth outcome of each child (live birth, miscarriage, still birth). Therefore, diagnoses were not necessarily restricted to live born children. However, there is the possibility that some women would have terminated the pregnancy after the 12- or 20-week scans which would lead to an under-representation of congenital anomaly cases.

MoBa

Information on whether a child had a CHD or not was obtained though linkage to the Medical Birth Registry of Norway (MBRN). All maternity units in Norway must notify births to the MBRN. Further information can be found in the publication by Leirgul et al (https://doi.org/10.1016/j.ahj.2014.07.030). The notification form includes the name and personal identity number of the child and parents, as well as information about maternal health before and during pregnancy, and any complications during pregnancy or at birth, including the presence of any heart defects. The MBRN contains information on all births and pregnancies ended after the 12th week of gestation, including stillbirths and abortions after the 12th week, including on heart defects. Heart defects are registered in the MBRN through notifications from

clinical staff identifying these defects at delivery or any hospital in patient treatments occurring immediately after birth until the child is discharged. The medical notification is made at discharge, which can be several months after birth. Details of the notified heart defects, such as specific diagnosis or treatment are not provided. Whilst most of the heart defects would have been diagnosed at birth it is possible that some children were admitted to hospital after delivery for non-specific reasons of for diagnoses that at the time were not considered to be related to a heart defect. Therefore, I considered MoBa diagnoses to have been made between birth and 6 months (few would remain in hospital after this length).

Table S4.2. Subcategories of CHD.

Category	CHDs included/excl	ICD codes
All CHDs	Any CHD as defined by EUROCAT* Patent ductus arteriosus (PDA) with gestational age (GA) < 37 weeks not considered a CHD case. Peripheral pulmonary artery stenosis with GA < 37weeks not considered as a CHD case.	Q20-Q25, Q260, Q262-Q269**
* Definitions taken from here: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/EUROCAT-Guide-1.4-Section-3.3.pdf **Q250 and Q256 not a case if isolated and GA<37weeks		

Text S4.4. Describing the pregnancy phenotype data: maternal BMI, smoking, alcohol, education, parity, diabetes separated by each cohort.

ALSPAC

For ALSPAC, in the 2nd pregnancy questionnaire (12 weeks' gestation) women were asked to report their pre-pregnancy weight and height and these were used to calculate BMI. No definition of pre-pregnancy was provided in the question. Extracted first antenatal clinic measurements of weight correlated strongly with the women's self-report (Pearson correlation = 0.93).

For smoking, women were asked the number of cigarettes per day during pregnancy in questionnaires at around 18 weeks' and 32 weeks' gestation. Binary variable used any smoking during pregnancy.

For alcohol, women were asked whether they had consumed alcohol during months 1-3 of the pregnancy in a questionnaire administered at around 18 weeks' and 32 weeks' gestation. Women were also asked about how many units they consumed in a questionnaire at 32 weeks' gestation. Binary variable used any alcohol consumption during pregnancy.

Women were asked about their highest educational qualification in a questionnaire administered around 32 weeks' gestation. Education was defined according to the international classification (High: Short cycle tertiary, Bachelor, Masters, Doctoral or equivalent (ISCED-2011: 5-8, ISCED-97: 5-6) Medium: Upper secondary, Post-secondary non- tertiary (ISCED-2011: 3-4, ISCED-97: 3-4) Low: No education; early childhood; pre-primary; primary; lower secondary or second stage of basic education). A binary variable was used (yes = medium or high education, no = low education).

For parity previous stillbirths were included and abortions excluded. Women were asked about previous children in a questionnaire administered around 32 weeks' gestation. A binary variable was used signifying multiparous and nulliparous women.

For diabetes, women were asked about existing diabetes and pregnancy diabetes using pregnancy questionnaires. Binary variable used any diabetes yes/no.

BiB

For BiB, weight and height (unshod and in light clothing and following a standard protocol) were measured at the recruitment assessment. As women were recruited at the oral glucose tolerance test (26-

28 weeks of gestation for the majority) this would not provide an accurate measure of pre-/early-pregnancy weight, as it would include fetal and amniotic weight and pregnancy related weight gain. All measurements of weight from all antenatal clinics were extracted from the obstetric records and pre-/early-pregnancy BMI was calculated using weight from the first antenatal clinic (median 12 weeks' gestation) and height at recruitment (26-28 weeks' gestation).

Women were asked number of cigarettes per day during pregnancy in the first questionnaire (26-28 weeks' gestation). Binary variable used any smoking during pregnancy.

Women were asked whether they consumed alcohol during the first 3 months of pregnancy.

Women were asked about their highest educational qualification in the recruitment questionnaire. Education was defined according to the international classification (High: Short cycle tertiary, Bachelor, Masters, Doctoral or equivalent (ISCED-2011: 5-8, ISCED-97: 5-6) Medium: Upper secondary, Post-secondary non- tertiary (ISCED-2011: 3-4, ISCED-97: 3-4) Low: No education; early childhood; pre-primary; primary; lower secondary or second stage of basic education). A binary variable was used (yes = medium or high education, no = low education).

For parity previous stillbirths were included and abortions excluded. Women were asked about previous children in a questionnaire administered at recruitment. A binary variable was used signifying multiparous and nulliparous women.

For diabetes, women were diagnosed with gestational diabetes based on results from the oral glucose tolerance test at recruitment. This was defined according to modified World Health Organization (WHO) definition used in clinical practice at the time: fasting glucose ≥ 6.1 mmol/L or 2 h post-load glucose ≥ 7.8 mmol/L. I then used questionnaire data that asked about existing diabetes administered at recruitment and defined an "any diabetes" variable.

MoBa

For MoBa, pre-pregnancy weight and height were self-reported during the first questionnaire at around 15 weeks' gestation.

During questionnaires administered around 15- and 32-weeks' gestation, women were asked if they smoked now after becoming pregnant. A binary variable was used to signify any smoking during pregnancy.

During the questionnaire administered around 32 weeks' gestation, women were asked about their drinking habits at different time points in the pregnancy. The options were: never, less than once a

month, roughly 1-3 times a week, roughly once a week, roughly 2-3 times a week, roughly 4-5 times a week and roughly 6-7 times a week. A binary variable was used to define any drinking during pregnancy (no = those that answered “never”, yes = those that answered anything else). In a sensitivity analysis to check the robustness of the GRS, I defined drinking during pregnancy as: no = those that answered “never” or “less than once a month” and yes = those that answered anything else.

Women were asked about their education in the questionnaire administered around 15 weeks’ gestation. The options were: 1) 9-year secondary school, 2) 1-2 year high school, 3) Vocational high school, 4) 3-year high school general studies, junior college, 5) Regional technical college, 4-year university degree (Bachelor’s degree, nurse, teacher, engineer), 6) University, technical college, more than 4 years (Master’s degree, medical doctor, PhD). I created a binary variable for high education: yes = 5 or 6, no = 1,2,3 or 4.

For parity, women were asked about the number of “previous deliveries” in a questionnaire. A binary variable was used signifying multiparous and nulliparous women.

For diabetes, women were asked about existing diabetes and pregnancy diabetes using pregnancy questionnaires. Binary variable used any diabetes yes/no.

Text S4.5. Genetic risk scores for multivariable Mendelian randomisation (MVMR).

The BMI GRS associated with smoking, education and diabetes across all three cohorts (Table S2). The effect of BMI on diabetes is well established, including from previous MR studies [92,245,246]. MR evidence suggests that higher education is causally related to lower BMI [247] whereas previous MR analyses show a potential causal effect of higher BMI on initiating smoking and other smoking traits [248,249] as well as smoking causing a reduction in BMI [250]. These findings would suggest that diabetes is a mediating path from BMI to CHD rather than a cause of horizontal pleiotropy, whereas education might be a source of horizontal pleiotropy and smoking, potentially with a bidirectional relationship could be both a horizontal pleiotropic and/or mediating path. Thus, I undertook MVMR adjusting the effects of the BMI GRS by a GRS predicting education (details below), and separately a smoking GRS, in additional analyses of the potential effect of BMI on CHDs, with caution in interpreting any change with adjustment for the smoking GRS.

The smoking GRS associated with BMI and education across the cohorts (Table S3). As discussed above the bidirectional relationship between BMI and smoking make it difficult to decide whether BMI is a potential biasing path, here, between the smoking GRS and CHD or mediates an effect of smoking. I undertook MVMR adjusting for a GRS predicting education (details below), and separately the BMI GRS, in additional analyses of the potential effect of smoking on CHDs.

The alcohol GRS showed consistent association with smoking across the cohorts (Table S4), and I used MVMR to adjust for the smoking GRS, to explore evidence that this might bias any effects of alcohol on CHD. There was evidence of the alcohol GRS relating to smoking and parity in BiB but given the weak statistical evidence and presence only in one of the cohorts I did not explore this further.

Education GRS

We used a recent large-scale GWAS on educational attainment [251] (~1.1 million participants, N = 481 independent SNPs in ALSPAC and BiB and 410 independent SNPs in MoBa ($r=0.01$ and $p<5.0\times10^{-8}$)). I generated the GRS for education using the same methods as described above (Text S2) and then included the GRS in the MR regression models.

STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies[1 2]

Item No.	Section		Checklist item	Page No.	Relevant text from manuscript
1	**TITLE and ABSTRACT**		Indicate Mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study	Complete.	
	INTRODUCTION				
2	**Background**		Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question	In the Introduction (paragraphs 2 and 3), we introduce the exposures of interest and the rationale for using MR to explore the causal question.	
3	**Objectives**		State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects	Complete - Introduction paragraphs 2 and 3.	
	METHODS				
4	**Study design and data sources**		Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:	Complete - Figure 1 shows the included cohorts and how the analysis populations were selected.	
		a)	Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.	Complete - Methods subheading "inclusion criteria and participating cohorts".	
		b)	Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis	Complete - Figure 1 shows the included cohorts and how the analysis populations were selected.	
		c)	Describe measurement, quality control and selection of genetic variants	Complete - genetic methods are described for each cohort with additional information provided in the supplementary material.	
		d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	Complete - Information provided under subheading "congenital heart disease and other phenotype data".	
		e)	Provide details of ethics committee approval and participant informed consent, if relevant	Complete - relevant information provided in cohort descriptions under "inclusion criteria and participating cohorts".	
5	**Assumptions**		Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis	Complete - Assumptions introduced in the Introduction and then described in relation to the analyses in the "statistical "analyses" section of methods.	
6	**Statistical methods: main analysis**		Describe statistical methods and statistics used		

		a)	Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)	Complete where applicable - Statistical analyses section.
		b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	Complete - see “genetic risk score generation” and “statistical analyses”.
		c)	Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples	Complete - see “statistical analyses”: we used logistic regression to test for the presence of a causal effect (i.e., we did not use an estimator to try and quantify the causal effect).
		d)	Explain how missing data were addressed	Complete - see Figure 1. We selected on all participants with maternal genotype data and offspring CHD data.
		e)	If applicable, indicate how multiple testing was addressed	NA.
7	**Assessment of assumptions**		Describe any methods or prior knowledge used to assess the assumptions or justify their validity	Complete - Statistical analyses in relation to the verification of MR assumptions are provided in the statistical analyses section.
8	**Sensitivity analyses and additional analyses**		Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations)	Complete - All analyses and additional analyses are provided in the “statistical analyses” section.
9	**Software and pre-registration**			
		a)	Name statistical software and package(s), including version and settings used	Complete - See “statistical analyses”.
		b)	State whether the study protocol and details were pre-registered (as well as when and where)	Complete - A pre-specified project analysis plan and registration was created on June 3rd 2021: https://doi.org/10.17605/OSF.IO/W62BG
	RESULTS			
10	**Descriptive data**			
		a)	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram	Complete - See Figure 1.
		b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	Complete - See Table 1.
		c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	NA.
		d)	For two-sample MR: i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples	NA.

			ii. Provide information on the number of individuals who overlap between the exposure and outcome studies	
11	**Main results**			
		a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	Complete - See Table 2 and Figure 2.
		b)	Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference	Complete - See Table 2 and Figure 2.
		c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA.
		d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	Complete - See Figure 2.
12	**Assessment of assumptions**			
		a)	Report the assessment of the validity of the assumptions	Complete - See Table 2 and text results.
		b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	NA.
13	**Sensitivity analyses and additional analyses**			
		a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	Complete - See results and Supplementary Figures. We performed: analyses additional adjusting for fetal genotype, analyses excluding BiB (due to unique ethnic structure) and multivariable MR analyses,
		b)	Report results from other sensitivity analyses or additional analyses	Complete - see comment above.
		c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	NA.
		d)	When relevant, report and compare with estimates from non-MR analyses	NA.
		e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	NA.
	DISCUSSION			
14	**Key results**		Summarize key results with reference to study objectives	Complete - Discussion paragraph 1.
15	**Limitations**		Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them	Complete - Limitations are discussed throughout the discussion and notably in paragraph 5.

16	**Interpretation**			
		a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	Complete - Done throughout the discussion.
		b)	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions	Possible mechanisms are not discussed in this paper. A key rationale for this work was to try and replicate previous results using negative control analyses where we do touch upon potential mechanisms: https://doi.org/10.1161/JAHA.120.020051.
		c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	Policy relevance is touched upon within the Discussion, but effect sizes are not discussed. As mentioned, we aimed to explore the direction of effects and compare these with previous observational negative control estimates.
17	**Generalizability**		Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure	Complete - See discussion paragraph 5.
	OTHER INFORMATION			
18	**Funding**		Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based	Funding requirements completed in accordance with journal guidelines.
19	**Data and data sharing**		Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where	Data and data sharing requirements completed in accordance with journal guidelines.
20	**Conflicts of Interest**		All authors should declare all potential conflicts of interest	Conflicts of interest statement completed.

1. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) Statement. JAMA. 2021;under review.

2. Skrivankova VW, Richmond RC, Woolf BAR, Davies NM, Swanson SA, VanderWeele TJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomisation (STROBE-MR): Explanation and Elaboration. BMJ. 2021;375:n2233.

Exploring associations between the GRSs (BMI, lifetime smoking index, drinks per week) and risk factors for CHDs.

Table S4.3. Exploring associations between the BMI GRS and risk factors for CHDs. I also include the association of the BMI GRS with BMI (also shown in main table within the chapter) for comparison.

Risk factor	N	Coefficient (95% CI) [a]	P-value	R^2 / Pseudo R^2 [b]	F statistic [c]	AUC
ALSPAC						
BMI	6,253	0.24 (0.21, 0.26)	1×10^{-80}	5.6%	372	-
Education	6,806	0.87 (0.81, 0.93)	2×10^{-5}	0.45%	-	0.54
Parity	6,982	1.03 (0.98, 1.08)	0.25	0.03%	-	0.51
Diabetes	6,786	1.15 (0.83, 1.60)	0.17	0.16%	-	0.54
Smoking	6,428	1.14 (1.08, 1.21)	4×10^{-6}	0.49%	-	0.54
Alcohol	6,087	0.96 (0.91, 1.03)	0.27	0.03%	-	0.51
BiB						
BMI	6,196	0.20 (0.18, 0.23)	5×10^{-59}	4.1%	268	-
Education	6,483	0.92 (0.88, 0.97)	0.002	0.2%	-	0.52
Parity	7,259	1.04 (0.99, 1.09)	0.15	0.03%	-	0.51
Diabetes	7,133	1.10 (1.01, 1.18)	0.04	0.1%	-	0.52
Smoking	6,482	1.09 (1.02, 1.16)	0.01	0.2%	-	0.52
Alcohol	2,110	1.07 (0.98, 1.16)	0.15	0.1%	-	0.52
MoBa						
BMI	22,533	0.25 (0.24, 0.27)	$< 1 \times 10^{-100}$	6.5%	1,555	-
Education	21,921	0.90 (0.87, 0.92)	3×10^{14}	0.4%	-	0.53
Parity	23,869	1.00 (0.97, 1.02)	0.80	0.0004%	-	0.50
Diabetes	23,869	1.24 (1.12, 1.39)	8×10^{-5}	0.5%	-	0.56
Smoking	20,981	1.18 (1.12, 1.24)	2×10^{-11}	0.5%	-	0.55
Alcohol	19,737	0.97 (0.94, 1.00)	0.03	0.03%	-	0.51

[a] Effect estimates (coefficient) are difference in mean (BMI SD units) or odds ratio per SD increase in genetic risk score; [b] for the binary outcomes pseudo-R^2 are presented; [c] for BMI F-statistic is presented; for binary outcomes AUC is presented. Education = high education vs low education around the time of pregnancy; Parity = multiparous vs nulliparous; Diabetes = Any diabetes vs none; Smoking = Any smoking during pregnancy yes vs no; Alcohol = Any alcohol consumption during pregnancy yes vs no. Abbreviations: BMI, body mass index; GRS, genetic risk score; CI, confidence interval; ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa. Norwegian Mother, Father and Child Cohort Study.

Table S4.4. Exploring associations between the smoking GRS (lifetime smoking index) and risk factors for CHDs. I also include the association of the smoking GRS with smoking (also shown in main table within the chapter) for comparison.

Risk factor	N	Coefficient (95% CI)[a]	P-value	R^2 / Pseudo R^2 [b]	F statistic [c]	AUC
ALSPAC						
Smoking	6,428	1.27 (1.20, 1.35)	1×10^{-16}	1.6%	-	0.56
Education	6,806	0.84 (0.79, 0.90)	3×10^{-7}	0.68%	-	0.55
Parity	6,982	1.01 (0.96, 1.05)	0.81	0.001%	-	0.50
Diabetes	6,786	0.92 (0.66, 1.28)	0.61	0.06%	-	0.54
Alcohol	6,087	1.00 (0.94, 1.06)	0.92	0.0002%	-	0.50
BMI	6,253	0.06 (0.03, 0.08)	3×10^{-6}	0.35%	22	-
BiB						
Smoking	6,482	1.36 (1.27, 1.45)	2×10^{-20}	2.2%	-	0.59
Education	6,483	0.95 (0.90, 1.00)	0.04	0.09%	-	0.52
Parity	7,259	0.96 (0.91, 1.00)	0.06	0.06%	-	0.51
Diabetes	7,133	0.93 (0.86, 1.01)	0.10	0.08%	-	0.52
Alcohol	2,110	0.98 (0.90, 1.07)	0.67	0.01%	-	0.50
BMI	6,196	0.03 (0.001, 0.05)	0.04	0.07%	4	-
MoBa						
Smoking	20,981	1.23 (1.17, 1.29)	7×10^{-17}	0.8%	-	0.56
Education	21,921	0.87 (0.85, 0.90)	9×10^{-22}	0.6%	-	0.54
Parity	23,869	1.01 (0.98, 1.04)	0.48	0.003%	-	0.50
Diabetes	23,869	1.02 (0.91, 1.13)	0.75	0.003%	-	0.51
Alcohol	19,737	0.99 (0.96, 1.02)	0.48	0.004%	-	0.50
BMI	22,533	0.04 (0.03, 0.06)	1×10^{-10}	0.2%	42	-

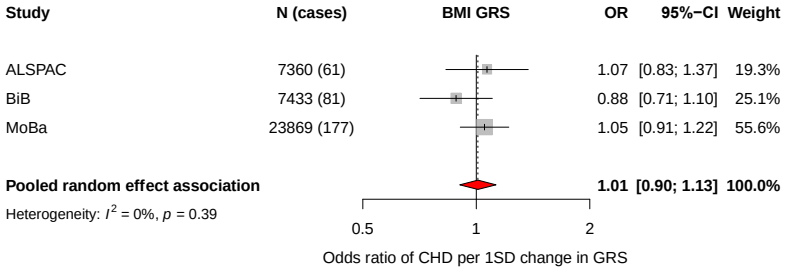
[a] Effect estimates (coefficient) are difference in mean (BMI SD units) or odds ratio per SD increase in genetic risk score; [b] for the binary outcomes pseudo-R^2 are presented; [c] for BMI F-statistic is presented; for binary outcomes AUC is presented. Education = high education vs low education around the time of pregnancy; Parity = multiparous vs nulliparous; Diabetes = Any diabetes vs none; Smoking = Any smoking during pregnancy yes vs no; Alcohol = Any alcohol consumption during pregnancy yes vs no. Abbreviations: BMI, body mass index; GRS, genetic risk score; CI, confidence interval; ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa. Norwegian Mother, Father and Child Cohort Study.

Table S4.5. Exploring associations between the alcohol GRS (drinks per week) and risk factors for CHDs. I also include the association of the alcohol GRS with alcohol (also shown in main table within the chapter) for comparison.

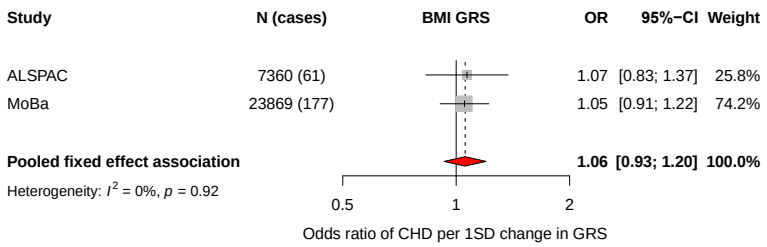
Risk factor	N	Coefficient (95% CI)[a]	P-value	R^2 / Pseudo R^2 [b]	F statistic [c]	AUC
ALSPAC						
Alcohol	6,087	1.14 (1.07, 1.21)	3×10^{-5}	0.4%	-	0.53
Education	6,806	1.05 (0.98, 1.12)	0.19	0.05%	-	0.51
Parity	6,982	1.02 (0.98, 1.07)	0.32	0.02%	-	0.51
Diabetes	6,786	1.22 (0.86, 1.73)	0.26	0.31%	-	0.57
Smoking	6,428	1.08 (1.02, 1.14)	0.01	0.15%	-	0.52
BMI	6,253	0.001 (-0.02, 0.03)	0.92	0.0002%	0.01	-
BiB						
Alcohol	2,110	1.08 (0.99, 1.18)	0.09	0.2%	-	0.52
Education	6,483	0.98 (0.93, 1.03)	0.34	0.02%	-	0.51
Parity	7,259	1.05 (1.00, 1.10)	0.03	0.09%	-	0.52
Diabetes	7,133	1.07 (0.99, 1.17)	0.09	0.09%	-	0.52
Smoking	6,482	0.84 (0.79, 0.89)	4×10^{-8}	0.8%	-	0.55
BMI	6,196	-0.02 (-0.05, 0.01)	0.16	0.03%	2	-
MoBa						
Alcohol	19,737	1.02 (0.99, 1.05)	0.13	0.02%	-	0.51
Alcohol sensitivity [d]	19,737	1.06 (1.01, 1.10)	0.01	0.07%	-	0.52
Education	21,921	1.00 (0.96, 1.02)	0.58	0.002%	-	0.50
Parity	23,869	1.00 (0.98, 1.03)	0.93	<0.0001%	-	0.50
Diabetes	23,869	1.00 (0.89, 1.10)	0.88	0.0007%	-	0.50
Smoking	20,981	1.02 (0.97, 1.07)	0.37	0.009%	-	0.51
BMI	22,533	-0.007 (-0.019, 0.006)	0.32	0.004%	1	-

[a] Effect estimates (coefficient) are difference in mean (BMI SD units) or odds ratio per SD increase in genetic risk score; [b] for the binary outcomes pseudo-R^2 are presented; [c] for BMI F-statistic is presented; for binary outcomes AUC is presented. Education = high education vs low education around the time of pregnancy; Parity = multiparous vs nulliparous; Diabetes = Any diabetes vs none; Smoking = Any smoking during pregnancy yes vs no; Alcohol = Any alcohol consumption during pregnancy yes vs no. Abbreviations: BMI, body mass index; GRS, genetic risk score; CI, confidence interval; ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa. Norwegian Mother, Father and Child Cohort Study.

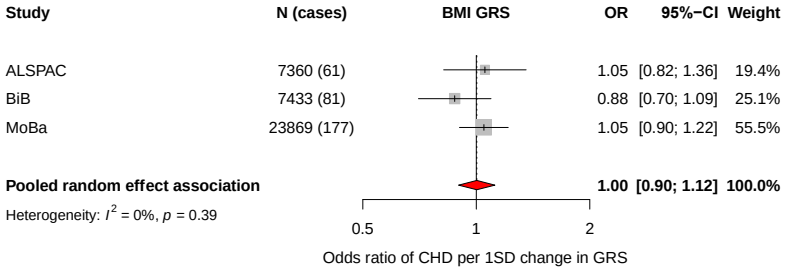
A: Main analyses



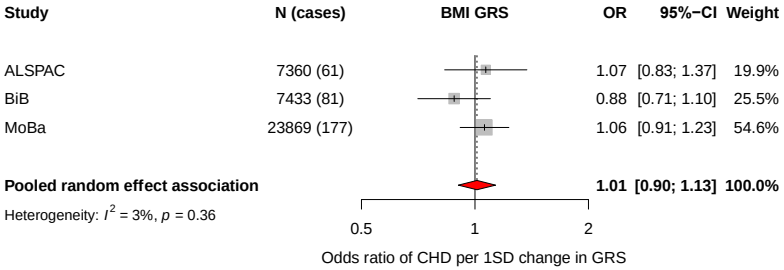
B: Main analyses excluding BiB



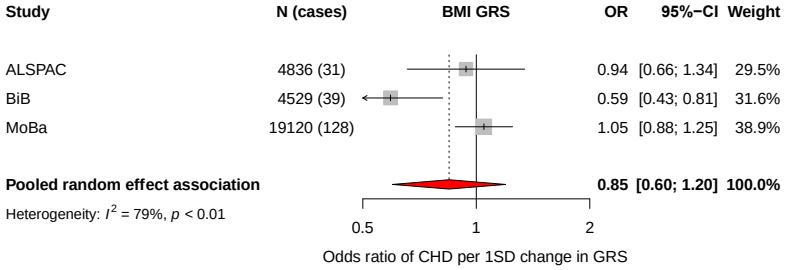
C: MVMR − Education GRS



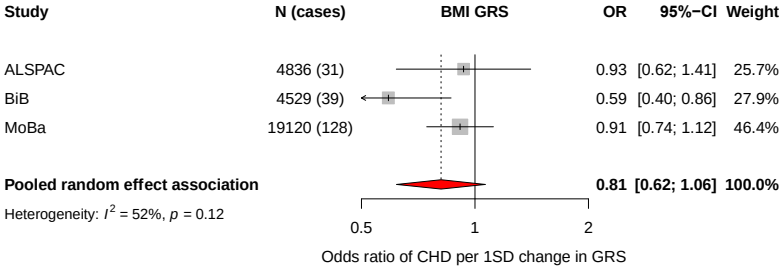
D: MVMR − Smoking GRS



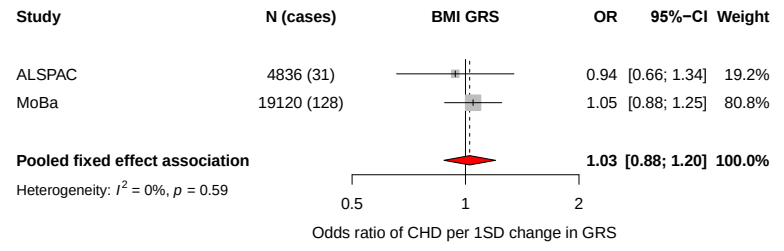
E: Main analyses in fetal genotype sub−population



F: Main analyses with adjustment for fetal genotype



G: Main analyses in fetal genotype sub−population excluding BiB



H: Main analyses with adjustment for fetal genotype excluding BiB

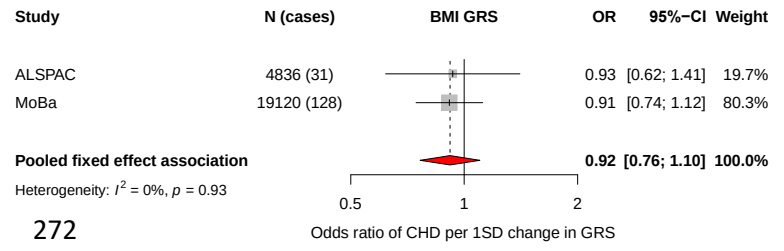
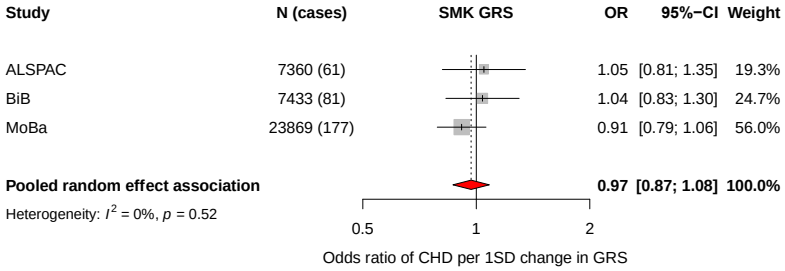
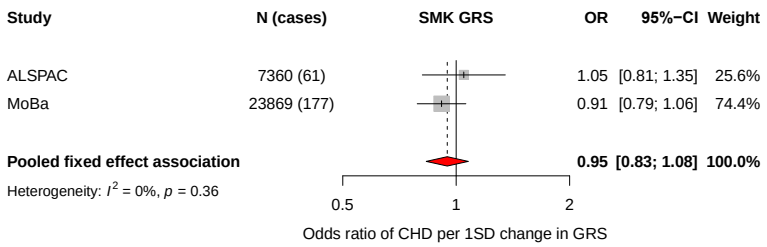


Figure S4.1. Showing the main results and results from additional analyses for the MR analyses of genetically predicted maternal BMI and offspring CHDs. Odds ratios (ORs) of CHD for a 1SD difference in maternal GRS in each study and pooled across studies using random effects meta-analysis or fixed-effects analyses when excluding BiB (panels B, G, H). Adjusted for top 10 genetic principal components in all cohorts with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. **Panel A:** Main analyses as shown in the main manuscript. **Panel B:** Main analyses excluding BiB. **Panel C:** Main analyses with additional adjustment for genetically predicted educational attainment (Multivariable Mendelian randomisation analyses). **Panel D:** Main analyses with additional adjustment for genetically predicted smoking (Multivariable Mendelian randomisation analyses). **Panel E:** Main analysis results in the sub-population with fetal genotype. **Panel F:** As Panel E, but with additional adjustment for fetal genotype. **Panel G:** As panel E but excluding BiB. **Panel H:** As panel F but excluding BiB. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort Study; BMI, body mass index; CI, confidence interval; CHD, congenital heart disease; SD, standard deviation; GRS, genetic risk score; MVMR, multivariable Mendelian randomisation.

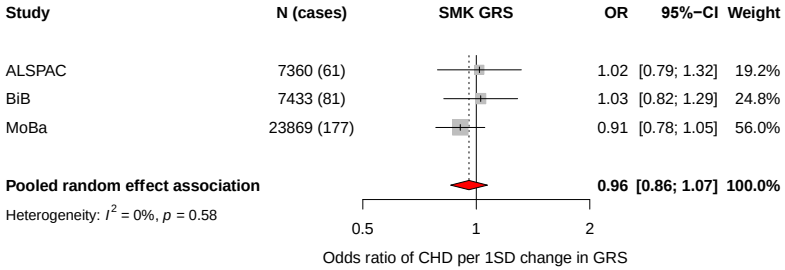
A: Main analyses



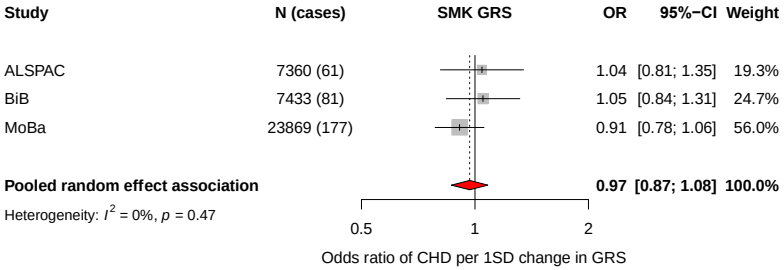
B: Main analyses excluding BiB



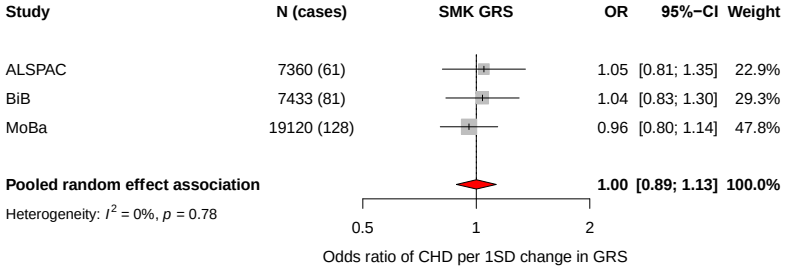
C: MVMR − Education GRS



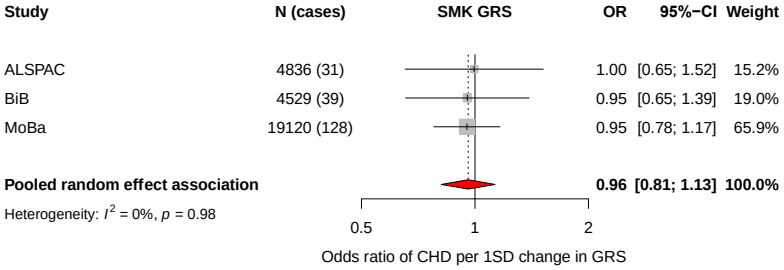
D: MVMR − BMI GRS



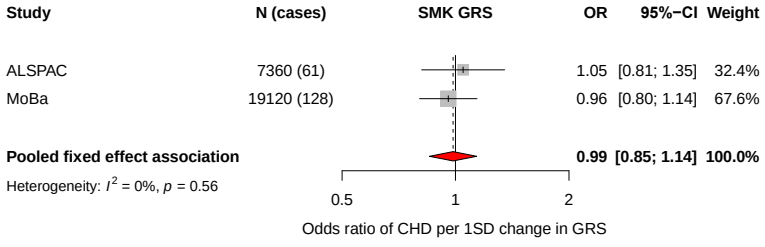
E: Main analyses in fetal genotype sub−population



F: Main analyses with adjustment for fetal genotype



G: Main analyses in fetal genotype sub−population excluding BiB



H: Main analyses with adjustment for fetal genotype excluding BiB

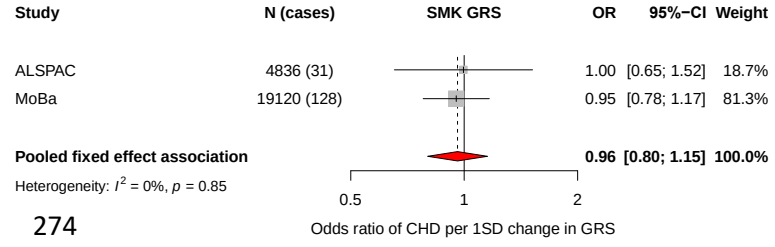
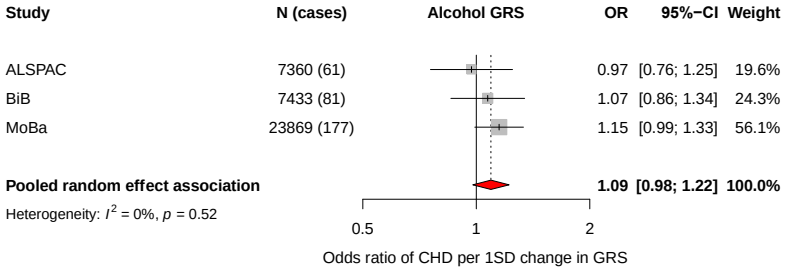
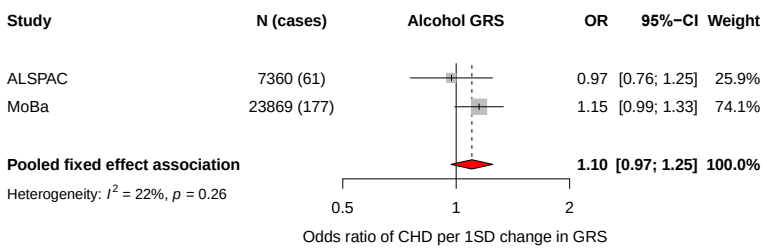


Figure S4.2. Showing the main results and results from additional analyses for the MR analyses of genetically predicted maternal smoking (using a genetic risk score of a lifetime smoking index) and offspring CHDs. Odds ratios (ORs) of CHD for a 1SD difference in maternal GRS in each study and pooled across studies using random effects meta-analysis or fixed-effects analyses when excluding BiB (panels B, G, H). Adjusted for top 10 genetic principal components in all cohorts with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. **Panel A:** Main analyses as shown in the main manuscript. **Panel B:** Main analyses excluding BiB. **Panel C:** Main analyses with additional adjustment for genetically predicted educational attainment (Multivariable Mendelian randomisation analyses). **Panel D:** Main analyses with additional adjustment for genetically predicted body mass index (Multivariable Mendelian randomisation analyses). **Panel E:** Main analysis results in the sub-population with fetal genotype. **Panel F:** As Panel E, but with additional adjustment for fetal genotype. **Panel G:** As panel E but excluding BiB. **Panel H:** As panel F but excluding BiB. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort Study; BMI, body mass index; CI, confidence interval; CHD, congenital heart disease; SD, standard deviation; GRS, genetic risk score; MVMR, multivariable Mendelian randomisation.

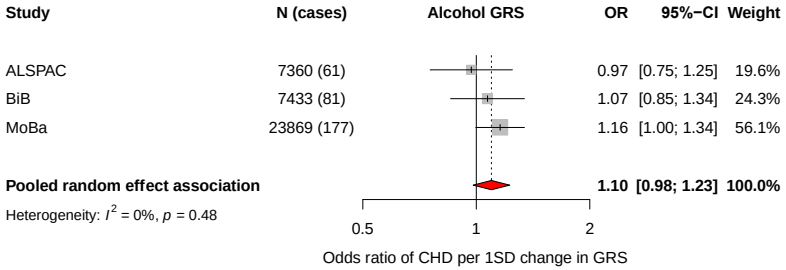
A: Main analyses



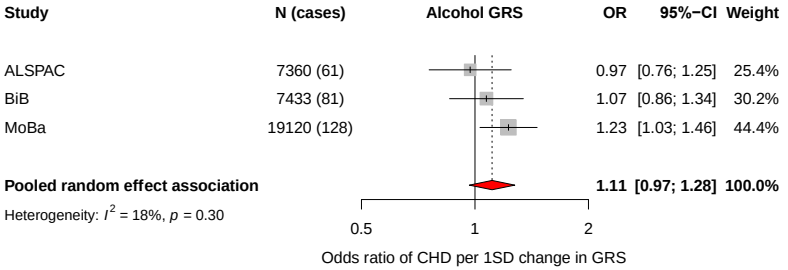
B: Main analyses excluding BiB



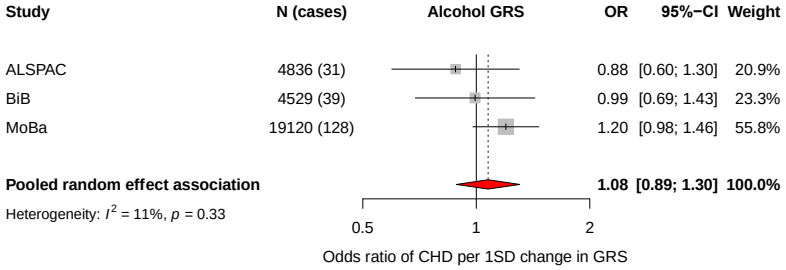
C: MVMR − Smoking GRS



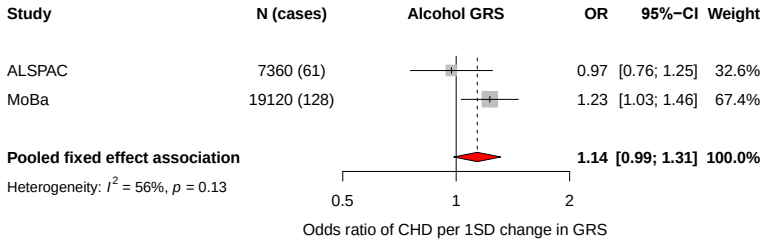
D: Main analyses in fetal genotype sub−population



E: Main analyses with adjustment for fetal genotype



F: Main analyses in fetal genotype sub−population excluding BiB



G: Main analyses with adjustment for fetal genotype excluding BiB

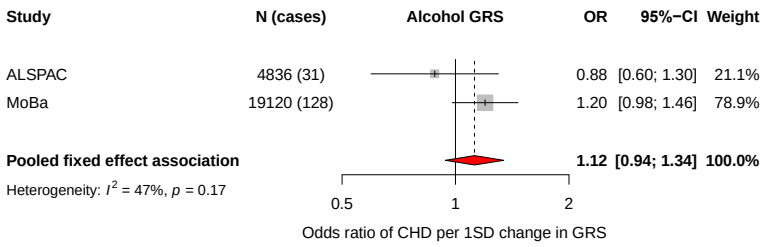


Figure S4.3. Showing the main results and results from additional analyses for the MR analyses of genetically predicted maternal alcohol consumption (using a genetic risk score of drinks per week) and offspring CHDs. Odds ratios (ORs) of CHD for a 1SD difference in maternal GRS in each study and pooled across studies using random effects meta-analysis or fixed-effects analyses when excluding BiB (panels B, G, H). Adjusted for top 10 genetic principal components in all cohorts with additional adjustment for genetic chip, genetic batch, and imputation batch in MoBa. **Panel A:** Main analyses as shown in the main manuscript. **Panel B:** Main analyses excluding BiB. **Panel C:** Main analyses with additional adjustment for genetically predicted smoking (Multivariable Mendelian randomisation analyses). **Panel D:** Main analysis results in the sub-population with fetal genotype. **Panel E:** As Panel D, but with additional adjustment for fetal genotype. **Panel F:** As panel D but excluding BiB. **Panel G:** As panel E but excluding BiB. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; BiB, Born in Bradford; MoBa, Norwegian Mother, Father and Child Cohort Study; BMI, body mass index; CI, confidence interval; CHD, congenital heart disease; SD, standard deviation; GRS, genetic risk score; MVMR, multivariable Mendelian randomisation.

Metabolomics data in the Born in Bradford Cohort

This work has been published:

Taylor K, McBride N, Goulding N, Burrows K, Mason D, Pembrey L, Yang TC, Azad R, Wright J & Lawlor DA. Metabolomics datasets in the Born in Bradford cohort. *Wellcome Open Research* (2021). https://doi.org/10.12688/wellcomeopenres.16341.2.

This paper has been included in appendices for the interested reader. It includes extensive detail on the methods and quality control of the Born in Bradford Metabolomics data.

I am joint first author with another (then) PhD student, Nancy McBride. We contributed equally to this publication. I was fortunate enough to be one of the first researchers to use the Born in Bradford (BiB) metabolomics data and these data are an important aspect of this thesis. As more people began to use the data, it became clear that there would be value in describing the metabolomics data available in the BiB cohort for our theses and the wider research community. Nancy and I conceptualised the idea together, undertook data curation, wrote the first draft and subsequent drafts in response to co-authors and reviewers' comments.

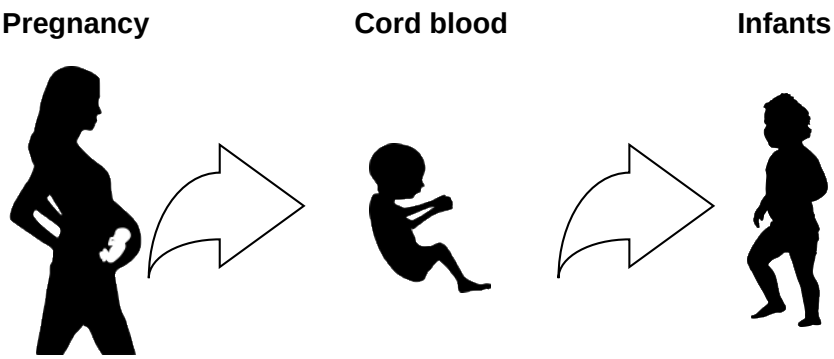
Introduction

Metabolomics is the quantification of small molecules resulting from metabolic processes. The metabolome is influenced by both genotype and environment, and dynamically responds to environmental influences. Developments in high-throughput technologies have allowed the efficient and accurate quantification of metabolites. This has revolutionised our ability to understand the causes and consequences of variation in human metabolism, and the contribution that multiple metabolites can make to risk prediction, using large-scale epidemiological studies [76,183–185]. Lipids and lipoproteins, which are measured in most high-throughput platforms used in epidemiology, are larger than the threshold used to define metabolites (<1.5k Daltons) and are therefore metabolomic traits. For simplicity in this paper, I refer to these as metabolites.

Birth cohorts can be useful for exploring prenatal influences on birth and later life outcomes. Recently, studies have shown metabolomic profiling can aid us in our understanding of maternal health during pregnancy [78,184,185] and of the influence of in utero exposures on subsequent offspring health [186,187]. The Born in Bradford (BiB) study is a UK longitudinal birth cohort [134]. Nuclear magnetic resonance (NMR) and mass spectrometry (MS) data are available in BiB including measurements during pregnancy, cord blood and early life in the offspring. MS offers a truly untargeted approach with comprehensive coverage of the metabolome (>1,000 metabolites) due to its high sensitivity. However, MS only provides relative quantification based on peak area in these approaches without comparison to a metabolite reference standard. NMR offers less coverage of the metabolome, but with absolute quantification possible in clinically meaningful units (e.g., mmol/L).

The range of metabolomics data in BiB, coupled with the substantial data obtained through questionnaires, research clinic assessments, linkage to medical records, educational and social records, genome-wide (mothers, offspring and a subgroup of fathers) and epigenome-wide (mother and offspring) profiling makes BiB a valuable resource for metabolomics research. This data note describes the metabolomics data currently available in BiB – how these were obtained, quantified, utilised, as well as potential future uses, strengths and limitations. **Figure 1** provides an illustrative summary of which type of metabolomic data have been collected on which cohort participants and when, up to 2020. Planned further metabolomic data collection is also described (see *Using the BiB metabolomic data*).

Born in Bradford Metabolomics Data



Nuclear Magnetic Resonance Metabolomics (N = 227 metabolic traits)		
NMR-Dataset 1: 11,480 pregnancies with NMR Metabolomics from fasting serum at ~26-28 weeks' gestation.	***NMR-Dataset 2:*** 7,980 children with NMR Metabolomics using cord blood.	***NMR-Dataset 3***: 2,108 infants with NMR Metabolomics from serum at either age 12- or 24-months.
Mass Spectrometry Metabolomics (N = ~1,000 metabolites)		
MS-Dataset 1a: 1,000 pregnancies with Mass Spectrometry Metabolomics from fasting EDTA plasma at ~26-28 weeks' gestation.	***MS-Dataset 1b***: 1,000 children paired with women from dataset 1a. Mass Spectrometry Metabolomics using cord blood.	***MS-Dataset 2***: 2,000 pregnancies with Mass Spectrometry Metabolomics from fasting EDTA plasma at ~26-28 weeks' gestation.

Figure 1. Summary illustration of the Born in Bradford metabolomics data.

Abbreviations: NMR, nuclear magnetic resonance; MS, mass spectrometry; EDTA, ethylenediaminetetraacetic acid.

Methods

Ethical approval and consent

Ethical approval for the study was granted by the Bradford National Health Service Research Ethics Committee (ref 06/Q1202/48), and all participants gave written informed consent. The ALL IN sub-study had ethical approval from the London School of Hygiene & Tropical Medicine ethics committee (ref: 5320) and the Bradford Research Ethics committee (ref: 08/H1302/21). Parents (usually the mother) gave informed, written consent to take part in the study.

Cohort

The BiB study is a population-based prospective birth cohort. In total, 12,453 women who experienced 13,776 pregnancies were recruited at their oral glucose tolerance test (OGTT) at approximately 26–28 weeks' gestation, which was offered to all women booked for delivery at Bradford Royal Infirmary (BRI) (with the exception of those with pre-existing diabetes (N = 70 – 0.5% of BiB pregnancies)). Eligible women had an expected delivery between March 2007 and December 2010. The study is unique because it includes high proportions of White European and South Asian families, all residing in Bradford, UK. Bradford is a city in the North of England with high levels of socioeconomic deprivation, and the cohort was started due to a high prevalence of poor child health in the city [134]. Full details of the study methodology were reported previously [134]. The study website provides more information, including protocols, questionnaires and information on how researchers can access data and a full list of all available data. Mothers and their partners, who were recruited into the study, provided detailed interview questionnaire data, measurements, and biological samples. They also consented to the linkage of their and their child's data to routine (primary and secondary care) health and education data.

Blood sampling

Maternal overnight-fasted blood was taken during the OGTT and processed and stored at -80°C for further research and analyses. Infant cord blood samples were taken whenever possible (i.e. so long as staff were available, and collection of an umbilical vein sample did not interfere with care of the mother

or infant) and immediately processed and stored at -80°C. Samples were taken in a subgroup of offspring in early childhood for a specific project on childhood viral infection [188]. I describe the processes of taking, processing, and storing samples at each time point before moving on to describe the NMR and MS metabolomic profiling.

Pregnancy blood samples

Of the 13,776 pregnancies in the BiB cohort, 11,480 had a fasting blood sample taken during the OGTT (n = 10,574 [92%] between 26–28 weeks' gestation, with the remaining women being within 11–39 weeks' gestation). Samples were taken by trained phlebotomists working in the antenatal clinic of the BRI and sent immediately to the hospital laboratory.

Venous blood was collected in GEL tubes to obtain serum and plasma. The following processing steps were undertaken prior to storage at -80°C.

1) Storage racks were prepared.
2) Participant details were checked, making sure that both the BiB study ID and hospital number on the specimen bottles matched those on the participant tracking forms.
3) Tubes were centrifuged at 3500 rpm for 10 minutes at room temperature.
4) A 1 ml automatic pipette was used to aliquot samples into 1.5 ml aliquots (1-4 aliquots dependent on sample volume).
5) Vials were labelled with appropriate BiB study labels and the duplicate barcode label was placed in the corresponding space marked on BiB tracking form.
6) Aliquots were then placed in racks in a -80°C freezer.

All samples were processed within 2.5 hours and then placed in -80°C freezers. There were no freeze-thaw events of the samples prior to their use for the pregnancy metabolomic profiling. Serum samples were used for NMR metabolomic profiling, except for five (0.04%) samples which were plasma. For MS pregnancy metabolomics, ethylenediaminetetraacetic acid (EDTA) (a sample tube anticoagulant) plasma samples were used. Previous work has shown that reproducibility in both serum and plasma is good. As long as the same blood sample procedures are used (as in BiB), either matrix should yield similar results [189].

Cord blood samples

Venous cord blood samples were all obtained at delivery by the attending midwife at the BRI, following research protocols. Cord blood sampling was not attempted for women delivering outside of the BRI, if the attending midwife was too busy, or if attempting to collect a research cord blood sample would interfere with postnatal care. Samples were refrigerated at 4°C in EDTA tubes until collected by BRI laboratory staff within 12 hours. Samples were then spun, frozen and stored at -80°C. In total, the BiB study collected 9,604 cord blood EDTA plasma samples. There were no freeze-thaw events of the cord blood samples.

Infant blood samples

Infant metabolomics were performed on blood samples that were collected on a subsample of the BiB cohort; those enrolled into the Allergy and Infection Study (ALL IN) [188] Children enrolled in the BiB cohort, and born on or after 1 March 2008 with a maternal baseline questionnaire were eligible to take part in ALL IN. Mothers were invited to participate in ALL IN one month before their child's first birthday. A questionnaire was completed by those who consented, and a 5ml venous blood sample was taken from the child, centrifuged, and stored at -80°C. This was repeated one year later to provide questionnaire data and serum from a ~12-month visit (mean age of 14 months, ranging from 9–18 months) and a ~24-month visit (mean age of 26 months, ranging from 23–33 months). Trained community research administrators (CRAs) recruited participants, obtained consent, and collected data, including blood samples, at each visit. They received training in phlebotomy and were assessed by the senior paediatric phlebotomist at the BRI. Ametop cream or Cryogesic spray were used to anaesthetise the venepuncture site. Only two attempts at venepuncture were permitted for each child. There was a fridge in the clinic for storing bloods before transfer to the lab. The blood samples taken on home visits were kept in a cool bag with an ice pack and then taken straight to the laboratory at BRI within 1–2 hours. The times of each step (blood taken, arrived at lab, centrifuged, aliquoted, frozen) were recorded on the blood form and were entered onto a database (so that researchers can check distribution of times if needed). For home or clinic visits outside normal working hours, the CRA who took the blood sample would centrifuge the blood at the lab and leave it in the lab fridge for processing the next day. All infant metabolomics were performed on serum samples. There was a maximum of two freeze-thaw events prior to metabolomics analyses of the infant samples.

Metabolomics datasets in BiB

There are six metabolomics datasets including different populations and timepoints available in BiB. These are described below and summarised in **Table 1** and **Figure 1**. I have divided the methods between the two main platforms (NMR and MS). I describe the methods used to generate each dataset and use flow charts to illustrate how selection was performed.

Table 1. Metabolomics datasets in the BiB cohort separated by platform.

#	Data source	Brief description
Nuclear magnetic resonance		
1	Pregnancy NMR – Dataset 1	N = 11,480 pregnancies. Single timepoint using maternal serum taken from a fasted blood sample around 26–28 weeks' gestation. Of the 11,480, 37% are White British (40% White European) mothers and 44% Pakistani (49% South Asian).
2	Cord blood NMR – Dataset 2	N = 7,980 children. Single timepoint using cord blood, EDTA plasma.
3	Infants NMR (aged 12 or 24 months) – Dataset 3	N = 2,108 at either 12- or 24-months using serum samples. N = 1,690 at 12 months. N = 1,536 at 24 months. N = 1,118 at both timepoints.
Mass spectrometry		
4	Pregnancy MS – Dataset 1a	N = 1,000 pregnancies. Single timepoint using EDTA plasma taken from a fasted blood sample between 26–28 weeks' gestation. Of the 1,000, 50% are White British and the other 50% are Pakistani ethnicity.
5	Cord blood MS – Dataset 1b	N = 1,000 children (paired with women from Dataset 1a). Single timepoint using cord blood, EDTA plasma.
6	Pregnancy MS – Dataset 2	N = 2,000 pregnancies within a case-cohort design. EDTA plasma sample taken between 26–28 weeks' gestation. Of the 2,000 women, 47% are White British and 53% are Pakistani.
Abbreviations: NMR, nuclear magnetic resonance; MS, mass spectrometry; EDTA, ethylenediaminetetraacetic acid.		

NMR metabolomics

NMR methods

I describe the NMR methods which apply to all the NMR datasets described in **Table 1**. Profiling of circulating lipids, fatty acids, and metabolites was done by a high-throughput targeted NMR platform

(Nightingale Health© (Helsinki, Finland)) at the University of Bristol, providing quantitative information on 227 metabolic traits (including ratios and other traits derived from the quantified NMR spectra) [76].

The Nightingale NMR metabolite quantification was achieved through measurements of three molecular windows from each serum/plasma sample. Two of the spectra (LIPO and LMWM windows) are acquired from native serum/plasma and one spectrum from serum lipid/plasma extracts (LIPID window). The NMR spectra are measured using Bruker AVANCE III spectrometer operating at 600 MHz. Measurements of native serum/plasma samples and serum/plasma lipid extracts are conducted at 37°C and 22°C, respectively.

The NMR spectra were analysed for metabolite quantification (molar concentrations) in an automated fashion. For each metabolite, a ridge regression model was applied for quantification to overcome the problems of heavily overlapping spectral data. In the case of the lipid data, quantification models were calibrated using high-performance liquid chromatography methods, and individually cross-validated against NMR-independent lipid data. Low-molecular-weight metabolites, as well as lipid extract measures, were quantified as mmol/L based on regression modelling calibrated against a set of manually fitted metabolite measures. The calibration data were quantified based on iterative line-shape fitting analysis using PERCH NMR software (PERCH Solutions Ltd., Kuopio, Finland). Quantification could not be directly established for the lipid extract measures due to experimental variation in the lipid extraction protocol. Therefore, serum/plasma lipid extract were scaled to total a standard serum cholesterol sample from the LIPO spectrum.

Validation of the NMR platform

Quality control (QC) of the data were undertaken by Nightingale Health© prior to returning metabolite concentrations to BiB. Their QC procedures check various issues related to the sample integrity and the biomarker quantification.

I also undertook validation of some of the NMR measures by comparing concentrations of fasting glucose, total cholesterol, high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), and triglycerides from the NMR platform to the same measures from the same samples assessed by clinical chemistry measurements (**Figure 2**). Clinical chemistry measurements were completed at the BRI laboratory (fasting glucose) or Glasgow Royal Infirmary (lipids). Glucose was measured using a glucose oxidase method that does not cross-react with insulin. Total cholesterol, HDLc and triglycerides were

measured following the standard Lipid Research Clinics Protocol using enzymatic reagents. LDLc was estimated from total cholesterol, HDLc and triglycerides (LDLc = [Total cholesterol in mmol/l] – [HDLc in mmol/l] – [Triglycerides in mmol/l ÷ 2.2]). The correlation between fasting glucose measured by clinical chemistry and by NMR was 0.73 and for all four lipids was between 0.85 and 0.93, with the intercepts of the regression line close to zero for HDLc, LDLc, and triglycerides, but higher for glucose (1.85) and total cholesterol (1.21). This suggests that the NMR platform systematically underestimates glucose and total cholesterol levels. However, the high levels of correlation, particularly for the lipid measures, is reassuring and suggests association analyses would have validity. It is evident from **Figure 2** that there are outliers for some of the measures, notably for glucose, total cholesterol and triglycerides (**Figures 2A, 2B, 2E**, respectively). I would recommend for researchers using the data to consider these potential outliers before commencing analyses. Determining how to deal with outliers will depend on the research question and the personal preference of the research group undertaking analyses. To further test the validity of the NMR measures, I compared associations of maternal early pregnancy body mass index (BMI), treated as an exposure, with fasting glucose, and the four lipid measures from clinical chemistry and NMR as the outcome. I also compared associations between the five metabolic measures (from clinical chemistry and NMR) as exposures, with hypertensive disorder of pregnancy (HDP; either gestational hypertension or pre-eclampsia, defined based on international criteria applied to all measures of blood pressure and proteinuria extracted from clinical records) [190] as the outcome. Associations of BMI with the five outcomes were directionally consistent between clinical chemistry and NMR measurements. However, the NMR associations were weaker (closer to the null) and there were clear differences in magnitudes of association between the two methods for the associations of BMI with glucose and HDLc (**Figure 3A**). By contrast, results were concordant between the two methods for the associations of metabolites with odds ratios of HDP (**Figure 3B**). Given the relatively modest correlation of glucose from the Metabolon MS analyses with the clinical chemistry levels on the same samples, I explored this further comparing results from two regression analyses – one of the difference in mean glucose per 1SD higher BMI (glucose as outcome) and one of the odds ratio for HDP per 1SD higher glucose (glucose as exposure).

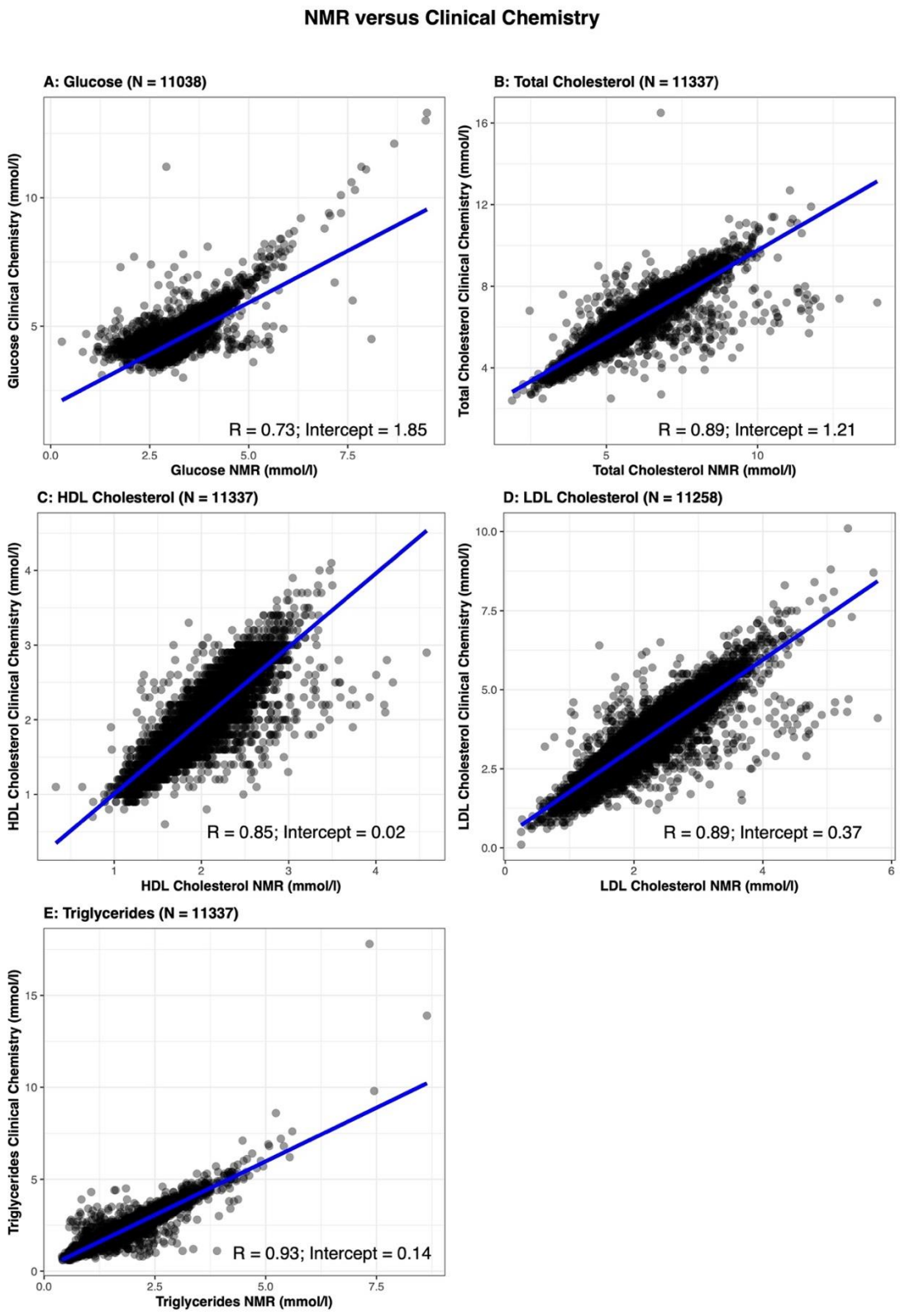


Figure 2. Comparison of glucose (2A), total cholesterol (2B), high-density lipoprotein cholesterol (2C), low-density lipoprotein cholesterol (2D) and triglycerides (2E) concentrations between Nightingale Health© nuclear magnetic resonance (NMR) (x-axis) and routine clinical chemistry assays (y-axis) (N= 11,036 to 11,337). R = Pearson correlation coefficient.

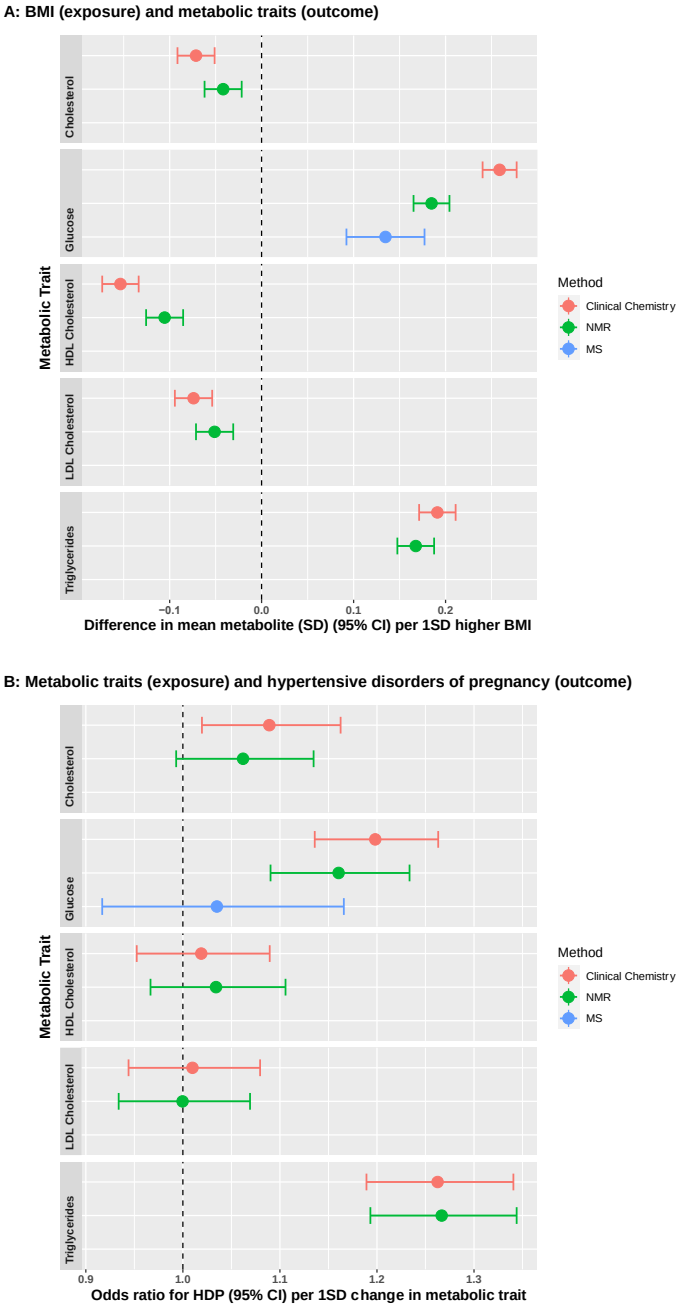


Figure 3. Comparisons of the associations of early pregnancy body mass index (BMI) with fasting glucose and lipids measured by routine clinical chemistry assays, Nightingale Health© nuclear magnetic resonance (NMR) and mass spectrometry (MS, glucose only) (3A), fasting glucose and lipids measured by routine clinical chemistry assays, Nightingale Health© NMR and MS (glucose only) with hypertensive disorder of pregnancy (HDP) (3B).

Associations in 3A are from unadjusted linear regression and data points show standard deviation (SD) differences in mean metabolite per one standard deviation (1SD) higher BMI. Associations in 3B are from unadjusted logistic regression and data points show unadjusted odds ratios for HDP per 1SD higher in metabolic trait. Error bars = 95% confidence intervals.

Participant selection and characteristics of those with NMR data

In this subsection, I present flow charts to illustrate selection and inclusion into the NMR participant groups (**Figure 4**) and describe participant characteristics for the BiB NMR datasets (**Table 2).** All three of the samples of BiB participants with NMR data (maternal pregnancy N = 11,480, offspring cord blood N = 7,980, and offspring 12–24 months N = 2,108) had very similar distributions of maternal age, parity, early pregnancy BMI, residential area deprivation, offspring sex and birth weight to those seen in the whole cohort of 13,776 participants (**Table 2**).

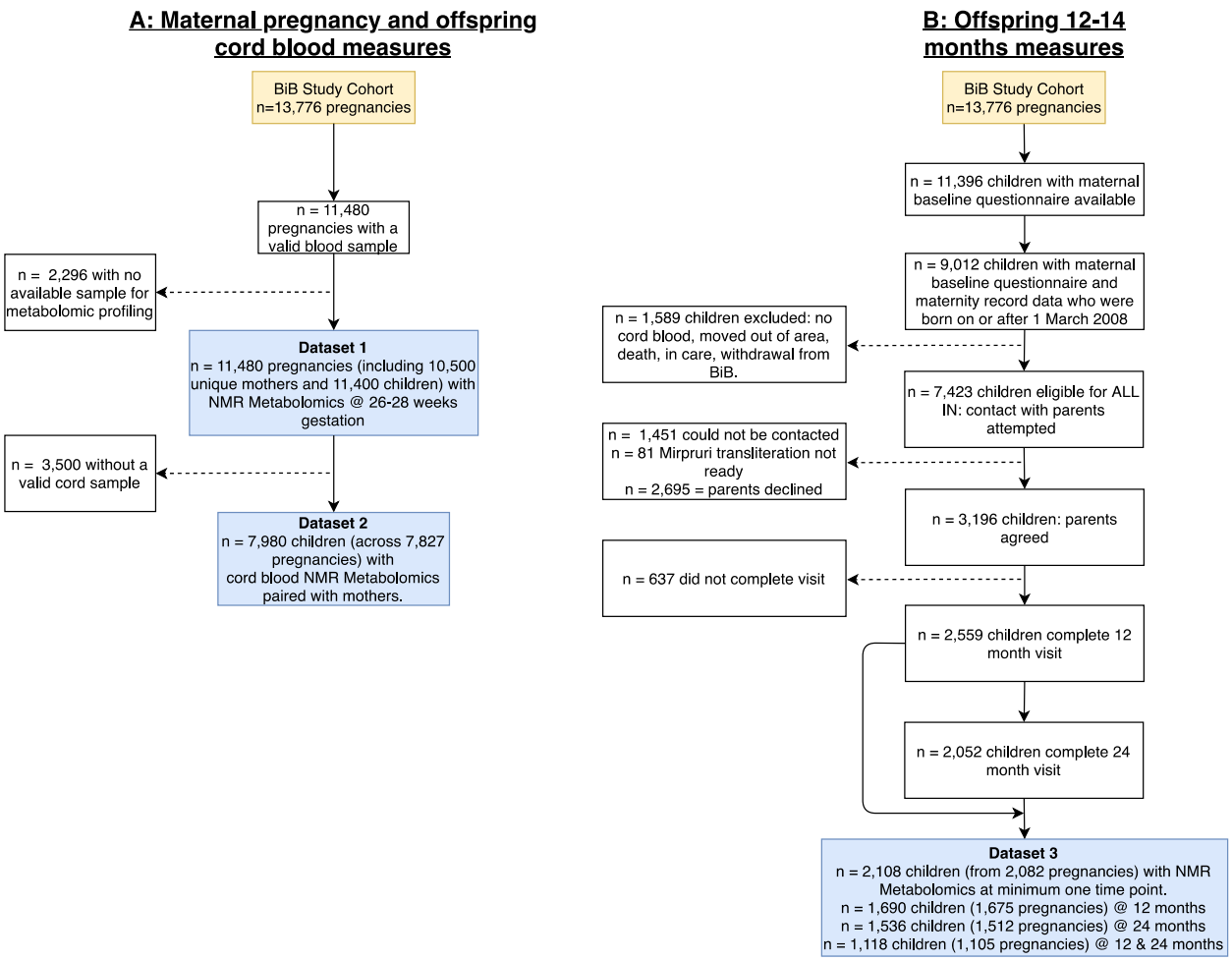


Figure 4. Illustrating the flow of participants into the NMR datasets in the Born in Bradford cohort. Figure 4A shows the maternal pregnancy (Dataset 1: NMR metabolomics at 26–28 weeks' gestation) and offspring cord blood samples (Dataset 2: NMR metabolomics taken from the umbilical vein shortly after delivery). Figure 4B shows the offspring 12–24 months NMR metabolomic sample (Dataset 3). Abbreviations: NMR, nuclear magnetic resonance; BiB, Born in Bradford; ALL IN, Allergy and Infection study.

Table 2. Participant characteristics for NMR datasets in the BiB cohort.

		Maternal pregnancy NMR dataset (n=11,480)	Offspring cord blood NMR dataset (n=7,890)	Offspring 12- or 24-months NMR dataset (n = 2,108)	BiB cohort (n=13,776)
Characteristics	**Unit / Category**				
Maternal Age	Years	27.3 (5.6)	27.5 (5.6)	28.3 (5.7)	27.3 (5.6)
	Missing	410 (3.6)	627 (7.9)	60 (2.9)	1445 (10.5)
Maternal Parity	Nulliparous	4310 (37.5)	2765 (36.6)	819(39.9)	5101 (37.0)
	Multiparous	6428 (55.9)	5125 (65.0)	1,233(58.4)	7773 (56.4)
	Missing	742 (6.5)	344 (4.4)	56 (2.7)	902 (6.5)
Maternal BMI	kg/m^2	26.1 (5.7)	26.2 (5.7)	26.2(5.5)	26.0 (5.7)
	Missing	2160 (18.8)	1464 (18.5)	106 (5.0)	3281 (23.8)
Maternal ethnicity	White British	4268 (37.2)	2902 (37.7)	769 (49.7)	5055 (37.8)
	Pakistani	4995 (43.5)	3596 (46.7)	1048 (49.7)	6088 (45.5)
	Other	1887(16.4)	1206 (15.7)	291 (13.8)	2223 (16.6)
	Missing	330 (2.4)	186 (2.4)	0	410 (3.0)
Index of multiple deprivation	Quintile 1 (most deprived)	6646 (65.9)	4439 (65.8)	1400(66.4)	7566 (66.4)
	Quintile 2	1830 (18.2)	1220 (18.1)	355 (16.8)	2052 (18.0)
	Quintile 3	1124 (11.2)	778 (11.5)	248 (11.8)	1250 (11.0)
	Quintile 4	306 (3.0)	187 (2.8)	70 (3.3)	334 (2.9)
	Quintile 5 (least deprived)	173 (1.7)	118 (1.8)	34(1.6)	190 (1.7)
	Missing	1401 (12.2)	1148 (14.6)	1 (0.0)	2384 (17.3)
Offspring sex	Male	5705 (49.7)	4095 (51.9)	1065(50.2)	6891 (50.0)
	Female	5420 (48.7)	3795 (48.1)	1029(48.1)	6470 (48.4)
	Missing	355 (3.1)	3 (0.0)	14 (0.7)	415 (3.0)
Birth weight	Grams	3226 (565)	3266 (522)	3224 (558)	3216 (565)
	Missing	356 (3.1)	4 (0.1)	14 (0.7)	416 (3.0)

Data are mean ± SD or n (%) unless stated. Abbreviations: NMR, nuclear magnetic resonance; BiB, Born in Bradford; BMI, body mass index; kg, kilogram; IMD, Index of Multiple Deprivation (taken from 2010 national quintiles). There were 9 ethnic groups, of which White British and Pakistani were the main homogeneous groups. The 'Other' ethnicity category comprises: White Other, Mixed-White and Black, Mixed-White and South Asian, Black, Indian, Bangladeshi or Other ethnicity.

Mass spectrometry metabolomics

Mass spectrometry methods

The untargeted MS metabolomics analysis of over 1,000 metabolites was performed at Metabolon, Inc. (Durham, North Carolina, USA). Samples were sent to Metabolon in two separate batches. Dataset 1 was completed in December 2017 and consisted of 1,000 maternal pregnancy samples and 1,000 offspring paired cord blood samples. Dataset 2 was completed in December 2018 and consisted of 2,000 maternal pregnancy samples.

At Metabolon, samples were managed by a laboratory information management system and were kept at -80°C. Recovery standards were added to samples prior to monitor the extraction process. To remove proteins, dissociate small molecules bound to proteins, disassociate molecules trapped in the precipitated protein matrices, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods with positive ion mode electrospray ionization (ESI), one for analysis by reverse phase UPLC-MS/MS with negative ion mode ESI, one for analysis by hydrophilic interactive liquid chromatography (HILIC)/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

The instrument configuration, data acquisition, and metabolite identification and quantitation used by Metabolon have been described previously [191]. To summarise, the structure of metabolites were identified by matching the ion features (retention time, molecular weight (m/z), MS fragmentation pattern, preferred adducts, and in-source fragments) in the study samples to a reference library of chemical standard entries. The confidence of this metabolite identification met most stringent tier 1 criteria defined by Schrimpe-Rutledge et al [192]. Peaks were quantified using area-under-the-curve of primary MS ions. To adjust for instrument batch effects for each run day, the raw ion counts for each metabolite were divided by the median value for the run day. Missing values were assumed to be the result of falling below the detection sensitivity, and thus were imputed with the minimum detection value based on each metabolite.

This process provides relative quantification (i.e., multiples of the median (MoM) for the days run) of >1,000 metabolites in 10 key classes: amino acids, carbohydrates, lipids, nucleotides, microbiota metabolism, carbon metabolism, energy, cofactors & vitamins, xenobiotics, and unidentified metabolites.

Validation of the MS platform

Metabolon conducted data QC for the BiB datasets. Procedures were conducted to: (i) assure that all aspects of the Metabolon process are operating within specifications, (ii) assess the effect of a non-plasma matrix on the Metabolon process and distinguish biological variability from process variability, (iii) assess the contribution to compound signals from the process (using Process Blank) and (iv) segregate contamination sources in the extraction (using Solvent Blank).

As an additional data QC, I explored correlations between MS and both NMR and clinical chemistry fasting glucose measures (glucose is the only common trait I have data on for MS, NMR, and clinical chemistry). Pearson's correlation coefficient comparing MS to clinical chemistry (0.65) was modest and lower than that for NMR (0.73, see above and **Figure 2**) and the intercept was 0.11 (**Figure 5A**). Correlation between Metabolon and NMR was higher (0.77) and the intercept was 0.10 (**Figure 5B**).

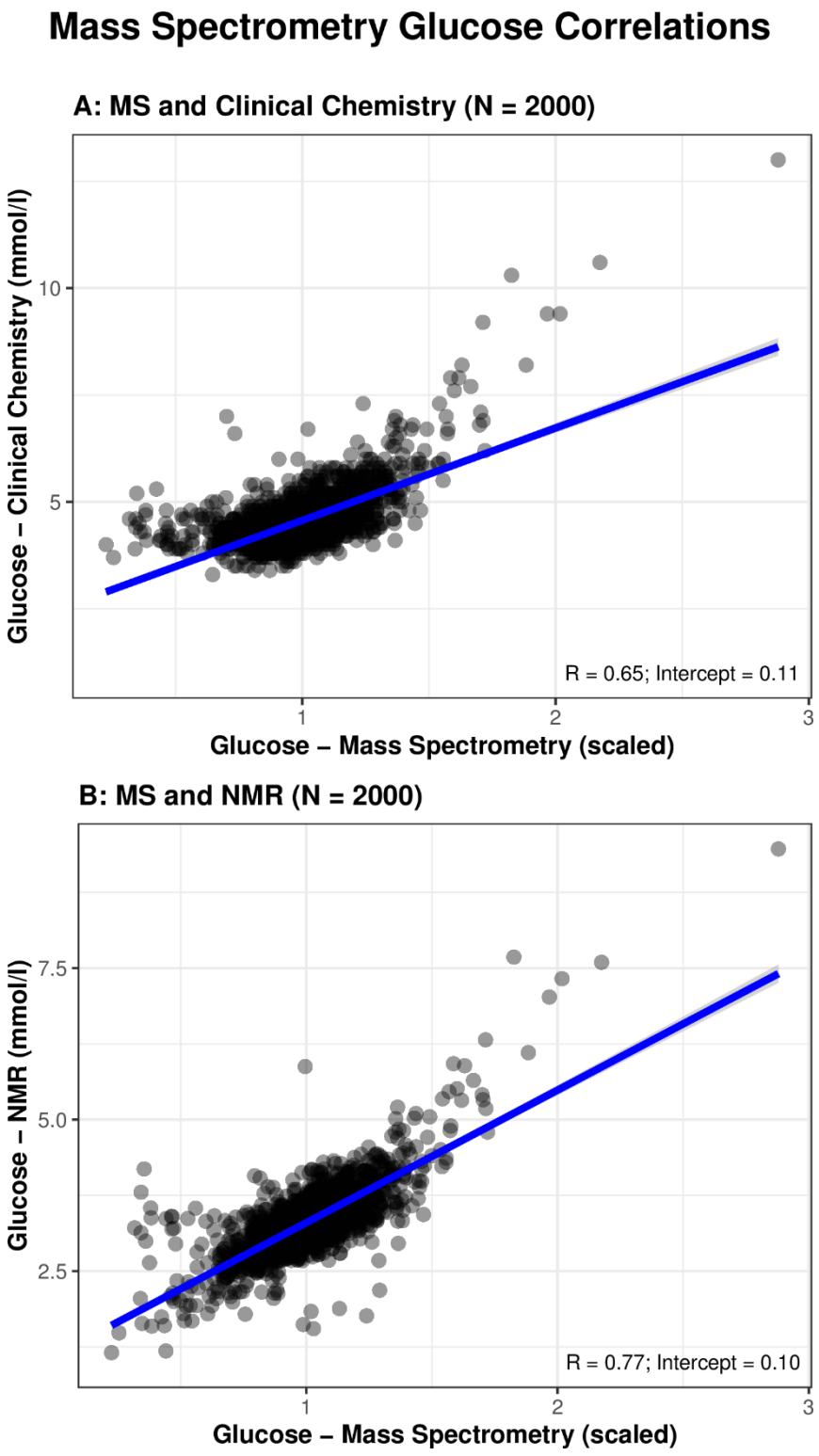


Figure 5. Comparisons of glucose concentrations for Metabolon mass spectrometry (MS) with routine glucose oxidase (5A) and Nightingale Health© nuclear magnetic resonance (NMR) (5B). R = Pearson correlation coefficient.

Participant selection and characteristics of those with MS data

The flow of participants into the MS datasets are illustrated in **Figure 6**, and the characteristics of participants included in the two MS datasets, together with characteristics of the whole BiB cohort are provided in **Table 3**. Selection processes for both MS datasets mean that I would not expect distributions of characteristics in these to reflect the whole cohort. Only women of either Pakistani or White British ethnic background were included in the MS datasets because, due to cost, BiB were only able to do this on a subset of the cohort. As these two groups represent ~85% of BiB it was felt the numbers for any other group would be too small for meaningful analyses. In Dataset 1, 1,000 women were selected on the basis that they had stored fasting plasma, a useable cord blood sample, genome wide data on both mother and offspring and were either of White British or Pakistani origin (**Figure 6A**). Following these inclusions, 500 women were selected at random from each ethnic group (White British and Pakistani). In Dataset 2, a case-cohort design was used [193,194]. A case-cohort design consists of a cohort with an over-sampling of all cases. The BiB case-cohort consists of 2,000 women (only pregnancy samples were assayed in Dataset 2). As with Dataset 1, women were selected based on certain characteristics shown in **Figure 6B**, including that they had not already had Metabolon MS analyses. From those who fulfilled these pre-specified criteria, six groups of cases were selected: women with (a) gestational diabetes; (b) gestational hypertension; (c) pre-eclampsia; (d) preterm birth; (e) congenital anomaly; (f) stillbirth. In total, 801 women had experienced one or more of these conditions. Having selected all cases these were then replaced into the eligible cohort and a sub-cohort of 1,199 women were randomly selected from the eligible cohort. Thus, the comparison group in this case-cohort study is representative of the eligible cohort (i.e., the cohort comparison group includes some of the cases in proportions that would reflect the whole cohort). The final BiB case-cohort sample consists of three groups (**Figure 6B**): 1) selected as comparison group (N = 1,199), 2) selected as cases only (N = 408), and 3) selected as a case and control (N = 393). The comparison group in any analyses will vary depending on the research question.

For the MS dataset, researchers are given the option of using the 'raw' data from Metabolon or a quantified (scaled) data set, in which missing data have been imputed and the multiple of median values transformed to SD- (z-) scores (by subtracting the sample mean value for each metabolite from the participant value and then dividing by the sample standard deviation for that metabolite). This transformation helps overcome the problem of high missing data in metabolomics [195]. This cohort for MS profiling were sampled on their ethnicity. It is almost 50% White British and Pakistani (there are slightly more Pakistani women in Dataset 2), as opposed to around 15% of the whole BiB cohort not belonging to

either of these ethnic groups. However aside from this, the sample is representative of the whole cohort (**Table 3**).

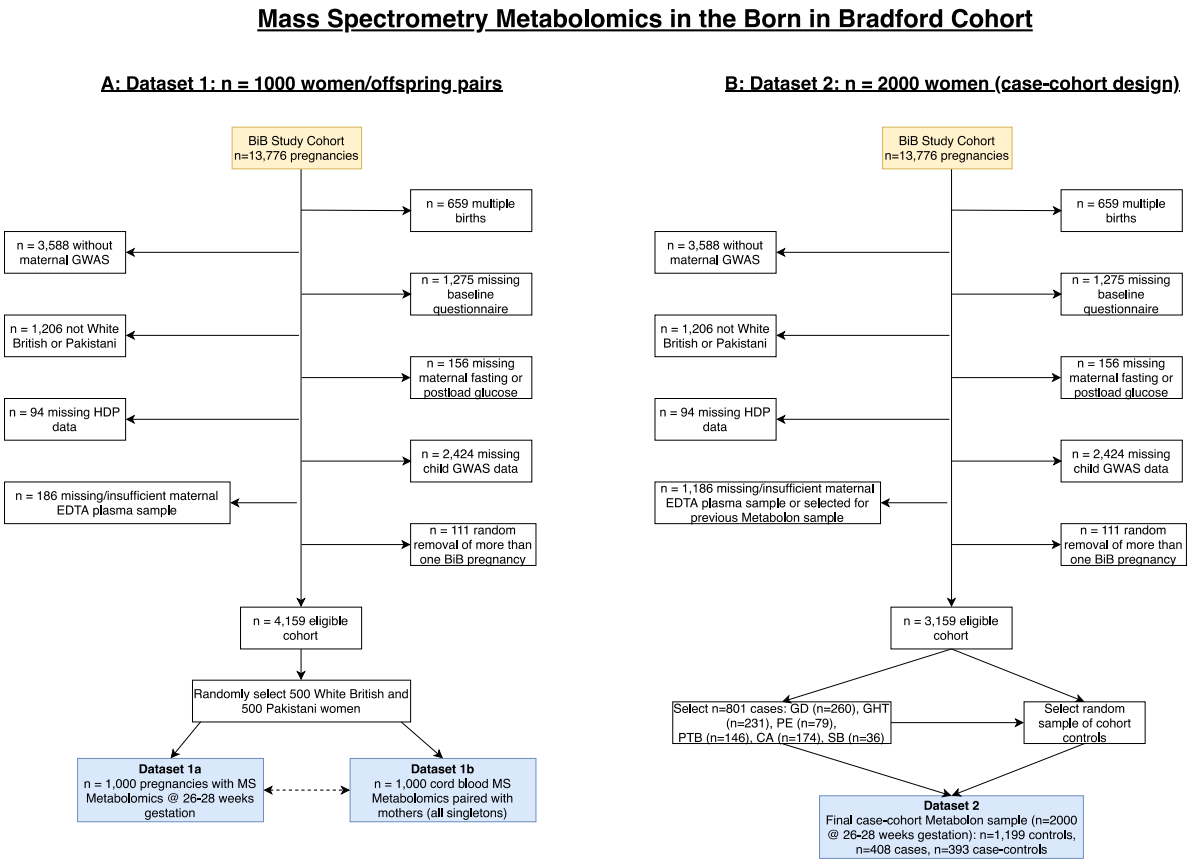


Figure 6. Illustrating the flow of participants into the Metabolon datasets in the Born in Bradford cohort.

Table 3. Participant characteristics of the mass spectrometry datasets in the BiB cohort.

		Dataset 1 (N = 1,000 mother/child pairs)	Dataset 2 case-cohort [a] (N = 2,000)	Dataset 2 random cohort sample only [b] (N = 1,199)	BiB cohort (N = 13,776)
Characteristics	**Category**	-	-		-
Maternal age	Years	27.5 (5.7)	27.5 (5.7)	26.91 (5.5)	27.3 (5.6)
	Missing	0 (0)	0 (0)	0 (0.0)	1445 (10.5)
Maternal parity	Nulliparous Multiparous *Missing*	359 (37.0) 611 (61.1) 30 (3.0)	745 (37.3) 1213 (60.1) 42 (2.1)	433 (37.4) 725 (60.5) 41 (3.4)	5101 (37.0) 7773 (56.4) 902 (6.5)
Maternal BMI	(kg/m^2) *Missing*	26.7 (6.0) 36 (3.6)	26.8 (5.9) 97 (4.9)	25.9 (5.4) 60 (5.0)	26.0 (5.7) 3281 (23.8)
Maternal ethnicity	White British Pakistani Other *Missing*	500 (50.0) 500 (50.0) 0 0	933 (46.7) 1067 (53.4) 0 0	537 (44.8) 662 (55.2) 0 0	5055 (37.8) 6088 (45.5) 2223 (16.6) 410 (3.0)
Index of multiple deprivation	Quintile 1 (most deprived) Quintile 2 Quintile 3 Quintile 4 Quintile 5 (least deprived) *Missing*	656 (65.6) 175 (17.5) 112 (11.2) 38 (3.8) 19 (1.9) 0 (0)	1340 (67.0) 358 (17.9) 212 (10.6) 53 (2.6) 37 (1.8) 0 (0)	823 (68.6) 203 (16.9) 123 (10.3) 31 (2.6) 19 (1.6) 0 (0)	7566 (66.4) 2052 (18.0) 1250 (11.0) 334 (2.9) 190 (1.7) 2384 (17.3)
Offspring sex	Male Female *Missing*	512 (51.2) 488 (48.8) 0 (0)	1053 (52.7) 947 (47.3) 0 (0)	625 (52.1) 574 (47.9) 0 (0)	6891 (50.0) 6470 (48.4) 415 (3.0)
Offspring birthweight	Grams *Missing*	3304 (517) 0 (0)	3232 (574) 1 (0)	3318 (486) 0 (0)	3216 (565) 416 (3.0)

[a] This column comprises of the full case-cohort dataset of 2,000 pregnancies. This includes 801 selected cases and the 1,199 random cohort.
[b] This column includes only the 1,199 random cohort to compare to the full case-cohort with the selected cases. Data are mean ± SD or n (%) unless stated.
Abbreviations: BiB, Born in Bradford; BMI, body mass index; kg, kilogram; IMD, Index of Multiple Deprivation (taken from 2010 national quintiles).
There were nine ethnic groups, of which White British and Pakistani were the main homogeneous groups. The 'Other' ethnicity category comprises: White Other, Mixed-White and Black, Mixed-White and South Asian, Black, Indian, Bangladeshi or Other ethnicity.
Please note because of the way participants were selected into the MS datasets I would not expect characteristics to match those of the whole cohort.

Overlap between metabolomics datasets

Having participants in multiple datasets (i.e., maternal pregnancy, offspring cord, offspring 12–24 months) and across the two metabolomic platforms provides scope for unique research opportunities. **Figure 7** illustrates the overlap between BiB metabolomic datasets. The numbers are all based around the offspring, for example the number of maternal pregnancy metabolite data in any cell refer to the number of offspring who have a mother with those samples. There were 11,557 children from 11,480 pregnancies whose mothers had a pregnancy NMR sample. Of these, 6,756 children also had a cord blood sample and 1,981 had at least one measurement from either the 12- or 24-month ALL IN subsample. All the mothers with a pregnancy MS sample (from either the first or second dataset) also have an NMR sample. There were 7,919 children in total with an NMR sample in cord blood with 1,275 of these also having at least one measure from the 12- or 24-month subsample. Of those with NMR cord blood data, 2,486 had a mother with MS pregnancy data (from either the first or second dataset) and 1,000 have MS cord blood data. There were 2,108 children with at least one NMR measure at either the 12- or 24-months assessment and of these, 690 have a mother with MS metabolite measures in pregnancy data (from either dataset) and 229 have MS cord blood data. Although the exclusion criteria for MS dataset 2 was no prior MS metabolomics (**Figure 6**), there was one mother with MS metabolomics in both datasets from different pregnancies.

	NMR Pregnancy Dataset 1	NMR Cord Dataset 2	NMR Infant Dataset 3	MS Pregnancy Dataset 1a	MS Cord Dataset 1b	MS Pregnancy Dataset 2
NMR Pregnancy Dataset 1	**11557**	**6756**	**1982**	**1000**	**1000**	**2000**
NMR Cord Dataset 2		**7919**	**1275**	**1000**	**1000**	**1486**
NMR Infant Dataset 3			**2108**	**229**	**229**	**461**
MS Pregnancy Dataset 1a				**1000**	**1000**	**0**
MS Cord Dataset 1b					**1000**	**0**
MS Pregnancy Dataset 2						**2000**

Figure 7. Showing the overlap between the metabolomic datasets in the Born in Bradford cohort presented at the offspring level.
Abbreviations: NMR, Nuclear magnetic resonance; MS, mass spectrometry.

Using the BiB metabolomic data, including a summary of published, ongoing and future research using these data

The current BiB metabolite data have been quantified on blood samples collected during pregnancy, cord blood at birth and in the offspring at 12- and 24-months. These are critical time periods for life-course research and the combination of these data with large amounts of genomic, epigenomic, social and health data makes BiB a platform which provides scope for unique research opportunities.

Issues for data users

Batch effects

The quantified NMR metabolites that have been measured in BiB are represented in clinically meaningful units, so can be compared to results from other studies. By contrast the Metabolon MS metabolites are quantified in relative abundance i.e., in relation to other quantified MS measurements that were run on the same day. The MS Dataset 1 and Dataset 2 were obtained ~2 years apart and have been normalised to different references, so are not directly comparable. For example, the value of a specific metabolite from a maternal pregnancy sample in Dataset 1 compared to the same metabolite in Dataset 2 may differ because they are from different batches. Because of the different selection process for the two datasets (Dataset 1 is paired pregnancy-offspring cord blood samples and Dataset 2 has a case-cohort sampling frame) it is not possible to normalize them to the same reference. I recommend running analyses separately in each of the two datasets and comparing results, then meta-analyse if appropriate.

Comparisons with clinical chemistry measurements

I have illustrated above strong correlations between glucose and lipids measured using clinical chemistry and the NMR platform. I found weaker (though directionally consistent) associations of BMI with these outcomes measured using NMR compared to those with clinical chemistry. In a second example, results were consistent between the two methods for the associations of pre-eclampsia with glucose and lipids. Researchers considering using these data might want to check for consistency with associations using the clinical chemistry measurement available in BiB. For the MS data I was only able to explore correlations with glucose and found this to be high between clinical chemistry and MS.

Summary of published research using the BiB metabolomics data

Bristol researchers undertook a collaboration between BiB and the UK Pregnancies Better Eating and Activity Trial (UPBEAT), a randomised control trial of obese pregnant women (BMI ≥ 30kg/m 2) [196]. They found evidence that maternal pregnancy NMR samples can improve prediction of pregnancy-related

disorders [196]. The prediction models consisting of NMR-derived metabolomics and established risk factors (maternal age, smoking, BMI, ethnicity, and parity) performed better than established risk factors alone for gestational diabetes, hypertensive disorders of pregnancy, small/large for gestational age but not preterm birth in BiB. Results were directionally consistent but attenuated in UPBEAT. The attenuated results in that validation sample may reflect the differences between the studies participants characteristics, model overfitting in BiB, or both.

In other work, I have shown that the distributions of most of the NMR metabolic measures differed by ethnicity [197]. White European women had higher levels of most lipoprotein subclasses, cholesterol, glycerides and phospholipids, monosaturated fatty acids, and creatinine but lower levels of glucose, linoleic acid, omega-6 and polyunsaturated fatty acids, and most amino acids, compared with South Asian women. This suggests a more lipidomic pregnancy metabolic profile in White Europeans and a stronger glycaemic metabolic profile in South Asian women. Higher BMI and having gestational diabetes were associated with higher levels of several lipoprotein subclasses, triglycerides, and other metabolites in both groups but with evidence of weaker magnitudes of association for most of these in the South Asian women.

In recent collaborations between the BiB cohort and the Pregnancy Outcome Prediction study (POPs) using Metabolon MS data, there was evidence that 4'-hydroxyglutamate improves prediction of pre-eclampsia compared to clinical risk factors alone 3 and that a ratio of four metabolites (1-(1-enyl-stearoyl)-2-oleoyl-GPC, 1,5-anhydroglucitol,5α-androstan-3α,17α-diol disulfate and N1,N12-diacetylspermine) together with the sFlt-1:PIGF ratio is a better predictor of fetal growth restriction/small for gestational age than sFlt-1:PIGF alone4 . Initial associations in POPs, a nulliparous, largely White European, affluent cohort from the Southeast of England, were validated in BiB. As I have outlined, BiB is a cohort of mixed ethnic background, with high levels of deprivation and including both nulliparous and multiparous women. The consistency of associations between POPs and BiB suggests that the prediction accuracy may be widely generalisable and that the metabolites predicting these outcomes may be causally related to them.

Furthermore, combining the MS metabolomics with genomic sequence data has enabled the establishment of metabolomic consequences of loss of functional rare variants in autozygous individuals and the health effects of this loss of function [198]. This has supported the development of the drug lumasiran for a rare kidney disease [199].

Ongoing and future research

Ongoing work using both the NMR and MS metabolomics data will explore how the pregnancy metabolic environment relates to fetal growth (using repeat ultrasound scan measures and birth weight), preterm delivery, and congenital heart disease. Potential causal effects in these studies will be explored where possible by replication, the use of Mendelian Randomisation (MR) and triangulation with other types of data and study designs. In ongoing work, teams are using data from both MS datasets to evaluate whether MS-derived metabolomics are better predictors of gestational diabetes, hypertensive disorders of pregnancy, small and large for gestational age and preterm birth, than risk factors alone (with external validation being undertaken in the POPs cohort). By combining both NMR and MS data, teams are exploring the relationships between maternal pregnancy metabolites and their offspring cord blood metabolites. To date, there is no published work using the offspring metabolomics data. Researchers can find information on planned follow up data elsewhere, to understand whether these data could be useful to their ongoing or future research [200].

BiB also contributes to metabolomic studies that are being undertaken by large collaborative efforts. This includes the European H2020 funded LifeCycle project [126], in which teams are exploring exposure to maternal hypertensive disorder of pregnancy, gestational diabetes, small and large for gestational age and preterm delivery on offspring subsequent metabolic profile. In the Consortium of Metabolomics Studies (COMETS) [183] there are ongoing projects including trans-ethnic genome-wide association analyses (GWAS), and exploring effects of BMI, smoking, dietary patterns and hypertension on maternal metabolomic profiles.

Discussion and future directions for metabolomic analyses in BiB

In this data note I have described multiple datasets with NMR and MS metabolomic measures in the BiB cohort. The wealth of metabolomics data available in BiB provides opportunities for addressing a range of research questions. In this section, I discuss the strengths and limitations of the data, together with some insights for using these data. I also provide information on plans for future measurements of metabolomics in BiB.

A key strength of these datasets is that they are based within a cohort that has very detailed information on 13,776 pregnancies. This includes detailed socioeconomic, education, cognitive, and mental and physical health data. BiB has OGTT results and fasting pregnancy blood samples on most (83%) of the mothers, genomics (genome wide and sequence) data and epigenomics data in maternal pregnancy

and offspring cord blood. Few studies have pregnancy metabolomics data or OGTT data in numbers of this size. It is possible to look at metabolomics and its role in prediction of adverse pregnancy/perinatal outcomes and health and development in children. BiB has large numbers of South Asian and White European families, residing in a city with high levels of socioeconomic deprivation. The ethnic diversity allows us to try and understand ethnic differences in the developmental origins of disease, for example, why South Asian populations have a higher risk of type two diabetes and coronary heart disease. There is also scope to explore how diet could relate to the range of metabolomic measurements that BiB possesses. Further information on dietary variables can be found online in the BiB data dictionary https://borninbradford.github.io/datadict/.

Having access to two metabolomics profiles (NMR and MS) is beneficial. The NMR platform mostly consists of lipids and lipoproteins, but also provides quantified fatty acids, amino acids, glycolysis metabolites, ketone bodies and glycoprotein acetyl (an inflammatory marker). It provides considerably more information than clinical chemistry measures that are conventionally measured in cohorts (e.g., glucose, total cholesterol, LDLc, HDLc and triglycerides) and at not much more cost (~£20 per sample). As a result, BiB has been able to obtain these data on large numbers of women in pregnancy, offspring cord blood and in samples taken in offspring at 12- and 24- months assessments. By contrast, the MS data covers more of the metabolome, including being able to assess energy metabolism (which might be important in pregnancy) and markers of medications such as paracetamol. However, it is more expensive (~£80-£200, depending on how many samples are assayed at a time). By having access to both datasets here, researchers can have broader coverage of the metabolome [201]. There are potential uses for both platforms – ranging from disease prediction to causal analyses using methods such as MR [202]. Both platforms have been used in previous GWAS of metabolites [203]. As such, BiB could be used to explore whether genetic instruments from GWAS can be related to NMR or MS metabolites in pregnancy.

Access to this unique metabolomic data is a big advantage in BiB. However, there has been difficulty in trying to validate findings in external cohorts. The work described above cannot be replicated because researchers cannot find other independent studies with relevant data [197]. It is hoped that this data note will encourage other studies to collect similar data in pregnancy, offspring cord blood, and in mothers and offspring postnatally throughout their life-course.

There are some additional important limitations of the data to consider. The impact of these limitations will depend on the research question. All the metabolomics datasets were collected on subsamples ranging from 11,480 with maternal pregnancy NMR samples (83% of the eligible 13,776 participants) to 1,000 (7%) with MS cord blood samples. Smaller sample sizes may be statistically

inefficient in some analyses and the selection processes (**Figure 4** and **Figure 6**) may result in selection bias in some analyses. It is notable and provides some reassurance that, even for the smaller samples, distributions of most characteristics are similar between participant groups with different types of metabolomics at different time points and the whole cohort (**Table 2** and **Table 3**). As Metabolon MS data have been collected only on White British and Pakistani women it cannot be assumed that analyses with these data would generalize to other ethnic groups. BiB cohort participants were largely recruited at the OGTT (with a small number recruited after that). This was opportunistic as there was no funding for initiating the cohort. After consultation with the community and health care providers, it was established that this would be a suitable time to obtain consent, interview pregnant women and collect a fasting blood sample for research. However, it means that women who did not attend the OGTT could have been missed and were not captured later in pregnancy or at delivery, and those who delivered pre-term before they attended their OGTT. BiB participants have previously compared to non-BiB births occurring between 2007–2011 [134]. Summary data from obstetric and delivery records were obtained for 11,761 non-BiB births, which would include some who moved to Bradford shortly before delivery (and would not have been eligible to recruit). The comparison showed a small number of differences. BiB participants were less likely to include younger mothers (age 20–24 years) and had a higher proportion of South Asian and nulliparous mothers. There were differences in gestational age and preterm delivery that reflected recruiting BiB participants relatively late in pregnancy [134]. This selection on gestational age may introduce selection bias in some BiB analyses, including those using the metabolomics data described here.

A limitation is that BiB only has pregnancy metabolomics at a single time point and does not have pre-pregnancy measurements. Previous research suggests metabolites change upon becoming pregnant and then revert to pre-pregnancy levels 5 and that they change during pregnancy [81]. Earlier measures would be valuable for prediction of future adverse outcomes to enable earlier antenatal monitoring and intervention.

This data note has focused on metabolomics data that have been quantified by high throughput commercial platforms (Nightingale Health© NMR and Metabolon MS). On a small subsample of BiB participants (N = 199) NMR urine and serum MS blood metabolites have been quantified at Imperial College, London, as part of the HELIX collaboration. HELIX aims to identify the human exposome in pregnancy and childhood. Metabolite measurements were undertaken alongside similar subsamples from five other cohorts (total N = 1,192). In all six cohorts, samples were from children aged between 6–11 years (BiB participants were mean age 6.6 years). 44 urine metabolites (24 semi-quantified) and 188 serum (56 fully quantified) metabolites were measured. I have not described these metabolomics datasets

here as the assays are unique to a small subgroup of BiB participants and any research on these participants is best done together with the other HELIX cohort subgroups on whom the same metabolomic data obtained at the same time and using the same methods is available. Further information about the samples and methods used can be found elsewhere [204].

Up until March 2020, BiB were undertaking a follow-up of BiB parents and offspring, including collecting further blood samples with funding available to complete the NMR analyses on offspring and parent serum/plasma collected at this follow-up. However, that follow-up stopped on the 16th March 2020 when restrictions on normal life due to the COVID-19 pandemic began in the UK. At the time of submitting this paper BiB did not know when face-to-face data collection will be possible to start again and what the best plans would be for further blood sample collection. At the relevant time BiB will discuss different potential scenarios for completing that planned follow-up with relevant scientific advisory groups. Whatever the decision, BiB should have some participants with serum/plasma NMR measures collected ~8–9 years after birth. BiB are also planning to measure metabolites on the available maternal pregnancy urine samples. Urine metabolites often provide a more accurate measure of dietary intake and medicine use than serum/plasma measures and would be a valuable addition to the existing datasets described here. Any new data will be made available to the wider research community.

Chapter 5

In the published version of this work, I included an Excel document containing all numerical results and refer to these in text. Due to the significant number of pages these tables would take up, I have not included them in this thesis. The Tables I am referring to are Tables S5.5-S5.9. For the interested reader, they can be found with the pre-print (doi: https://doi.org/10.1101/2022.02.04.22270425) and also within the Open Science Framework repository (doi: https://doi.org/10.17605/OSF.IO/U3C4N).

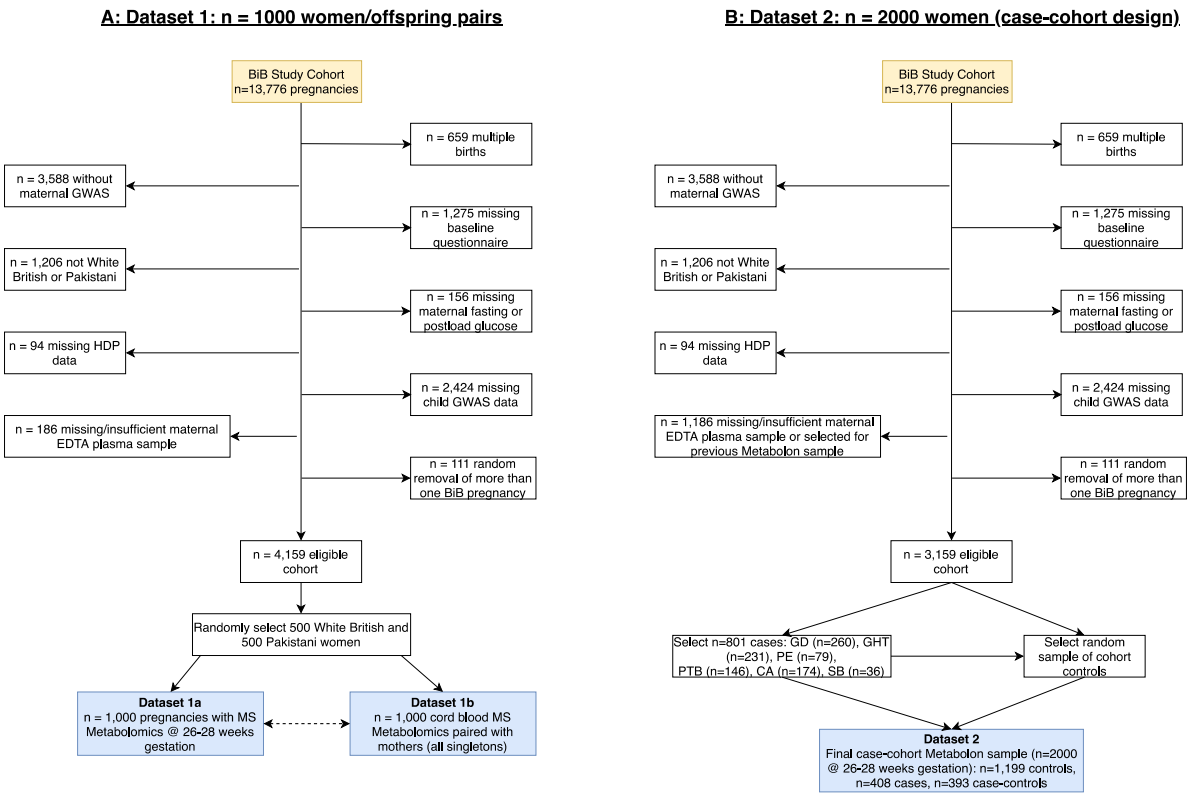


Figure S5.1. Illustrating the flow of participants into the Metabolon datasets in the Born in Bradford cohort. Panel A shows dataset 1 which includes 1,000 pregnancies and infants with MS metabolomics during pregnancy (26–28 weeks' gestation (dataset 1a)) and in cord blood (dataset 1b; dataset 1b is not included in any analyses in the present study). 1,000 women were selected on the basis that they had stored fasting plasma, a useable cord blood sample and genome wide data on both mother and offspring. Following these inclusion criteria, 500 women were selected at random from the two largest ethnic groups: White British and Pakistani, which make up 85% of the cohort. Panel B shows dataset 2 which includes 2,000 pregnancies (26–28 weeks' gestation) with MS metabolomics within a case-cohort design. This consisted of a cohort with an over-sampling of all cases of six pregnancy / perinatal outcomes. Abbreviations: MS, mass spectrometry; BiB, Born in Bradford; GWAS, genome wide association study; EDTA, ethylenediaminetetraacetic acid; HDP, hypertensive disorders of pregnancy; GD, gestational diabetes; GHT, gestational hypertension; PE, pre-eclampsia, PTB, preterm birth; CA, congenital anomaly; SB, still birth. Taken with permission from Taylor & McBride et al:

https://doi.org/10.12688/wellcomeopenres.16341.2.

Text S5.1. Confounder data

Age was obtained for all women at pregnancy booking. Ethnicity was self-reported by the mother at her recruitment questionnaire interview and based on the UK Office of National Statistics guidance. For women who did not have ethnicity data collected at the recruitment interview, data were abstracted from primary care medical records, which use a similar categorization. Women classified as South Asian included those who indicated they were Pakistani, Indian, or Bangladeshi. Women classified as White European included those who indicated that they were White British or other White European origin. Parity was categorised as having one or more previous pregnancies (multiparous (yes)) or no previous pregnancy (nulliparous (no)). I used the residential 2010 index of multiple deprivation (IMD) score presented as quintiles as a marker of socioeconomic position (SEP). Height was measured at recruitment (26–28 weeks' gestation) using a Leicester Height Measure (*Seca*, London, UK). Maternal BMI was calculated using the height measured at recruitment and weight measured at first antenatal clinic visit (approximately 12 weeks' gestation), and it was also extracted from medical records. All women were recruited from the same hospital which used *Seca* two-in-one scales (Harlow Healthcare Ltd., London, UK) to measure weight. For smoking and alcohol, women were asked the number of cigarettes smoked per day during pregnancy in the first questionnaire (26-28 weeks' gestation). I then assigned women as smokers and non-smokers. Women were asked whether they drank alcohol during pregnancy or 3 months before and assigned drinkers and non-drinkers.

Text S5.2. Defining congenital heart disease

Table S5.1. ICD codes for defining CHDs.

Methods to define CHDs were the same as those described above in Appendices for Chapter 4 (Text S4.3). Click here to navigate to them.

Text S5.3. Genetic Data.

Genetic data methods were the same as those described above in Appendices for Chapter 4 (Text S4.1). Click here to navigate to them.

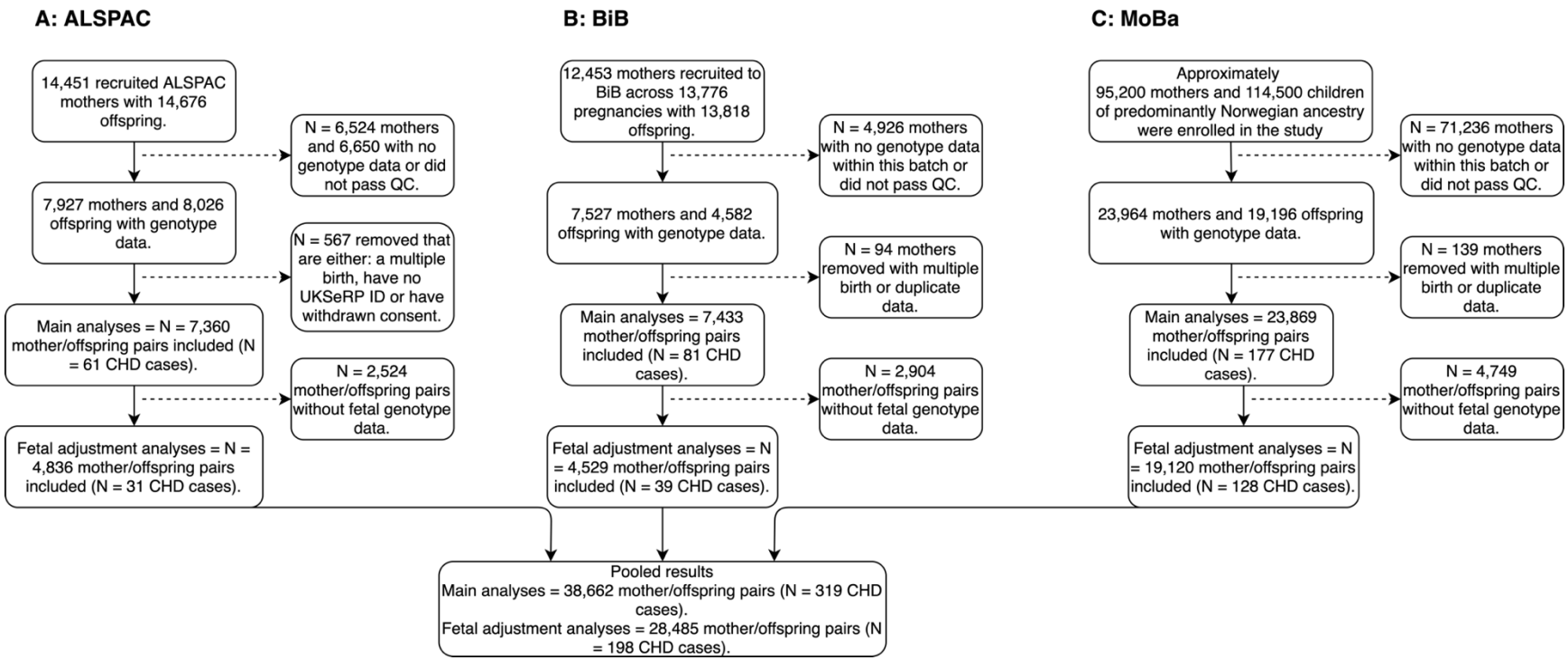


Figure S5.2. An overview of included cohorts and selection of study participants. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort; CHD, congenital heart disease; BiB, Born in Bradford; GWAS, genome-wise association study.

Table S5.2: Logistic regression results from the presence/absence xenobiotic analysis.

Metabolite	Sub pathway	OR_c (95% CI)	OR_a (95% CI)
saccharin	Food Component/Plant	1.82 (0.91, 4.03)	2.16 (1.02, 5.13)
salicyluric glucuronide	Drug - Analgesics, Anesthetics	2.01 (1.05, 3.70)	2.27 (1.16, 4.29)
alliin	Food Component/Plant	0.60 (0.33, 1.10)	0.35 (0.17, 0.73)
ferulic acid 4-sulfate	Food Component/Plant	0.74 (0.40, 1.34)	0.50 (0.25, 0.96)
naringenin 7-glucuronide	Food Component/Plant	0.28 (0.05, 0.93)	0.14 (0.01, 0.66)
glucuronide of piperine metabolite C17H21NO3 (5)	Food Component/Plant	0.42 (0.20, 0.81)	0.47 (0.22, 0.93)

Odds ratios are given for the presence of the metabolite during pregnancy and odds of having CHD in the offspring.
OR_c = unadjusted odds ratio (N = 2,605 [46 CHD cases]); OR_a = adjusted odds ratio (N = 2,426 [42 CHD cases]), adjusted for: maternal age, ethnicity, parity, SEP, BMI, smoking).

Table S5.3. Participant characteristics for the 3 studies included in Mendelian randomisation analyses.

Characteristic	Category	BiB (N = 7,433)	ALSPAC (N = 7,360)	MoBa (N = 23,869)
Offspring				
CHD	Yes	81 (1.1)	61 (0.8)	177 (0.7)
Sex	Male	3,818 (51.4)	3,703 (50.3)	12,139 (50.9)
	Female	3,615 (48.6)	3,657 (49.7)	11,704 (49.0)
Maternal				
Age, years		27.4 (5.6)	29.2 (4.6)	30.1 (4.5)
Parity	Nulliparous	2,963 (40.1)	3,257 (46.6)	11,288 (47.3)
BMI, kg/m^2		26.2 (5.7)	22.5 (4.2)	24.1 (4.3)
Ethnicity	White European	3,084 (42.6)	7,360 (100.0) [a]	NA [b]
	South Asian	3,503 (48.4)	-	-
	Other	656 (9.1)	-	-
Any smoking during pregnancy	Yes	1,175 (18.1)	1,679 (26.1)	1,814 (8.6)
Any alcohol during pregnancy	Yes	1,040 (49.3)	4,866 (79.9)	6,209 (31.5)

Data are means ± SD or n (%) unless stated. % are based on data available (data were not complete).

[a] All non-white European women with ethnicity data were not included in the analysis.

[b] There are no data available that describe ethnicity in MoBa, but it is believed that 99-100% are of White European origin.

Abbreviations: BiB, Born in Bradford; ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort Study; CHD, congenital heart disease; BMI, body mass index; kg, kilograms; m, meters.

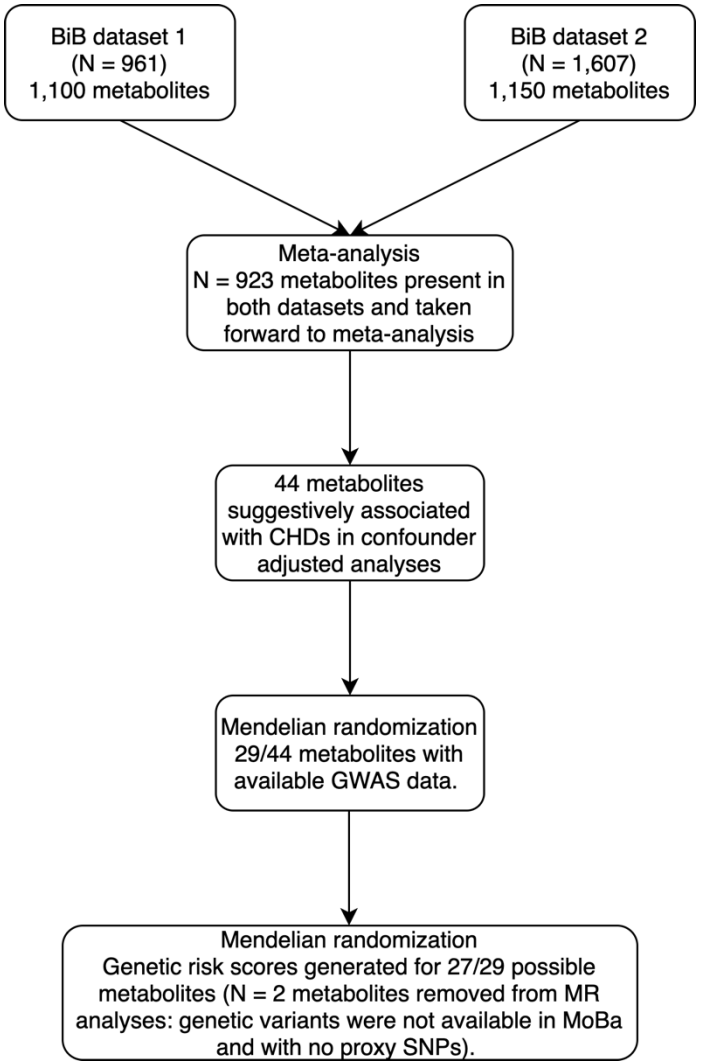
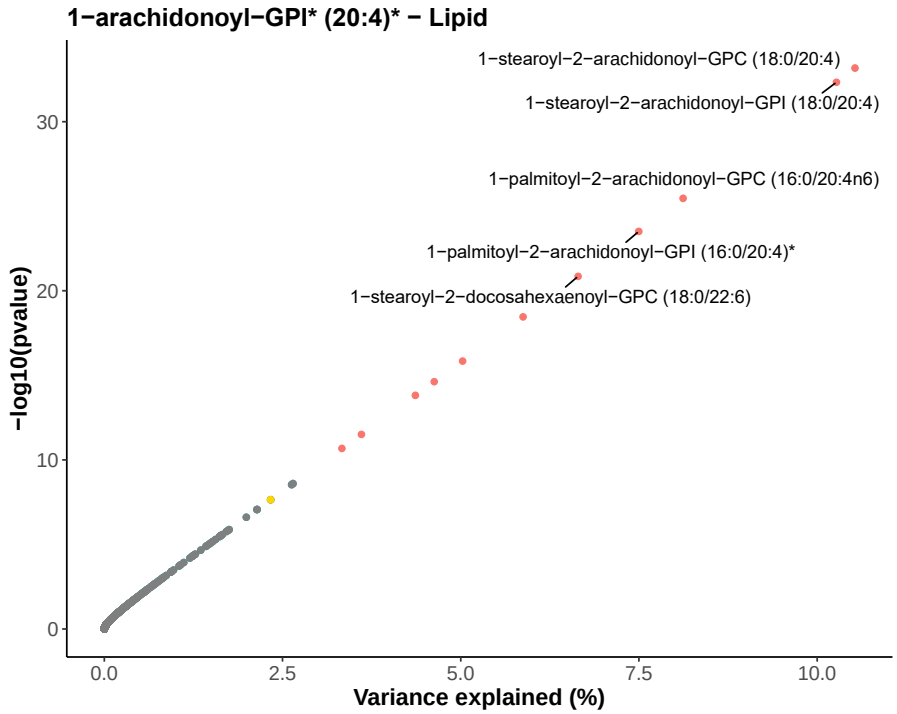


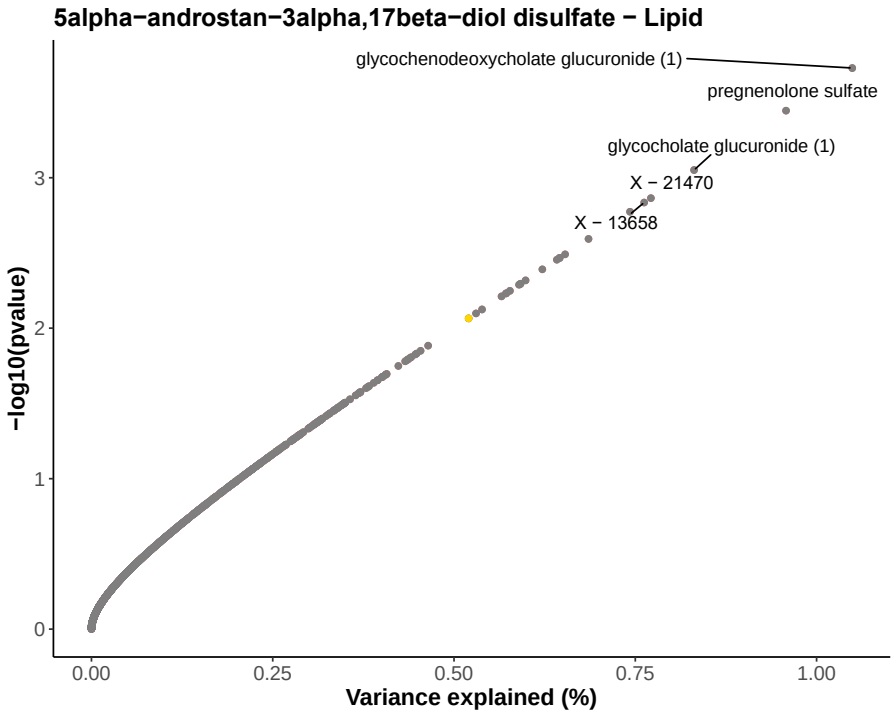
Figure S5.3. Flow chart to illustrate the analysis pipeline and selection of metabolites for Mendelian randomisation. The two metabolites (Glycerophosphoethanolamine and X-24295) were the two metabolites removed from MR analyses because they had a small number of SNPs and these were not present in MoBa and had no proxy's.

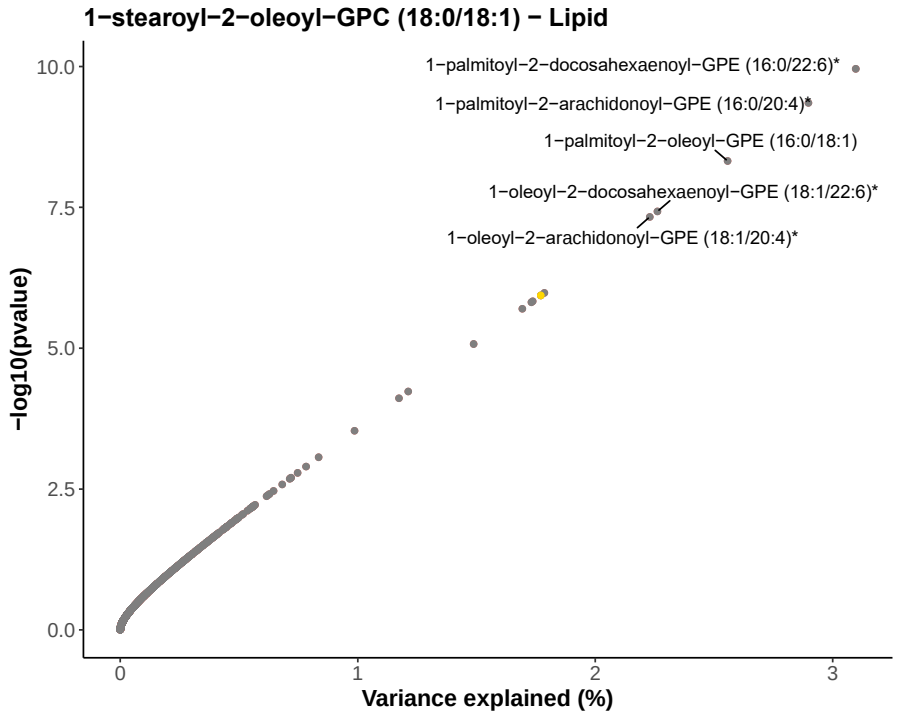
Table S5.4. Characteristics of maternal GRS and associations with the corresponding metabolite in BiB (N = 1,326).

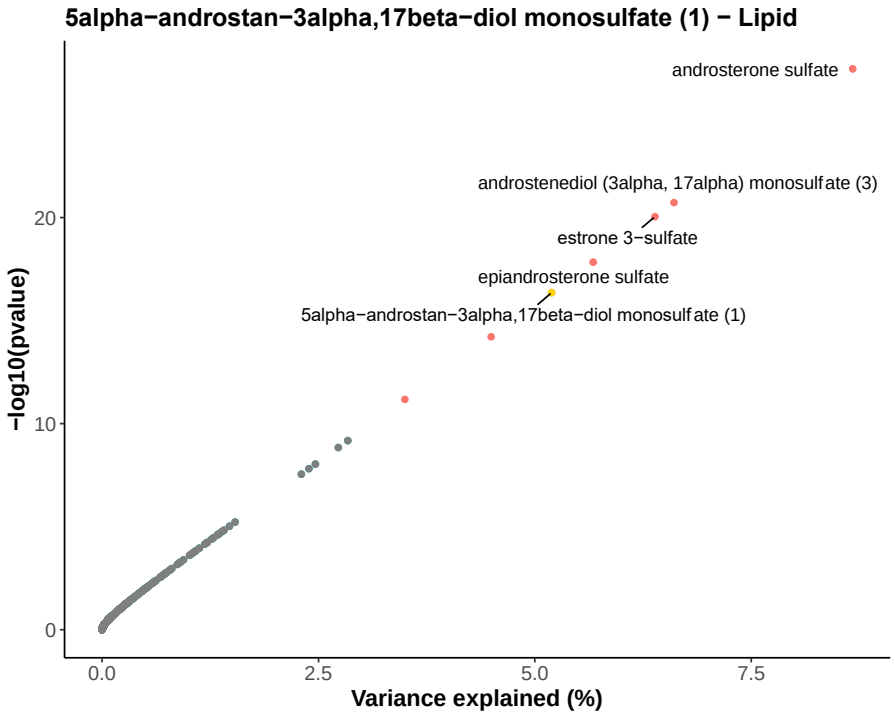
Metabolite	Super pathway	HMDB	N SNPs in GRS[a]	Coefficient (95% CI)[b]	P-Value	R^2 (%)	F-statistic
Isoleucine	Amino Acid	HMDB00172	5	0.06 (0.0006, 0.11)	0.05	0.3	3.9
N-Acetylleucine	Amino Acid	HMDB11756	2	0.37 (0.32, 0.43)	9.38E-41	12.6	191.4
Indolelactate	Amino Acid	HMDB00671	1	0.04 (-0.01, 0.09)	0.15	0.2	2.0
Betaine	Amino Acid	HMDB00043	9	0.19 (0.14, 0.25)	1.23E-11	3.4	46.7
N-Acetylarginine	Amino Acid	HMDB04620	10	0.59 (0.55, 0.63)	8.55E-122	34.0	683.3
N-Acetylcarnosine	Amino Acid	HMDB12881	9	0.25 (0.20, 0.30)	1.57E-20	6.3	89.2
Leucine	Amino Acid	HMDB00687	6	0.02 (-0.04, 0.07)	0.55	0.3	0.4
Myo-Inositol	Lipid	HMDB00211	2	0.02 (-0.04, 0.08)	0.56	0.03	0.3
Phosphoethanolamine (Pe)	Lipid	HMDB00224	2	0.07 (0.02, 0.12)	0.01	0.5	6.4
Androsterone Sulfate	Lipid	HMDB02759	11	0.30 (0.25, 0.36)	1.12E-28	8.9	129.4
Glycolithocholate Sulfate*	Lipid	HMDB02639	2	0.10 (0.05, 0.16)	0.0004	1.0	12.7
Epiandrosterone Sulfate	Lipid		10	0.25 (0.20, 0.31)	1.69E-20	6.3	89.0
1-Arachidonoyl-Gpi* (20:4)*	Lipid	HMDB61690	2	0.15 (0.10, 0.21)	2.27E-08	2.3	31.6
Phosphocholine	Lipid	HMDB01565	6	0.15 (0.10, 0.20)	1.98E-09	2.7	36.5
Taurolithocholate 3-Sulfate	Lipid	HMDB02580	4	0.10 (0.04, 0.15)	0.0004	0.9	12.5
5alpha-Androstan-3alpha,17beta-Diol Disulfate	Lipid	-	6	0.07 (0.02, 0.13)	0.009	0.5	6.9
5alpha-Androstan-3alpha,17beta-Diol Monosulfate (1)	Lipid	-	14	0.23 (0.18, 0.29)	4.41E-17	5.2	72.5
5alpha-Sndrostan-3beta,17beta-Diol Disulfate	Lipid	HMDB00493	11	0.24 (0.19, 0.30)	6.05E-19	5.8	81.5
Androstenediol (3alpha, 17alpha) Monosulfate (3)	Lipid	-	13	0.31 (0.26, 0.37)	3.97E-30	9.4	136.7
Glycerol 3-Phosphate	Lipid	HMDB00126	3	0.12 (0.07, 0.17)	4.80E-06	1.6	21.1
1-Stearoyl-2-Oleoyl-GPC (18:0/18:1)	Lipid	HMDB08038	3	0.13 (0.08, 0.18)	1.16E-06	1.8	23.9
Glycodeoxycholate 3-Sulfate	Lipid	-	5	0.20 (0.15, 0.25)	2.18E-14	4.3	59.7
Biliverdin	Cofactors and Vitamins	HMDB01008	3	0.52 (0.48, 0.57)	3.01E-93	27.2	493.9
Succinylcarnitine (C4)	Energy	HMDB61717	7	0.35 (0.30, 0.40)	1.56E-45	14.1	216.7
X - 11787	NA	-	5	0.28 (0.23, 0.34)	3.93E-26	8.1	116.7
X - 18921	NA	-	3	0.27 (0.22, 0.33)	2.80E-25	7.8	112.5
X - 24544	NA	-	5	0.19 (0.14, 0.24)	2.03E-13	4.0	55.1

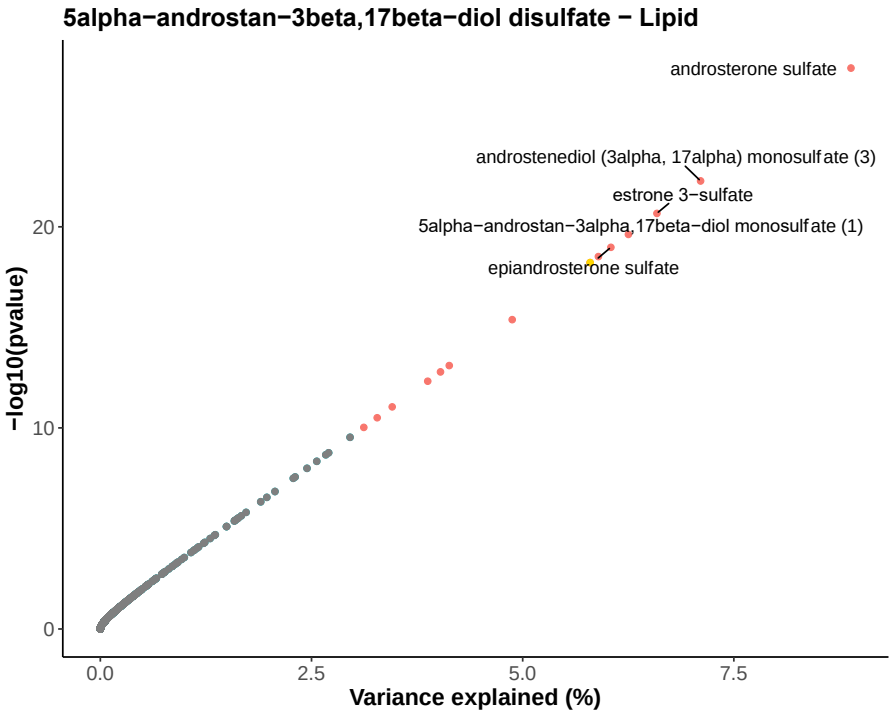
[a] SNPs for isoleucine and leucine taken from: https://www.nature.com/articles/s41588-020-00751-5; remainder of SNPs taken from a recent GWAS of metabolon metabolites (unpublished).
[b] Estimates from linear regression interpreted as difference in metabolite (scaled/imputed metabolites were log-transformed and presented in SD units as per manuscript methods) per SD increase in genetic risk score. MS-derived metabolomics measured using plasma taken during pregnancy around 26-28 weeks' gestation in the BiB cohort (see methods).
N = 1,326 is the number of women in BiB dataset 2 with Metabolon metabolomics data and GWAS data.
Abbreviations: HMDB, The Human Metabolome Database; SNP, single nucleotide polymorphism; GRS, genetic risk score; CI, confidence interval.

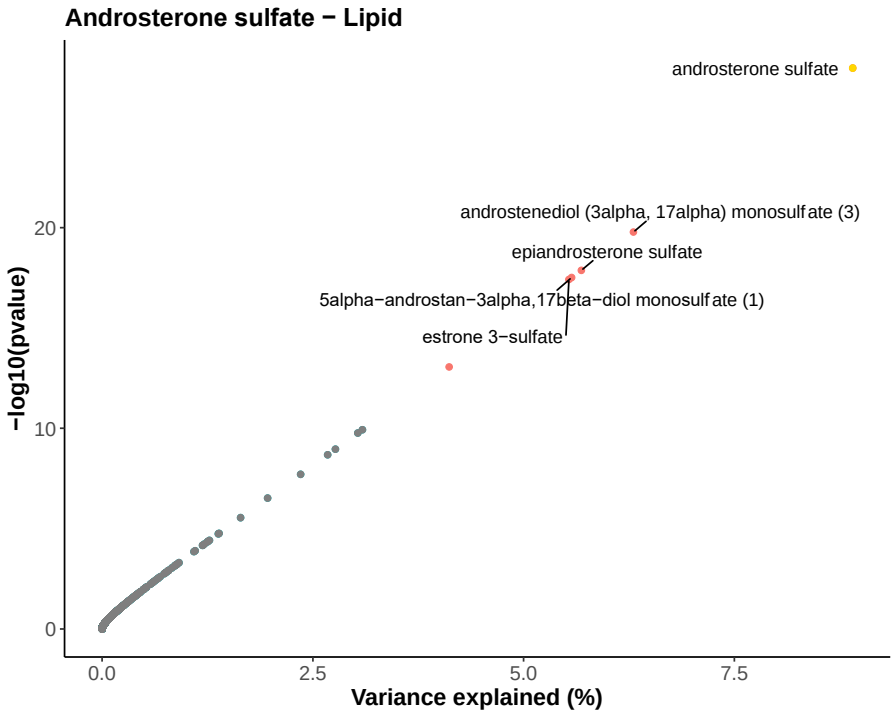

1−arachidonoyl−GPI* (20:4)* − Lipid
1−stearoyl−2−arachidonoyl−GPC (18:0/20:4)
1−stearoyl−2−arachidonoyl−GPI (18:0/20:4)
1−palmitoyl−2−arachidonoyl−GPC (16:0/20:4n6)
1−palmitoyl−2−arachidonoyl−GPI (16:0/20:4)*
1−stearoyl−2−docosahexaenoyl−GPC (18:0/22:6)
−log10(pvalue)
Variance explained (%)

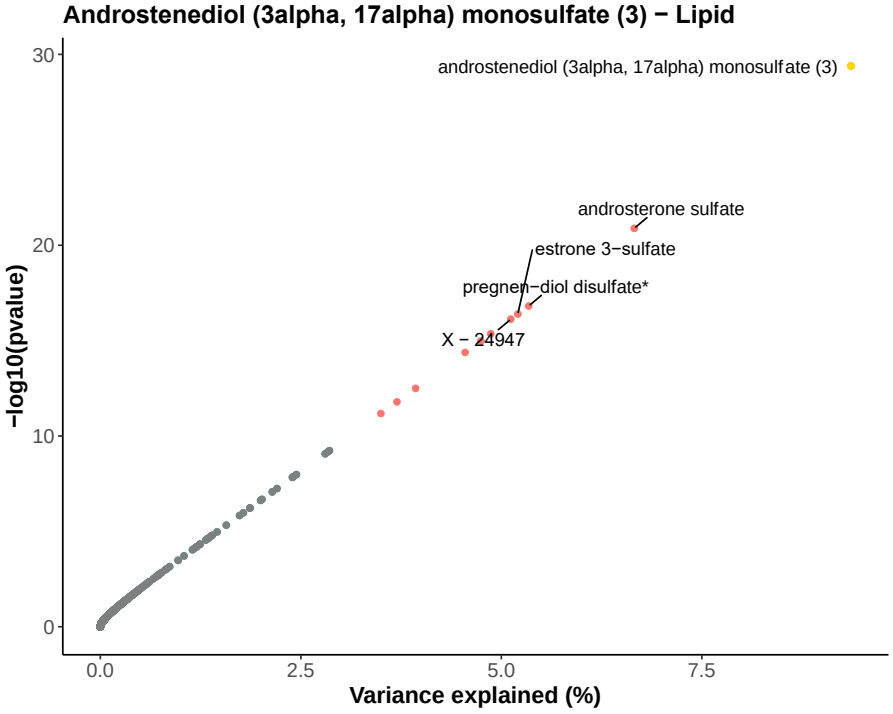

5alpha−androstan−3alpha,17beta−diol disulfate − Lipid
glycochenodeoxycholate glucuronide (1)
pregnenolone sulfate
glycocholate glucuronide (1)
X − 21470
X − 13658
−log10(pvalue)
Variance explained (%)

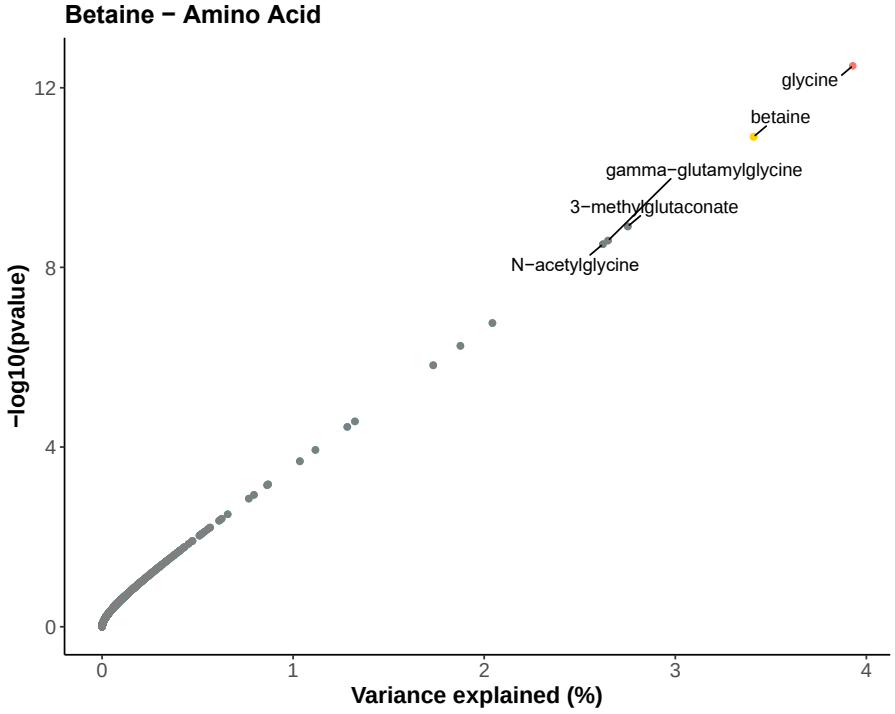

1−stearoyl−2−oleoyl−GPC (18:0/18:1) − Lipid
1−palmitoyl−2−docosahexaenoyl−GPE (16:0/22:6)*
1−palmitoyl−2−arachidonoyl−GPE (16:0/20:4)*
1−palmitoyl−2−oleoyl−GPE (16:0/18:1)
1−oleoyl−2−docosahexaenoyl−GPE (18:1/22:6)*
1−oleoyl−2−arachidonoyl−GPE (18:1/20:4)*
−log10(pvalue)
Variance explained (%)

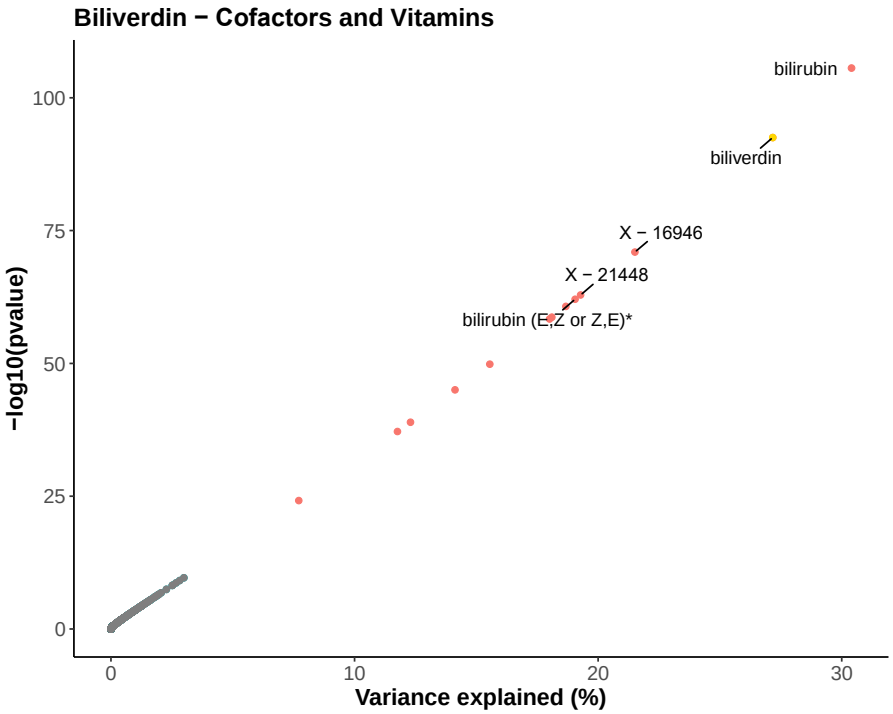

5alpha−androstan−3alpha,17beta−diol monosulfate (1) − Lipid
androsterone sulfate
androstenediol (3alpha, 17alpha) monosulfate (3)
estrone 3−sulfate
epiandrosterone sulfate
5alpha−androstan−3alpha,17beta−diol monosulfate (1)
−log10(pvalue)
Variance explained (%)

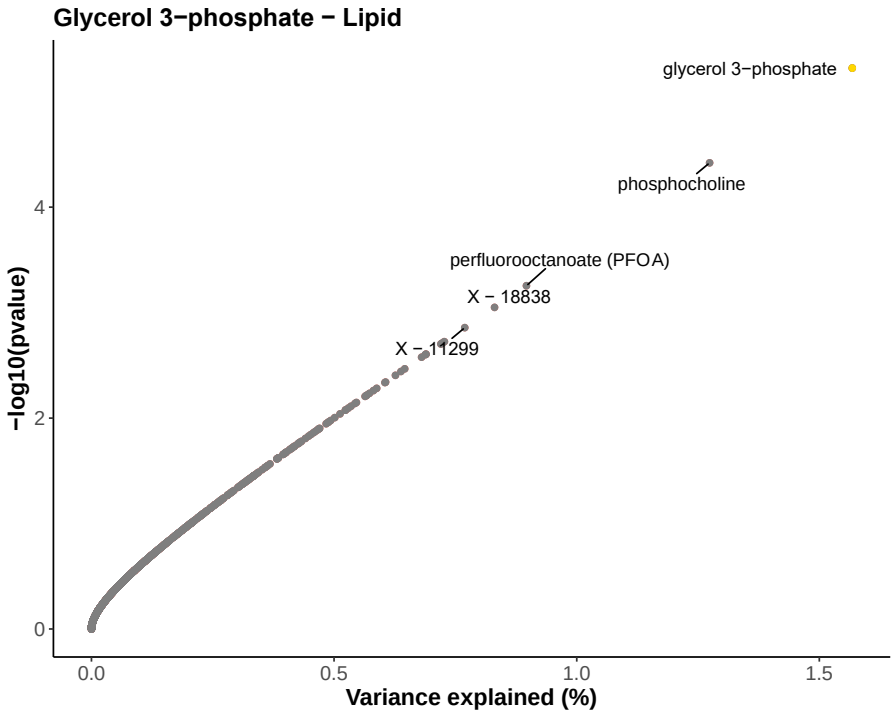

5alpha−androstan−3beta,17beta−diol disulfate − Lipid
androsterone sulfate
androstenediol (3alpha, 17alpha) monosulfate (3)
estrone 3−sulfate
5alpha−androstan−3alpha,17beta−diol monosulfate (1)
epiandrosterone sulfate
−log10(pvalue)
Variance explained (%)

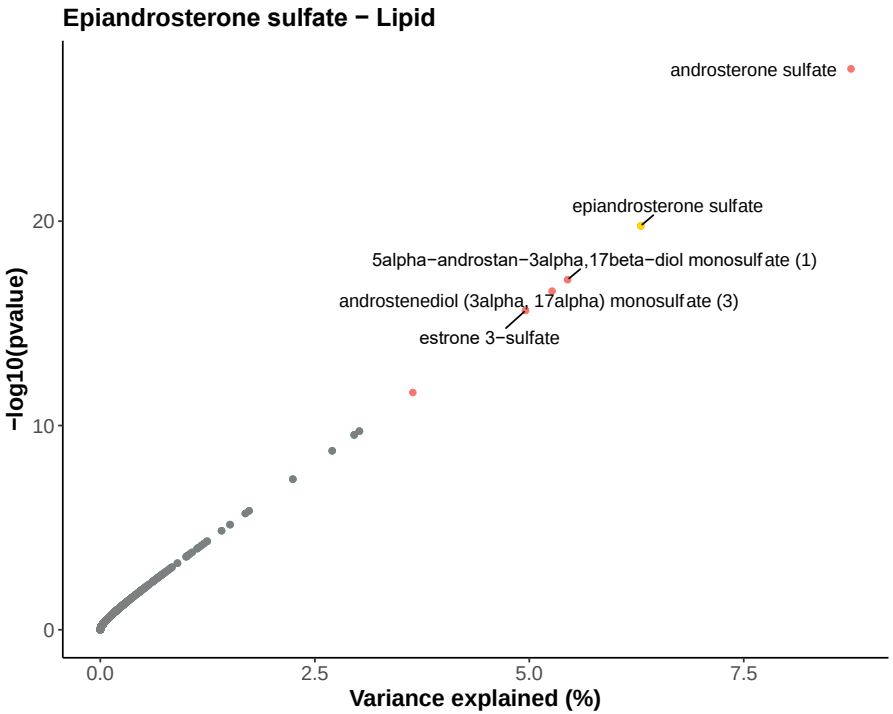

Androsterone sulfate − Lipid
androsterone sulfate
androstenediol (3alpha, 17alpha) monosulfate (3)
epiandrosterone sulfate
5alpha−androstan−3alpha,17beta−diol monosulfate (1)
estrone 3−sulfate
−log10(pvalue)
Variance explained (%)

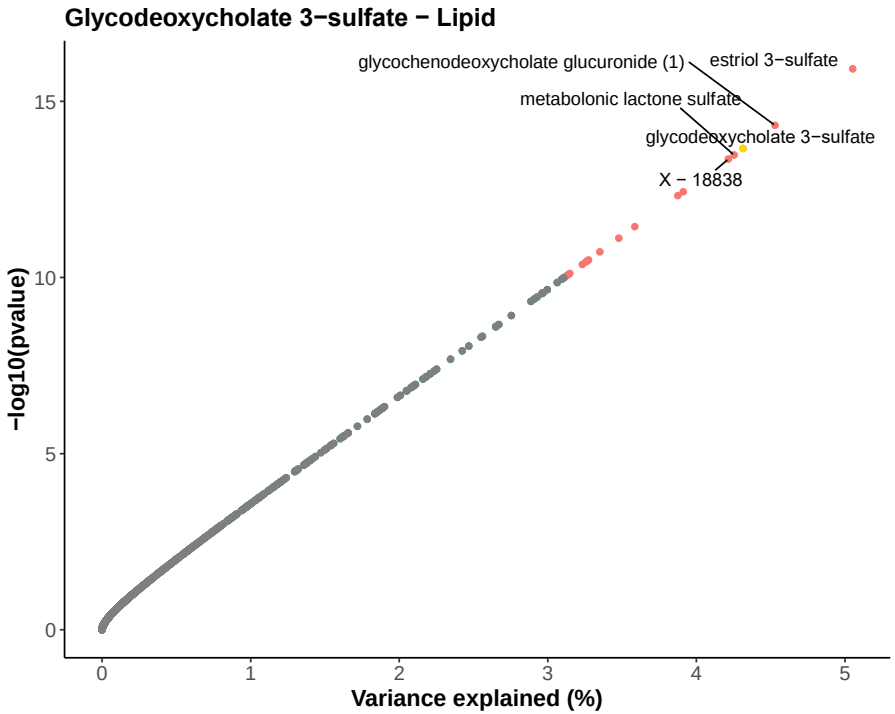

Androstenediol (3alpha, 17alpha) monosulfate (3) − Lipid
androstenediol (3alpha, 17alpha) monosulfate (3)
androsterone sulfate
estrone 3−sulfate
pregnen−diol disulfate*
X − 24947
−log10(pvalue)
Variance explained (%)

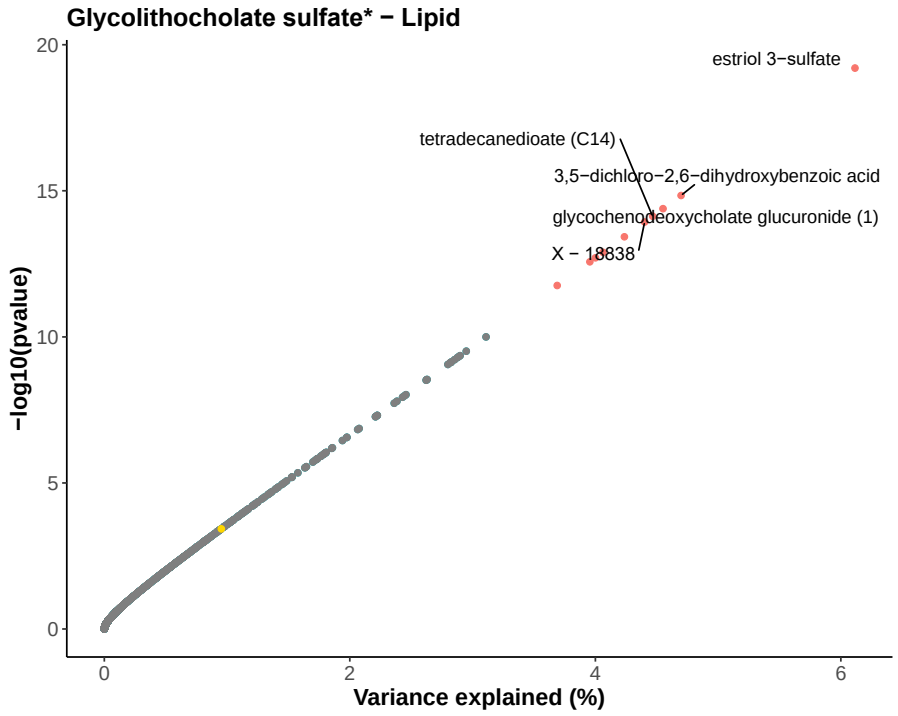

Betaine − Amino Acid
glycine
betaine
gamma−glutamylglycine
3−methylglutaconate
N−acetylglycine
−log10(pvalue)
Variance explained (%)

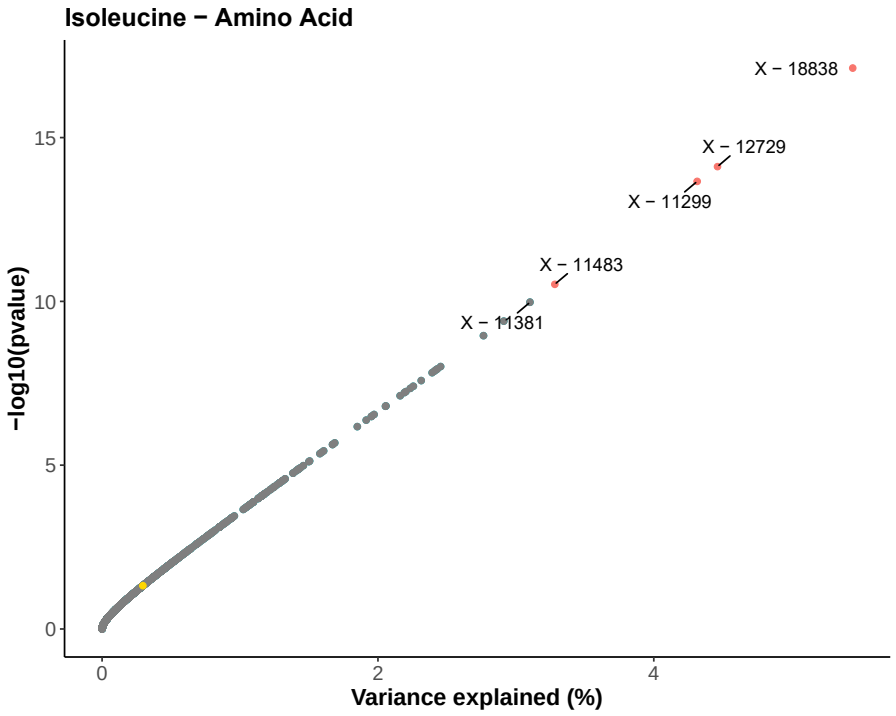

Biliverdin − Cofactors and Vitamins
bilirubin
biliverdin
X − 16946
X − 21448
bilirubin (E,Z or Z,E)*
−log10(pvalue)
Variance explained (%)

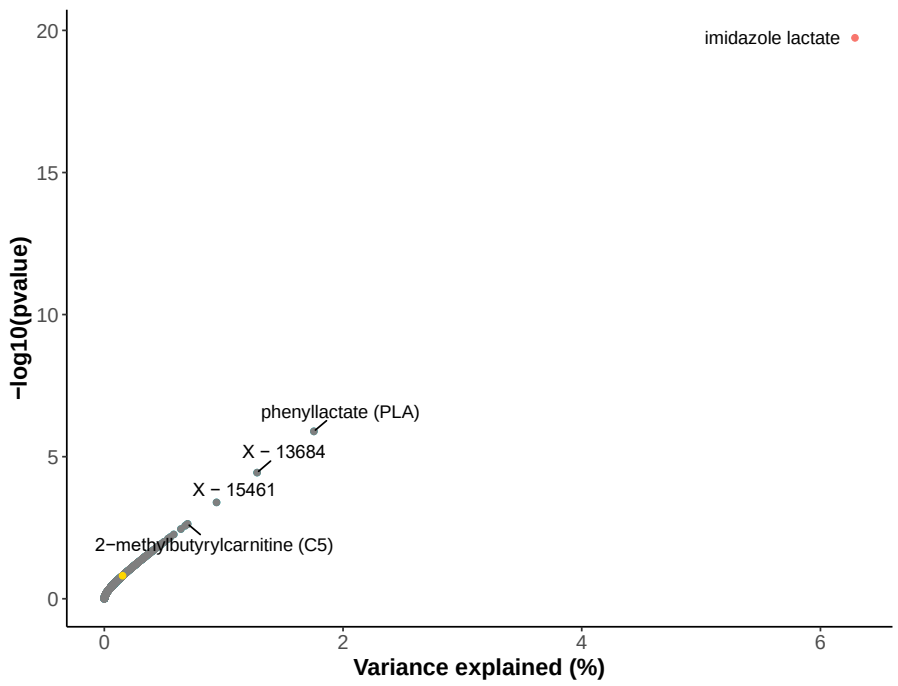

Glycerol 3−phosphate − Lipid
glycerol 3−phosphate
phosphocholine
perfluorooctanoate (PFOA)
X − 18838
X − 11299
−log10(pvalue)
Variance explained (%)

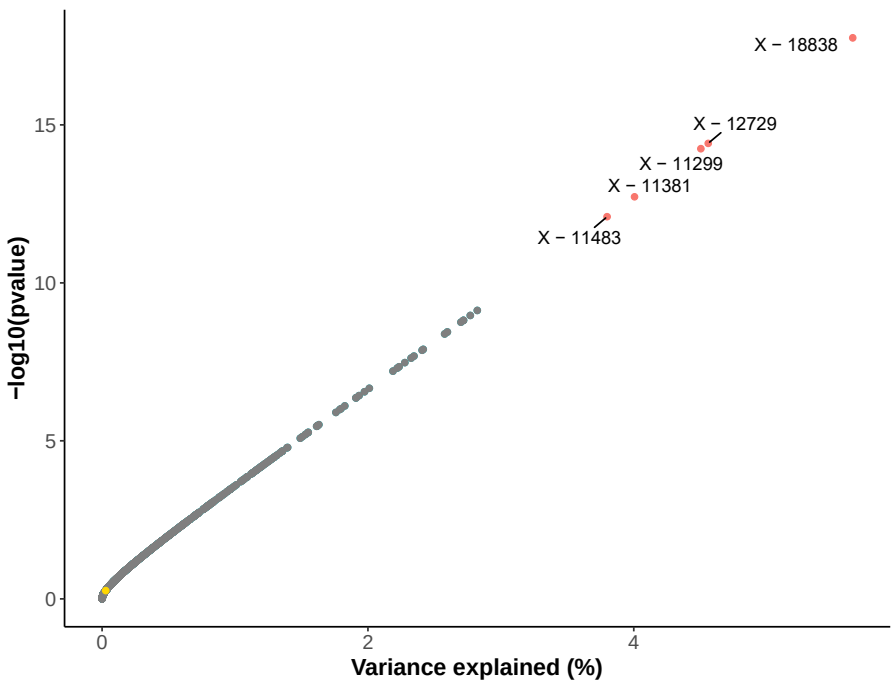

Epiandrosterone sulfate − Lipid
androsterone sulfate
epiandrosterone sulfate
5alpha−androstan−3alpha,17beta−diol monosulfate (1)
androstenediol (3alpha, 17alpha) monosulfate (3)
estrone 3−sulfate
−log10(pvalue)
Variance explained (%)

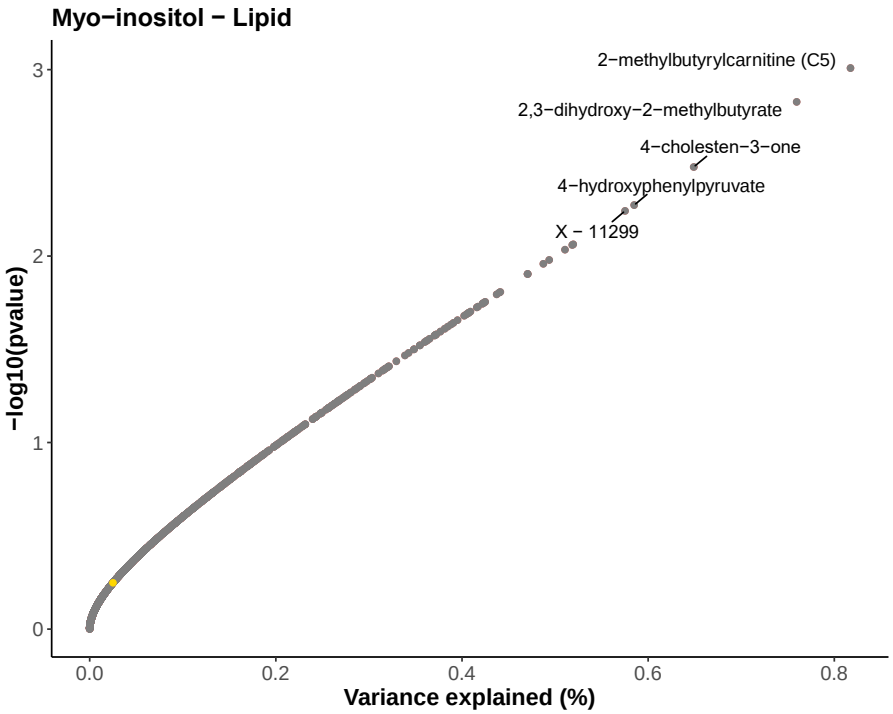

Glycodeoxycholate 3−sulfate − Lipid
glycochenodeoxycholate glucuronide (1)
estriol 3−sulfate
metabolonic lactone sulfate
glycodeoxycholate 3−sulfate
X − 18838
−log10(pvalue)
Variance explained (%)

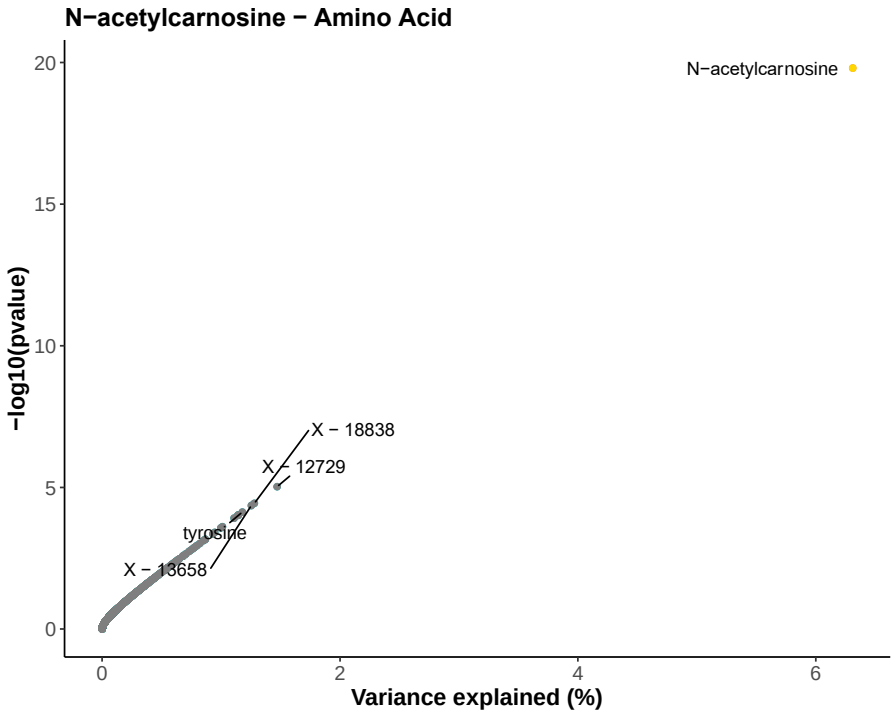

Glycolithocholate sulfate* − Lipid
estriol 3−sulfate
tetradecanedioate (C14)
3,5−dichloro−2,6−dihydroxybenzoic acid
glycochenodeoxycholate glucuronide (1)
X − 18838
−log10(pvalue)
Variance explained (%)

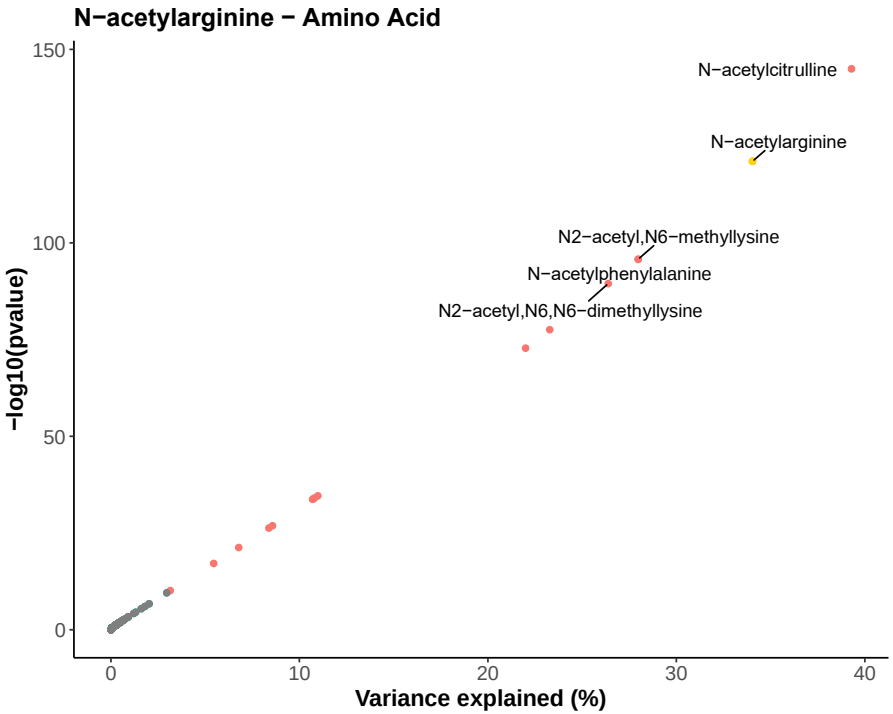

Isoleucine − Amino Acid
X − 18838
X − 12729
X − 11299
X − 11483
X − 11381
−log10(pvalue)
Variance explained (%)

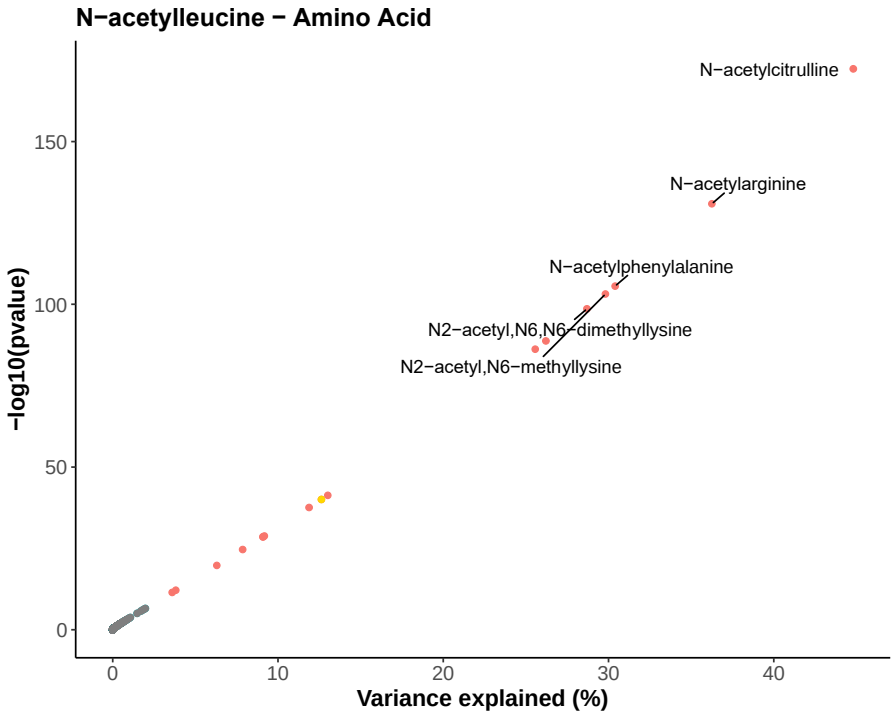

Indolelactate − Amino Acid
imidazole lactate
phenyllactate (PLA)
X − 13684
X − 15461
2−methylbutyrylcarnitine (C5)
−log10(pvalue)
Variance explained (%)

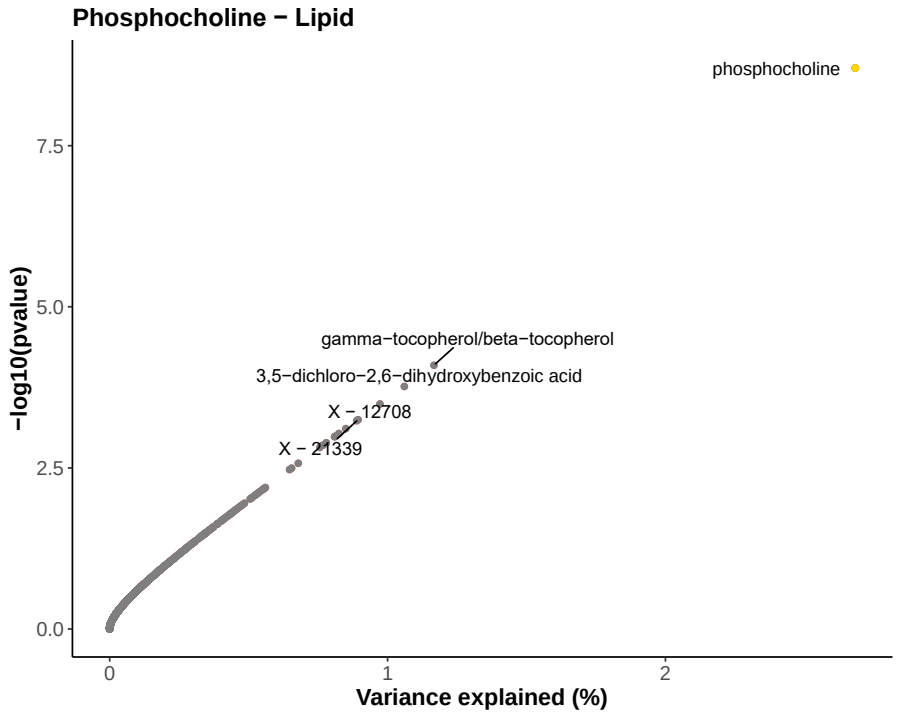

Leucine − Amino Acid
X − 18838
X − 12729
X − 11299
X − 11381
X − 11483
−log10(pvalue)
Variance explained (%)

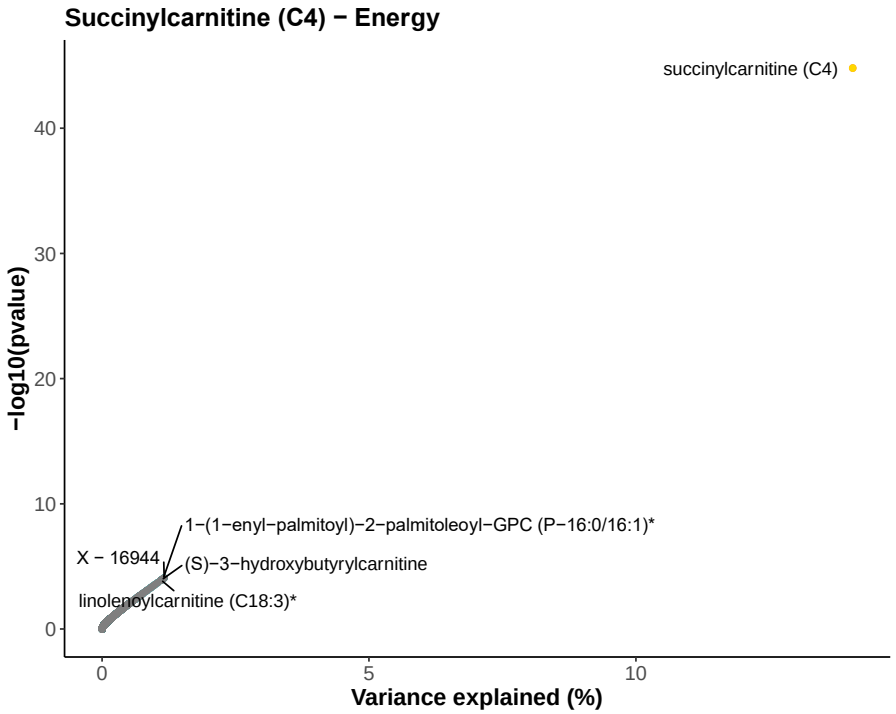

Myo−inositol − Lipid
2−methylbutyrylcarnitine (C5)
2,3−dihydroxy−2−methylbutyrate
4−cholesten−3−one
4−hydroxyphenylpyruvate
X − 11299
−log10(pvalue)
Variance explained (%)

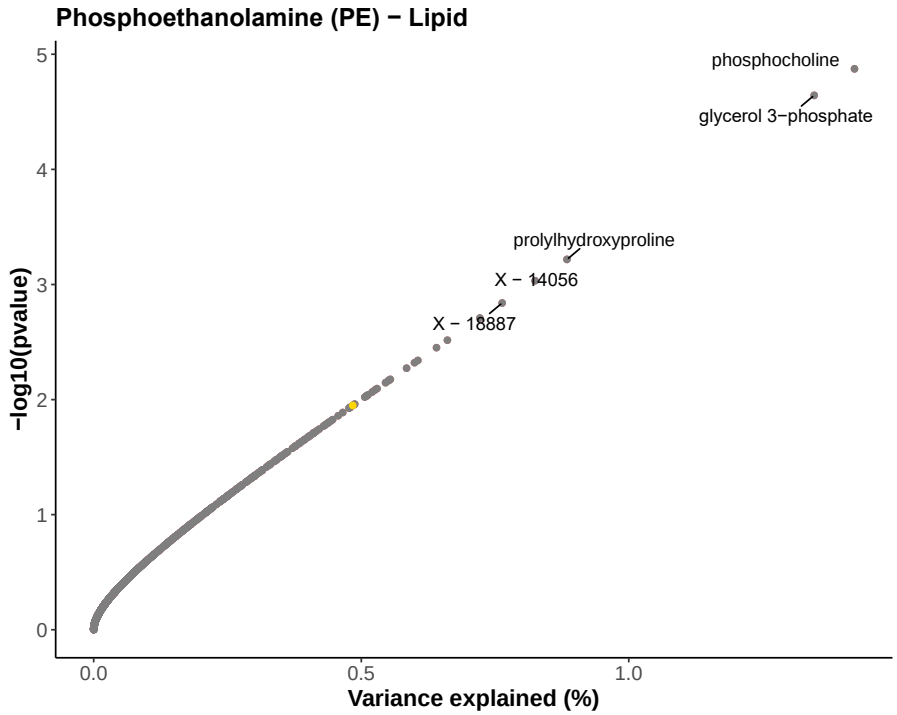

N−acetylcarnosine − Amino Acid
N−acetylcarnosine
X − 18838
X − 12729
tyrosine
X − 13658
−log10(pvalue)
Variance explained (%)

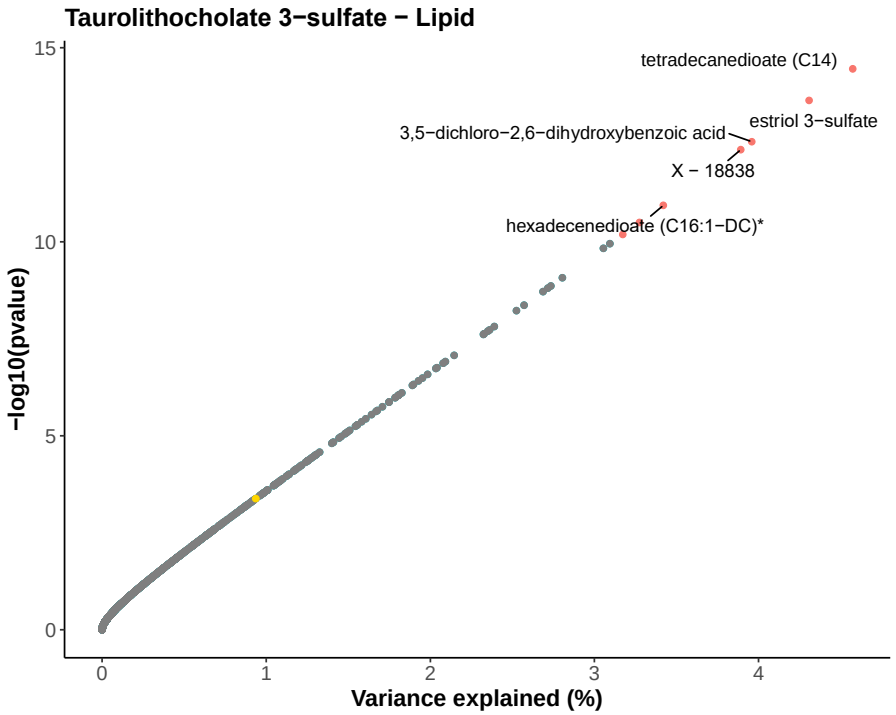

N−acetylarginine − Amino Acid
N−acetylcitrulline
N−acetylarginine
N2−acetyl,N6−methyllysine
N−acetylphenylalanine
N2−acetyl,N6,N6−dimethyllysine
−log10(pvalue)
Variance explained (%)

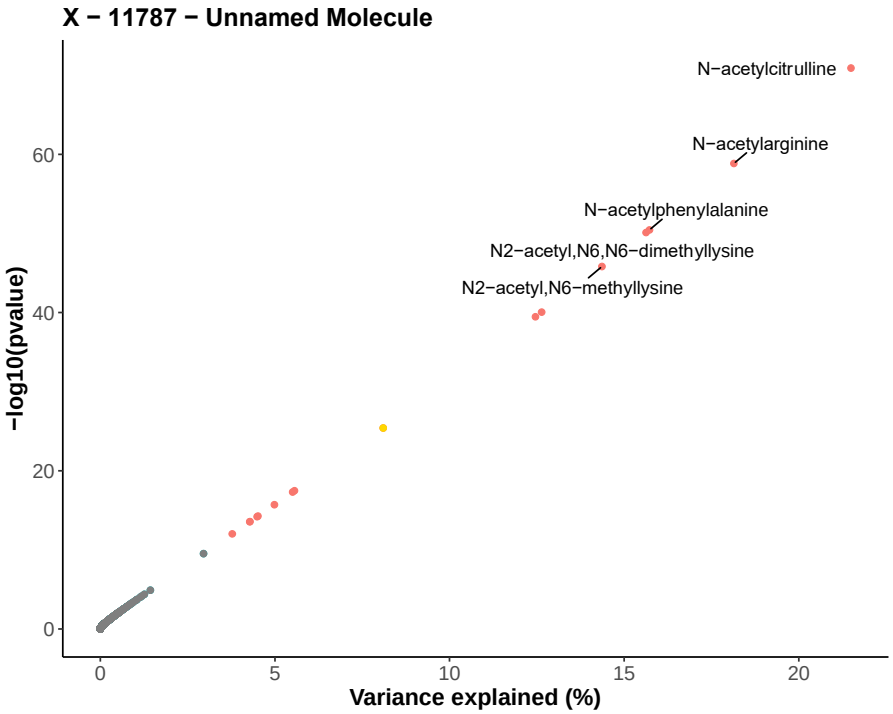

N−acetylleucine − Amino Acid
N−acetylcitrulline
N−acetylarginine
N−acetylphenylalanine
N2−acetyl,N6,N6−dimethyllysine
N2−acetyl,N6−methyllysine
−log10(pvalue)
Variance explained (%)

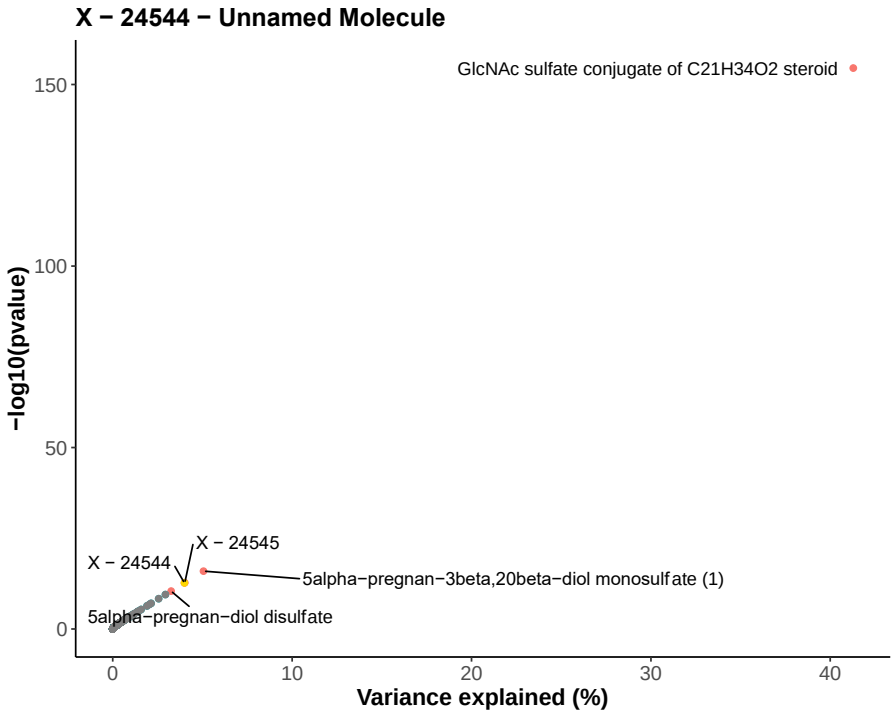

Phosphocholine − Lipid
phosphocholine
gamma−tocopherol/beta−tocopherol
3,5−dichloro−2,6−dihydroxybenzoic acid
X − 12708
X − 21339
−log10(pvalue)
Variance explained (%)


Succinylcarnitine (C4) − Energy
succinylcarnitine (C4)
1−(1−enyl−palmitoyl)−2−palmitoleoyl−GPC (P−16:0/16:1)*
X − 16944
(S)−3−hydroxybutyrylcarnitine
linolenoylcarnitine (C18:3)*
−log10(pvalue)
Variance explained (%)


Phosphoethanolamine (PE) − Lipid
phosphocholine
glycerol 3−phosphate
prolylhydroxyproline
X − 14056
X − 18887
−log10(pvalue)
Variance explained (%)


Taurolithocholate 3−sulfate − Lipid
tetradecanedioate (C14)
estriol 3−sulfate
3,5−dichloro−2,6−dihydroxybenzoic acid
X − 18838
hexadecenedioate (C16:1−DC)*
−log10(pvalue)
Variance explained (%)


X − 11787 − Unnamed Molecule
N−acetylcitrulline
N−acetylarginine
N−acetylphenylalanine
N2−acetyl,N6,N6−dimethyllysine
N2−acetyl,N6−methyllysine
−log10(pvalue)
Variance explained (%)
0
20
40
60
0
5
10
15
20


X − 24544 − Unnamed Molecule
GlcNAc sulfate conjugate of C21H34O2 steroid
X − 24545
X − 24544
5alpha−pregnan−3beta,20beta−diol monosulfate (1)
5alpha−pregnan−diol disulfate
−log10(pvalue)
Variance explained (%)
0
50
100
150
0
10
20
30
40

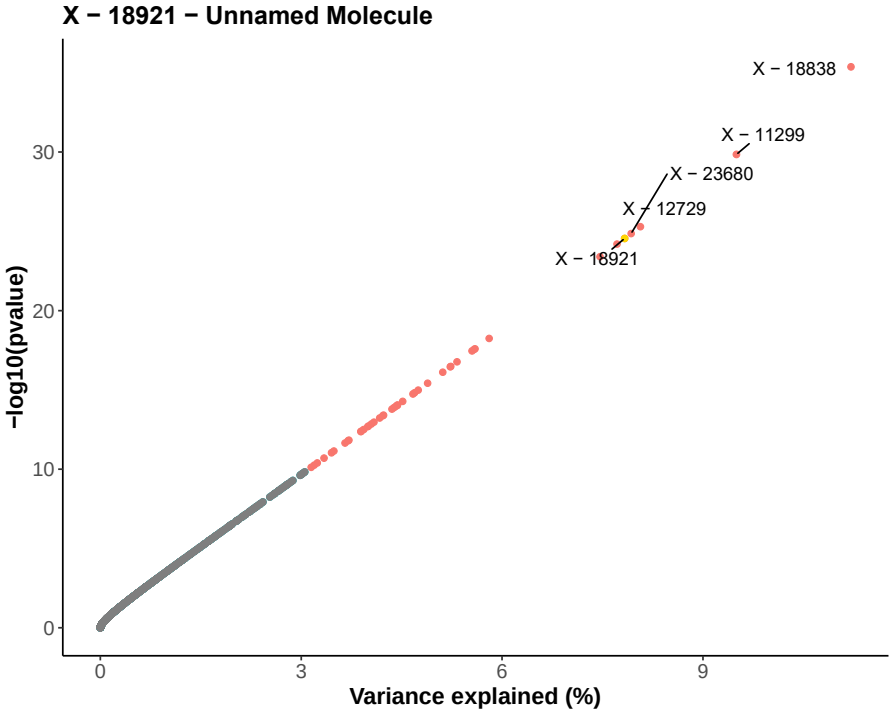

X − 18921 − Unnamed Molecule
X − 18838
X − 11299
X − 23680
X − 12729
X − 18921
−log10(pvalue)
Variance explained (%)
0
10
20
30
0
3
6
9

Figure S5.4. Scatter plots of the variance explained by the weighted genetic risk score for each of the 27 metabolites included in MR analyses. Results are from linear regression analyses of GRS against all metabolites (exposure: GRS, outcome: metabolite) in BiB dataset 2 (see flowchart above for BiB dataset 2). The x-axes are R^2 expressed as a percentage and the y-axes are the -log10 P-value. The 5 metabolites with the lowest p-values in each scatter plot are labelled. Those with a -log10 P-value >10 are filled in red, with the remainder filled grey. The metabolite I am trying to instrument with the GRS is filled with gold. A highly specific GRS for a given metabolite would produce a scatter plot with the gold-filled point in the top right corner (high variance explained and strongly associated) with the remainder of the metabolites lower down towards the left corner.

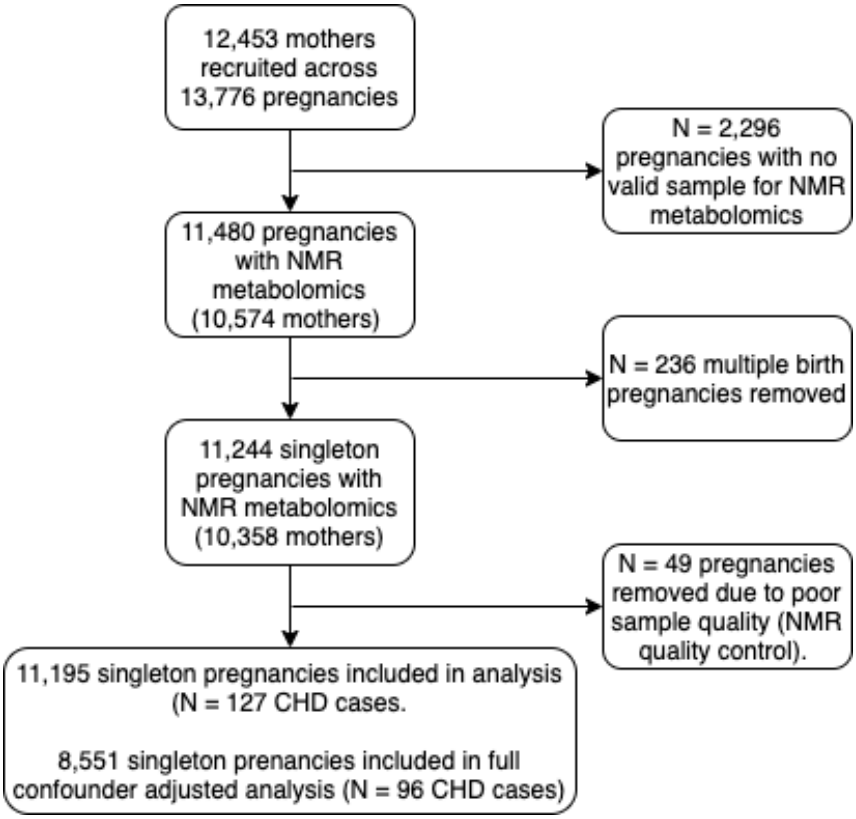


Figure S6.1. Illustrating the flow of participants into the NMR metabolomic analyses in the Born in Bradford cohort.

Text S6.1. Methods for confounder data.

Age was obtained for all women at pregnancy booking. Ethnicity was self-reported by the mother at her recruitment questionnaire interview and based on the UK Office of National Statistics guidance. For women who did not have ethnicity data collected at the recruitment interview, data were abstracted from primary care medical records, which use a similar categorization. Women classified as South Asian included those who indicated they were Pakistani, Indian, or Bangladeshi. Women classified as White European included those who indicated that they were White British or other White European origin. Parity was categorised as having one or more previous pregnancies (multiparous (yes)) or no previous pregnancy (nulliparous (no)). I used the residential 2010 index of multiple deprivation (IMD) score presented as quintiles as a marker of socioeconomic position (SEP). Height was measured at recruitment (26–28 weeks' gestation) using a Leicester Height Measure (*Seca*, London, UK). Maternal BMI was calculated using the height measured at recruitment and weight measured at first antenatal clinic visit (approximately 12 weeks' gestation), and it was also extracted from medical records. All women were recruited from the same hospital which used *Seca* two-in-one scales (Harlow Healthcare Ltd., London, UK) to measure weight. For smoking, women were asked the number of cigarettes smoked per day during pregnancy in the first questionnaire (26-28 weeks' gestation). I then assigned women as smokers and non-smokers.

Text S6.2. Defining congenital heart disease

Methods to define CHDs were the same as those described above in Appendices for Chapter 4 (Text S4.3). Click here to navigate to them.

Text S6.3. Genetic Data.

Genetic data methods were the same as those described above in Appendices for Chapter 4 (Text S4.1). Click here to navigate to them.

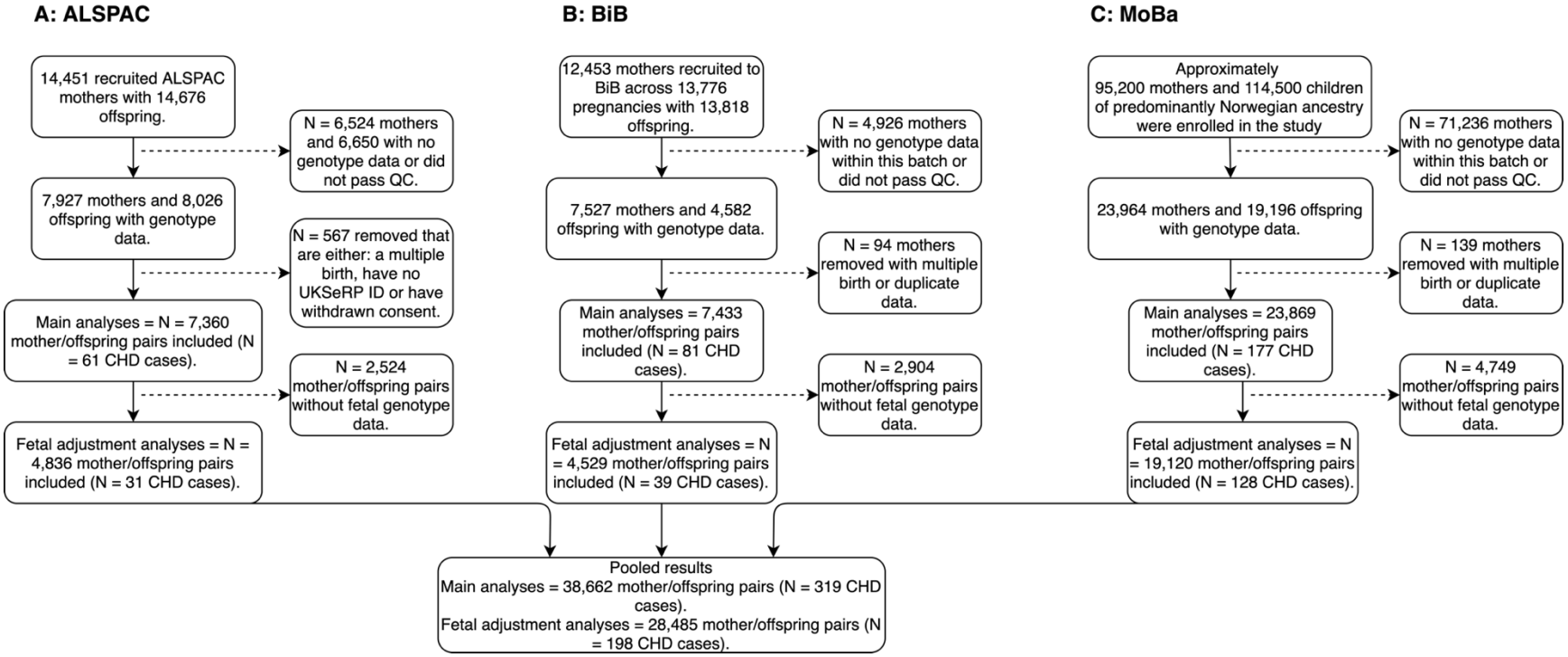
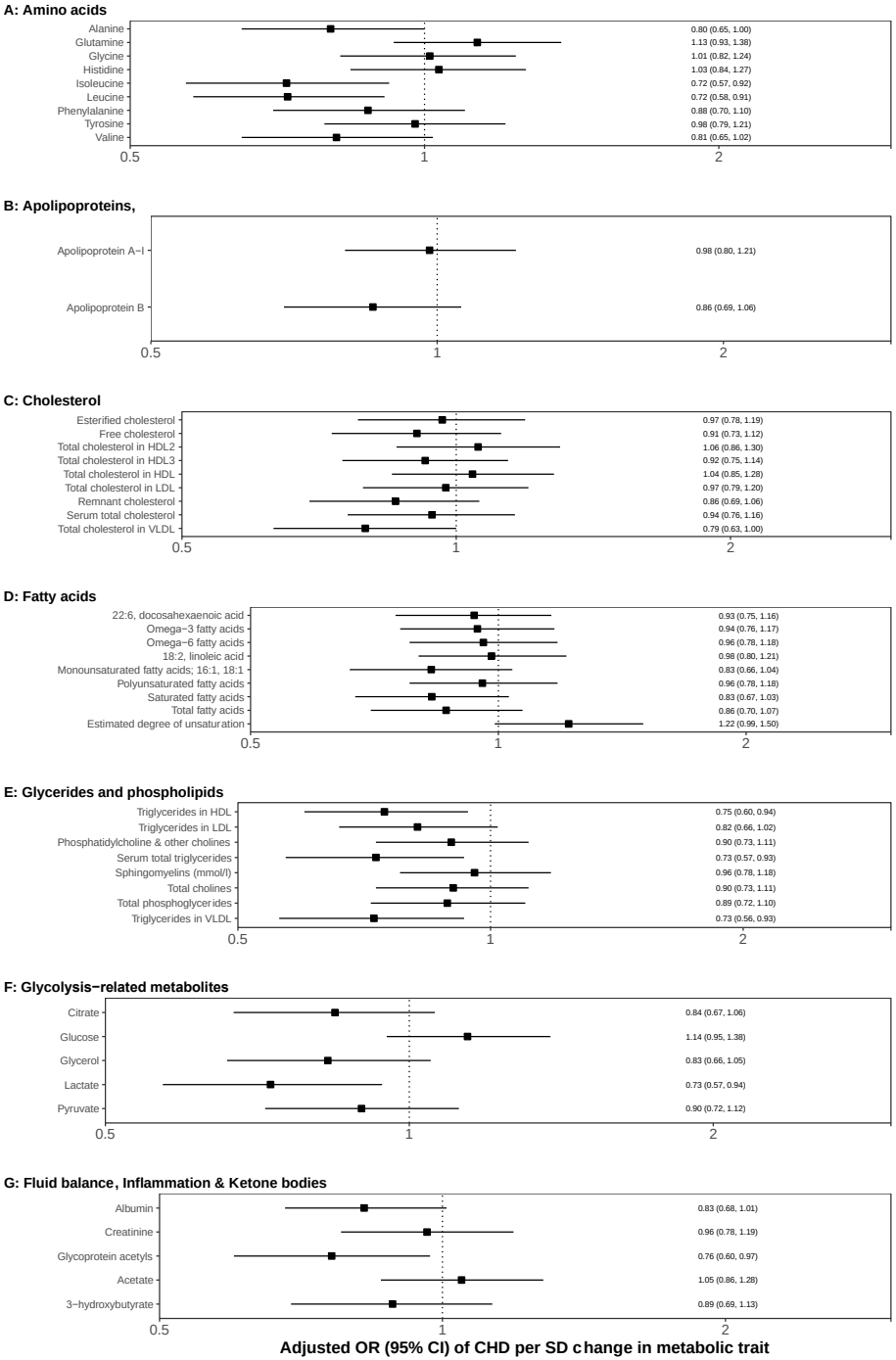


Figure S6.2. An overview of included cohorts and selection of study participants. Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; MoBa, Norwegian Mother, Father and Child Cohort; CHD, congenital heart disease; BiB, Born in Bradford; GWAS, genome-wise association study.


A: Amino acids
Alanine 0.80 (0.65, 1.00)
Glutamine 1.13 (0.93, 1.38)
Glycine 1.01 (0.82, 1.24)
Histidine 1.03 (0.84, 1.27)
Isoleucine 0.72 (0.57, 0.92)
Leucine 0.72 (0.58, 0.91)
Phenylalanine 0.88 (0.70, 1.10)
Tyrosine 0.98 (0.79, 1.21)
Valine 0.81 (0.65, 1.02)
0.5
1
2
B: Apolipoproteins,
Apolipoprotein A−I 0.98 (0.80, 1.21)
Apolipoprotein B 0.86 (0.69, 1.06)
0.5
1
2
C: Cholesterol
Esterified cholesterol 0.97 (0.78, 1.19)
Free cholesterol 0.91 (0.73, 1.12)
Total cholesterol in HDL2 1.06 (0.86, 1.30)
Total cholesterol in HDL3 0.92 (0.75, 1.14)
Total cholesterol in HDL 1.04 (0.85, 1.28)
Total cholesterol in LDL 0.97 (0.79, 1.20)
Remnant cholesterol 0.86 (0.69, 1.06)
Serum total cholesterol 0.94 (0.76, 1.16)
Total cholesterol in VLDL 0.79 (0.63, 1.00)
0.5
1
2
D: Fatty acids
22:6, docosahexaenoic acid 0.93 (0.75, 1.16)
Omega−3 fatty acids 0.94 (0.76, 1.17)
Omega−6 fatty acids 0.96 (0.78, 1.18)
18:2, linoleic acid 0.98 (0.80, 1.21)
Monounsaturated fatty acids; 16:1, 18:1 0.83 (0.66, 1.04)
Polyunsaturated fatty acids 0.96 (0.78, 1.18)
Saturated fatty acids 0.83 (0.67, 1.03)
Total fatty acids 0.86 (0.70, 1.07)
Estimated degree of unsaturation 1.22 (0.99, 1.50)
0.5
1
2
E: Glycerides and phospholipids
Triglycerides in HDL 0.75 (0.60, 0.94)
Triglycerides in LDL 0.82 (0.66, 1.02)
Phosphatidylcholine & other cholines 0.90 (0.73, 1.11)
Serum total triglycerides 0.73 (0.57, 0.93)
Sphingomyelins (mmol/l) 0.96 (0.78, 1.18)
Total cholines 0.90 (0.73, 1.11)
Total phosphoglycerides 0.89 (0.72, 1.10)
Triglycerides in VLDL 0.73 (0.56, 0.93)
0.5
1
2
F: Glycolysis−related metabolites
Citrate 0.84 (0.67, 1.06)
Glucose 1.14 (0.95, 1.38)
Glycerol 0.83 (0.66, 1.05)
Lactate 0.73 (0.57, 0.94)
Pyruvate 0.90 (0.72, 1.12)
0.5
1
2
G: Fluid balance, Inflammation & Ketone bodies
Albumin 0.83 (0.68, 1.01)
Creatinine 0.96 (0.78, 1.19)
Glycoprotein acetyls 0.76 (0.60, 0.97)
Acetate 1.05 (0.86, 1.28)
3−hydroxybutyrate 0.89 (0.69, 1.13)
0.5
1
2
Adjusted OR (95% CI) of CHD per SD change in metabolic trait

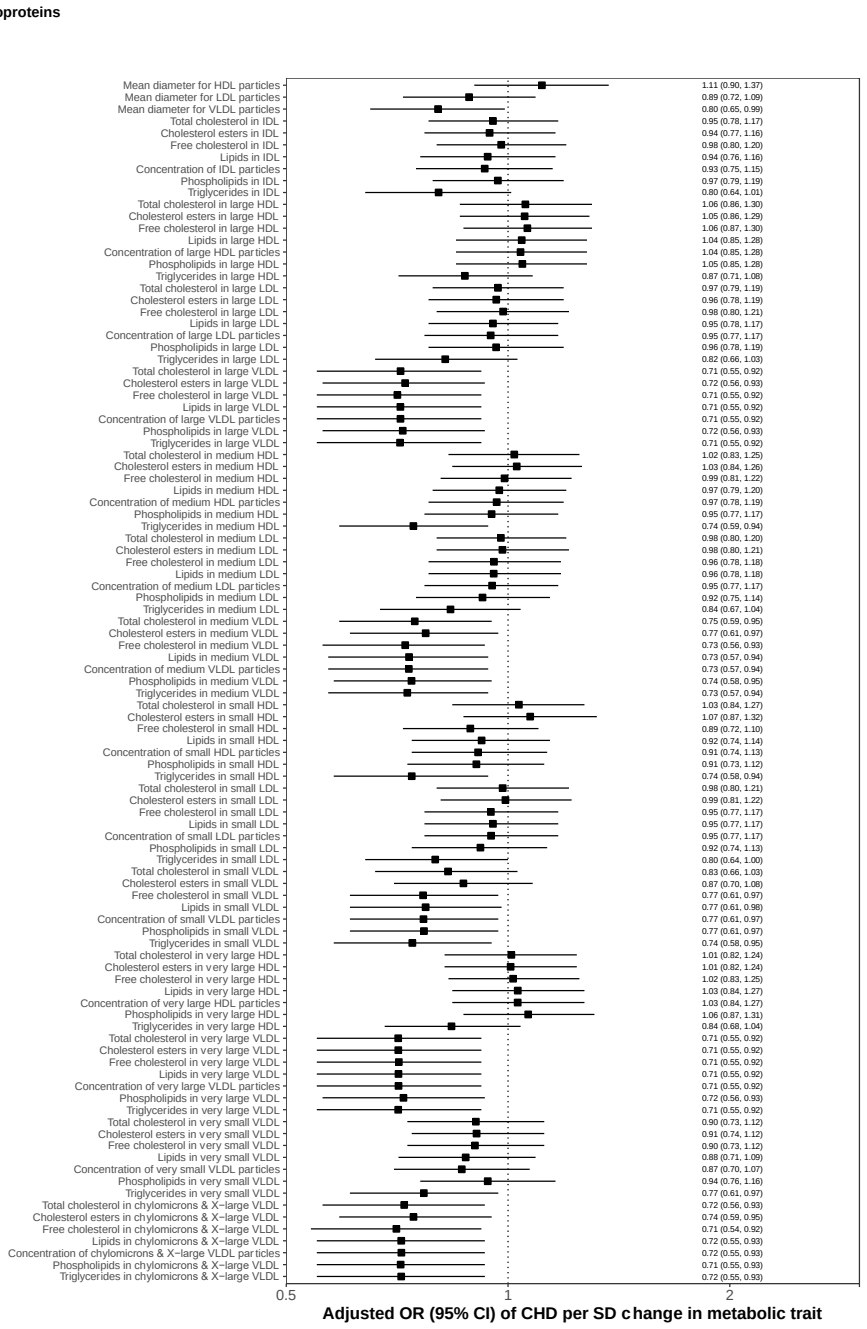

H: Lipoproteins
Mean diameter for HDL particles 1.11 (0.90, 1.37)
Mean diameter for LDL particles 0.89 (0.72, 1.09)
Mean diameter for VLDL particles 0.80 (0.65, 0.99)
Total cholesterol in IDL 0.95 (0.78, 1.17)
Cholesterol esters in IDL 0.94 (0.77, 1.16)
Free cholesterol in IDL 0.98 (0.80, 1.20)
Lipids in IDL 0.94 (0.76, 1.16)
Concentration of IDL particles 0.93 (0.75, 1.15)
Phospholipids in IDL 0.97 (0.79, 1.19)
Triglycerides in IDL 0.80 (0.64, 1.01)
Total cholesterol in large HDL 1.06 (0.86, 1.30)
Cholesterol esters in large HDL 1.05 (0.86, 1.29)
Free cholesterol in large HDL 1.06 (0.87, 1.30)
Lipids in large HDL 1.04 (0.85, 1.28)
Concentration of large HDL particles 1.04 (0.85, 1.28)
Phospholipids in large HDL 1.05 (0.85, 1.28)
Triglycerides in large HDL 0.87 (0.71, 1.08)
Total cholesterol in large LDL 0.97 (0.79, 1.19)
Cholesterol esters in large LDL 0.96 (0.78, 1.19)
Free cholesterol in large LDL 0.98 (0.80, 1.21)
Lipids in large LDL 0.95 (0.78, 1.17)
Concentration of large LDL particles 0.95 (0.77, 1.17)
Phospholipids in large LDL 0.96 (0.78, 1.19)
Triglycerides in large LDL 0.82 (0.66, 1.03)
Total cholesterol in large VLDL 0.71 (0.55, 0.92)
Cholesterol esters in large VLDL 0.72 (0.56, 0.93)
Free cholesterol in large VLDL 0.71 (0.55, 0.92)
Lipids in large VLDL 0.71 (0.55, 0.92)
Concentration of large VLDL particles 0.71 (0.55, 0.92)
Phospholipids in large VLDL 0.72 (0.56, 0.93)
Triglycerides in large VLDL 0.71 (0.55, 0.92)
Total cholesterol in medium HDL 1.02 (0.83, 1.25)
Cholesterol esters in medium HDL 1.03 (0.84, 1.26)
Free cholesterol in medium HDL 0.99 (0.81, 1.22)
Lipids in medium HDL 0.97 (0.79, 1.20)
Concentration of medium HDL particles 0.97 (0.78, 1.19)
Phospholipids in medium HDL 0.95 (0.77, 1.17)
Triglycerides in medium HDL 0.74 (0.59, 0.94)
Total cholesterol in medium LDL 0.98 (0.80, 1.20)
Cholesterol esters in medium LDL 0.98 (0.80, 1.21)
Free cholesterol in medium LDL 0.96 (0.78, 1.18)
Lipids in medium LDL 0.96 (0.78, 1.18)
Concentration of medium LDL particles 0.95 (0.77, 1.17)
Phospholipids in medium LDL 0.92 (0.75, 1.14)
Triglycerides in medium LDL 0.84 (0.67, 1.04)
Total cholesterol in medium VLDL 0.75 (0.59, 0.95)
Cholesterol esters in medium VLDL 0.77 (0.61, 0.97)
Free cholesterol in medium VLDL 0.73 (0.56, 0.93)
Lipids in medium VLDL 0.73 (0.57, 0.94)
Concentration of medium VLDL particles 0.73 (0.57, 0.94)
Phospholipids in medium VLDL 0.74 (0.58, 0.95)
Triglycerides in medium VLDL 0.73 (0.57, 0.94)
Total cholesterol in small HDL 1.03 (0.84, 1.27)
Cholesterol esters in small HDL 1.07 (0.87, 1.32)
Free cholesterol in small HDL 0.89 (0.72, 1.10)
Lipids in small HDL 0.92 (0.74, 1.14)
Concentration of small HDL particles 0.91 (0.74, 1.13)
Phospholipids in small HDL 0.91 (0.73, 1.12)
Triglycerides in small HDL 0.74 (0.58, 0.94)
Total cholesterol in small LDL 0.98 (0.80, 1.21)
Cholesterol esters in small LDL 0.99 (0.81, 1.22)
Free cholesterol in small LDL 0.95 (0.77, 1.17)
Lipids in small LDL 0.95 (0.77, 1.17)
Concentration of small LDL particles 0.95 (0.77, 1.17)
Phospholipids in small LDL 0.92 (0.74, 1.13)
Triglycerides in small LDL 0.80 (0.64, 1.00)
Total cholesterol in small VLDL 0.83 (0.66, 1.03)
Cholesterol esters in small VLDL 0.87 (0.70, 1.08)
Free cholesterol in small VLDL 0.77 (0.61, 0.97)
Lipids in small VLDL 0.77 (0.61, 0.96)
Concentration of small VLDL particles 0.77 (0.61, 0.97)
Phospholipids in small VLDL 0.77 (0.61, 0.97)
Triglycerides in small VLDL 0.74 (0.58, 0.95)
Total cholesterol in very large HDL 1.01 (0.82, 1.24)
Cholesterol esters in very large HDL 1.01 (0.82, 1.24)
Free cholesterol in very large HDL 1.02 (0.83, 1.25)
Lipids in very large HDL 1.03 (0.84, 1.27)
Concentration of very large HDL particles 1.03 (0.84, 1.27)
Phospholipids in very large HDL 1.06 (0.87, 1.31)
Triglycerides in very large HDL 0.84 (0.68, 1.04)
Total cholesterol in very large VLDL 0.71 (0.55, 0.92)
Cholesterol esters in very large VLDL 0.71 (0.55, 0.92)
Free cholesterol in very large VLDL 0.71 (0.55, 0.92)
Lipids in very large VLDL 0.71 (0.55, 0.92)
Concentration of very large VLDL particles 0.71 (0.55, 0.92)
Phospholipids in very large VLDL 0.72 (0.56, 0.93)
Triglycerides in very large VLDL 0.71 (0.55, 0.92)
Total cholesterol in very small VLDL 0.90 (0.73, 1.12)
Cholesterol esters in very small VLDL 0.91 (0.74, 1.12)
Free cholesterol in very small VLDL 0.90 (0.73, 1.12)
Lipids in very small VLDL 0.88 (0.71, 1.09)
Concentration of very small VLDL particles 0.87 (0.70, 1.07)
Phospholipids in very small VLDL 0.94 (0.76, 1.16)
Triglycerides in very small VLDL 0.77 (0.61, 0.97)
Total cholesterol in chylomicrons & X−large VLDL 0.72 (0.56, 0.93)
Cholesterol esters in chylomicrons & X−large VLDL 0.74 (0.59, 0.95)
Free cholesterol in chylomicrons & X−large VLDL 0.71 (0.54, 0.92)
Lipids in chylomicrons & X−large VLDL 0.72 (0.55, 0.93)
Concentration of chylomicrons & X−large VLDL particles 0.72 (0.55, 0.93)
Phospholipids in chylomicrons & X−large VLDL 0.71 (0.55, 0.93)
Triglycerides in chylomicrons & X−large VLDL 0.72 (0.55, 0.93)
0.5
1
2
Adjusted OR (95% CI) of CHD per SD change in metabolic trait

Figure S6.3. Confounder adjusted associations of maternal pregnancy metabolic traits with offspring congenital heart disease in the Born in Bradford cohort (N = 8,551 & N CHD cases = 96). The associations show confounder adjusted odds ratios of CHD per standard deviation change metabolic trait levels for 148 traits separated by the trait class. Metabolic traits were measured at ~26-28 weeks' gestation. Associations were adjusted for maternal age, ethnicity, parity, Index of Multiple Deprivation, body mass index, and smoking. Abbreviations: OR, odds ratio; CHD, congenital heart disease; SD, standard deviation.

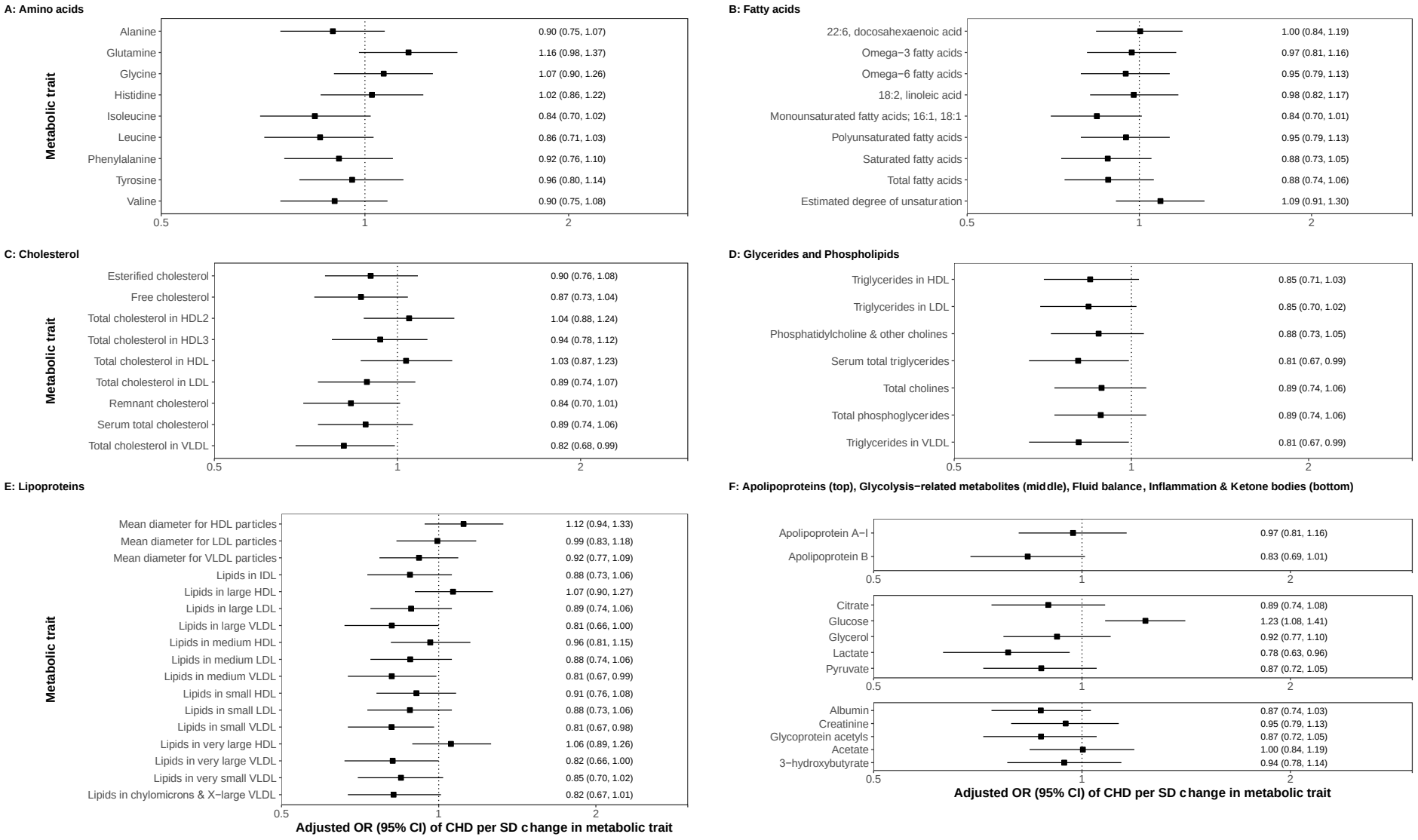


Figure S6.4. Unadjusted associations of maternal pregnancy metabolic traits with offspring congenital heart disease in the Born in Bradford cohort (N = 11,195 & N CHD cases = 127). The associations show odds ratios of CHD per standard deviation change metabolic trait levels for 63 (out of 148) key traits separated by the trait class. Metabolic traits were measured at ~26-28 weeks' gestation. Abbreviations: OR, odds ratio; CHD, congenital heart disease; SD, standard deviation.

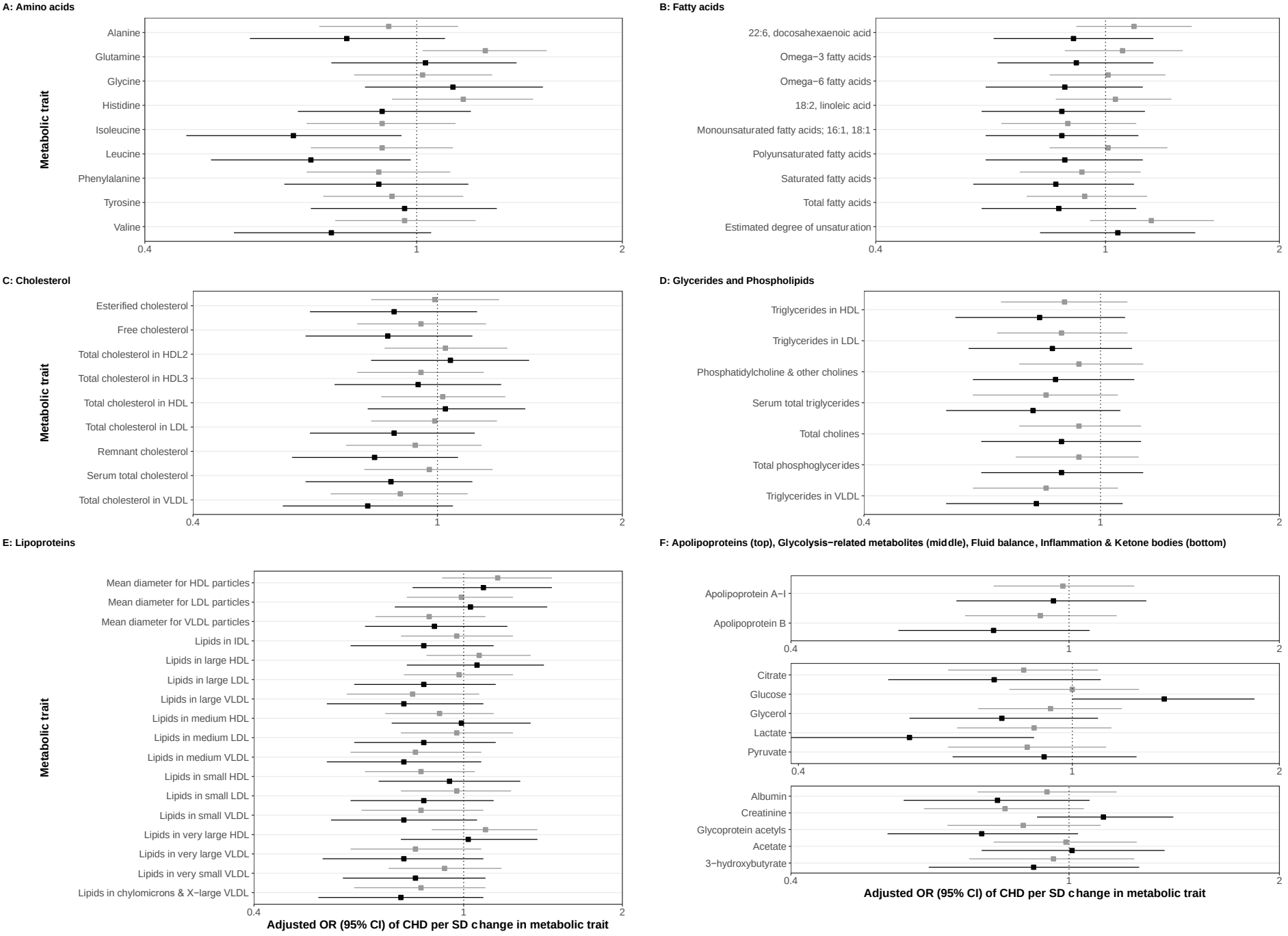

A: Amino acids
Metabolic trait
Alanine
Glutamine
Glycine
Histidine
Isoleucine
Leucine
Phenylalanine
Tyrosine
Valine
0.4
1
2
B: Fatty acids
22:6, docosahexaenoic acid
Omega−3 fatty acids
Omega−6 fatty acids
18:2, linoleic acid
Monounsaturated fatty acids; 16:1, 18:1
Polyunsaturated fatty acids
Saturated fatty acids
Total fatty acids
Estimated degree of unsaturation
C: Cholesterol
Esterified cholesterol
Free cholesterol
Total cholesterol in HDL2
Total cholesterol in HDL3
Total cholesterol in HDL
Total cholesterol in LDL
Remnant cholesterol
Serum total cholesterol
Total cholesterol in VLDL
D: Glycerides and Phospholipids
Triglycerides in HDL
Triglycerides in LDL
Phosphatidylcholine & other cholines
Serum total triglycerides
Total cholines
Total phosphoglycerides
Triglycerides in VLDL
E: Lipoproteins
Mean diameter for HDL particles
Mean diameter for LDL particles
Mean diameter for VLDL particles
Lipids in IDL
Lipids in large HDL
Lipids in large LDL
Lipids in large VLDL
Lipids in medium HDL
Lipids in medium LDL
Lipids in medium VLDL
Lipids in small HDL
Lipids in small LDL
Lipids in small VLDL
Lipids in very large HDL
Lipids in very large VLDL
Lipids in very small VLDL
Lipids in chylomicrons & X−large VLDL
Adjusted OR (95% CI) of CHD per SD change in metabolic trait
F: Apolipoproteins (top), Glycolysis−related metabolites (middle), Fluid balance, Inflammation & Ketone bodies (bottom)
Apolipoprotein A−I
Apolipoprotein B
Citrate
Glucose
Glycerol
Lactate
Pyruvate
Albumin
Creatinine
Glycoprotein acetyls
Acetate
3−hydroxybutyrate
Adjusted OR (95% CI) of CHD per SD change in metabolic trait

Figure S6.5. Confounder adjusted associations of maternal pregnancy metabolic traits with offspring congenital heart disease in the Born in Bradford cohort stratified by ethnicity **(grey fill = South Asian women, black fill = White European woman)**. The associations show confounder adjusted odds ratios of CHD per standard deviation change metabolic trait levels for 63 (out of 148) key traits separated by the trait class. Metabolic traits were measured at ~26-28 weeks' gestation. Associations were adjusted for maternal age, ethnicity, parity, Index of Multiple Deprivation, body mass index, and smoking. Abbreviations: OR, odds ratio; CHD, congenital heart disease; SD, standard deviation.

Table S6.1. Associations of genetic risk scores (N = 145) with the corresponding metabolic trait in BiB (N = 7,154 participants with genome-wide and NMR data).

Metabolite	Class	Coefficient (95% CI)[b]	P-Value	R^2 (%)	F-statistic
Alanine	Amino acids	0.008 (0.007, 0.009)	3.73E-44	2.7	196.9
Glutamine	Amino acids	0.010 (0.009, 0.011)	1.54E-87	5.4	404.4
Glycine	Amino acids	0.010 (0.010, 0.011)	6.88E-172	10.4	825.5
Histidine	Amino acids	0.000 (0.000, 0.001)	3.46E-05	0.2	17.2
Isoleucine	Amino acids	0.001 (0.000, 0.001)	2.32E-09	0.5	35.8
Leucine	Amino acids	0.000 (0.000, 0.001)	6.15E-05	0.2	16.1
Phenylalanine	Amino acids	0.000 (0.000, 0.001)	3.09E-03	0.1	8.8
Tyrosine	Amino acids	0.000 (0.000, 0.001)	1.51E-14	0.8	59.3
Valine	Amino acids	0.001 (0.000, 0.001)	4.76E-05	0.2	16.6
Apolipoprotein A-I	Apolipoproteins	0.042 (0.037, 0.047)	2.15E-61	3.8	278.6
Apolipoprotein B	Apolipoproteins	0.056 (0.051, 0.061)	2.36E-99	6.1	462
Esterified cholesterol	Cholesterol	0.219 (0.202, 0.237)	1.08E-124	7.6	587
Free cholesterol	Cholesterol	0.095 (0.088, 0.102)	1.18E-135	8.3	641.8
Cholesterol in HDL	Cholesterol	0.070 (0.062, 0.078)	1.77E-60	3.7	274.3
Cholesterol in LDL	Cholesterol	0.180 (0.167, 0.194)	7.11E-144	8.7	683
Remnant cholesterol (non-HDL, non-LDL -cholesterol)	Cholesterol	0.111 (0.100, 0.121)	5.02E-94	5.7	436
Serum total cholesterol	Cholesterol	0.317 (0.292, 0.342)	2.06E-127	7.7	600.5
Cholesterol in VLDL	Cholesterol	0.043 (0.037, 0.049)	2.41E-40	2.4	179
22:6, docosahexaenoic acid	Fatty acids	0.009 (0.008, 0.010)	6.45E-72	4.4	329
Omega-3 fatty acids	Fatty acids	0.025 (0.023, 0.028)	1.92E-93	5.7	433.2
Omega-6 fatty acids	Fatty acids	0.156 (0.137, 0.175)	5.74E-56	3.4	252.8
18:2, linoleic acid	Fatty acids	0.122 (0.105, 0.140)	3.42E-42	2.6	187.7
Monounsaturated fatty acids; 16:1, 18:1	Fatty acids	0.132 (0.109, 0.155)	2.06E-29	1.8	127.9
Polyunsaturated fatty acids	Fatty acids	0.169 (0.148, 0.190)	3.15E-55	3.4	249.3
Saturated fatty acids	Fatty acids	0.119 (0.094, 0.143)	8.69E-22	1.3	92.6
Total fatty acids	Fatty acids	0.345 (0.281, 0.409)	5.40E-26	1.6	112.1
Estimated degree of unsaturation	Fatty acids	0.014 (0.013, 0.015)	3.85E-159	9.7	760.5
Albumin	Fluid balance	0.000 (0.000, 0.000)	1.05E-06	0.3	23.9
Creatinine	Fluid balance	0.001 (0.001, 0.001)	2.66E-28	1.7	122.8
Triglycerides in HDL	Glycerides and phospholipids	0.002 (0.001, 0.003)	3.84E-06	0.3	21.4
Triglycerides in LDL	Glycerides and phospholipids	0.011 (0.010, 0.013)	1.78E-45	2.8	203.2
Phosphatidylcholine and other cholines	Glycerides and phospholipids	0.090 (0.080, 0.101)	1.27E-63	3.9	289.3
Serum total triglycerides	Glycerides and phospholipids	0.026 (0.012, 0.039)	1.69E-04	0.2	14.2
Sphingomyelins (mmol/l)	Glycerides and phospholipids	0.020 (0.018, 0.022)	9.75E-90	5.5	415.1

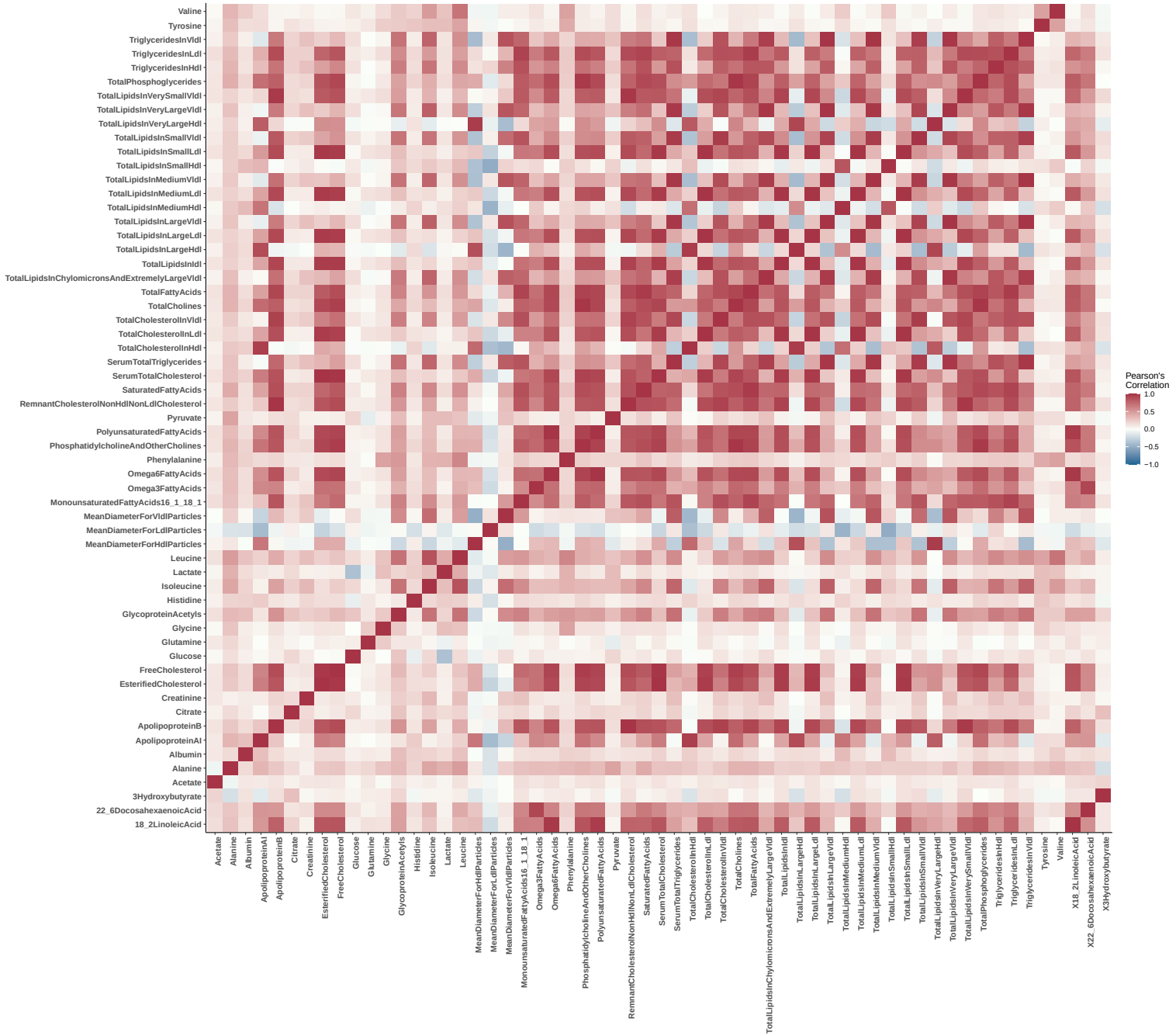
Total cholines	Glycerides and phospholipids	0.092 (0.081, 0.103)	9.84E-59	3.6	266
Total phosphoglycerides	Glycerides and phospholipids	0.077 (0.068, 0.086)	2.67E-61	3.8	278.2
Triglycerides in VLDL	Glycerides and phospholipids	0.028 (0.017, 0.038)	4.04E-07	0.4	25.7
Citrate	Glycolysis related metabolites	0.001 (0.001, 0.001)	1.06E-07	0.4	28.3
Glucose	Glycolysis related metabolites	0.037 (0.025, 0.048)	2.09E-10	0.6	40.5
Lactate	Glycolysis related metabolites	0.017 (0.005, 0.028)	4.31E-03	0.1	8.2
Pyruvate	Glycolysis related metabolites	0.002 (0.001, 0.002)	4.79E-10	0.5	38.9
Glycoprotein acetyls	Inflammation	0.011 (0.006, 0.015)	3.85E-06	0.3	21.4
Acetate	Ketone bodies	0.000 (-0.000, 0.000)	8.39E-01	0	0
3-hydroxybutyrate	Ketone bodies	0.004 (0.003, 0.005)	2.12E-16	0.9	67.8
Mean diameter for HDL particles	Lipoprotein particle size	0.054 (0.050, 0.059)	2.89E-114	7	535.3
Mean diameter for LDL particles	Lipoprotein particle size	-0.004 (-0.005, -0.003)	6.00E-08	0.4	29.4
Mean diameter for VLDL particles	Lipoprotein particle size	0.099 (0.076, 0.122)	6.47E-17	1	70.2
Cholesterol in IDL	Lipoprotein subclasses	0.066 (0.061, 0.071)	1.36E-141	8.6	671.5
Cholesterol esters in IDL	Lipoprotein subclasses	0.046 (0.042, 0.050)	9.04E-135	8.2	637.3
Free cholesterol in IDL	Lipoprotein subclasses	0.020 (0.018, 0.021)	3.05E-146	8.9	694.9
Total lipids in IDL	Lipoprotein subclasses	0.099 (0.092, 0.107)	3.43E-136	8.3	644.4
Concentration of IDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	8.48E-139	8.4	657.5
Phospholipids in IDL	Lipoprotein subclasses	0.026 (0.024, 0.028)	3.12E-141	8.6	669.7
Triglycerides in IDL	Lipoprotein subclasses	0.006 (0.005, 0.007)	1.38E-31	1.9	138.1
Cholesterol in large HDL	Lipoprotein subclasses	0.047 (0.043, 0.051)	3.83E-104	6.4	485.4
Cholesterol esters in large HDL	Lipoprotein subclasses	0.036 (0.032, 0.039)	3.64E-102	6.2	475.7
Free cholesterol in large HDL	Lipoprotein subclasses	0.009 (0.008, 0.010)	4.69E-71	4.3	324.8
Total lipids in large HDL	Lipoprotein subclasses	0.083 (0.075, 0.091)	3.12E-90	5.5	417.5
Concentration of large HDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	3.22E-72	4.4	330.4
Phospholipids in large HDL	Lipoprotein subclasses	0.035 (0.032, 0.039)	1.55E-85	5.2	394.7
Triglycerides in large HDL	Lipoprotein subclasses	0.003 (0.003, 0.004)	2.22E-77	4.7	355.3
Cholesterol in large LDL	Lipoprotein subclasses	0.091 (0.085, 0.098)	5.52E-148	9	703.7
Cholesterol esters in large LDL	Lipoprotein subclasses	0.068 (0.063, 0.073)	8.46E-143	8.7	677.6
Free cholesterol in large LDL	Lipoprotein subclasses	0.022 (0.021, 0.024)	7.71E-145	8.8	687.9
Total lipids in large LDL	Lipoprotein subclasses	0.126 (0.116, 0.135)	1.46E-146	8.9	696.6
Concentration of large LDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	8.78E-145	8.8	687.6
Phospholipids in large LDL	Lipoprotein subclasses	0.025 (0.023, 0.027)	1.77E-136	8.3	645.8
Triglycerides in large LDL	Lipoprotein subclasses	0.006 (0.006, 0.007)	2.83E-46	2.8	206.9
Cholesterol in large VLDL	Lipoprotein subclasses	0.004 (0.003, 0.005)	3.94E-15	0.9	62
Cholesterol esters in large VLDL	Lipoprotein subclasses	0.001 (0.001, 0.002)	8.37E-06	0.3	19.9
Free cholesterol in large VLDL	Lipoprotein subclasses	0.002 (0.001, 0.002)	2.87E-08	0.4	30.9
Total lipids in large VLDL	Lipoprotein subclasses	0.013 (0.009, 0.018)	2.15E-08	0.4	31.4

Concentration of large VLDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	5.31E-09	0.5	34.2
Phospholipids in large VLDL	Lipoprotein subclasses	0.003 (0.002, 0.004)	9.11E-11	0.6	42.1
Triglycerides in large VLDL	Lipoprotein subclasses	0.007 (0.004, 0.010)	2.11E-07	0.4	27
Cholesterol in medium HDL	Lipoprotein subclasses	0.019 (0.016, 0.021)	1.83E-50	3.1	226.7
Cholesterol esters in medium HDL	Lipoprotein subclasses	0.015 (0.013, 0.016)	6.80E-48	2.9	214.6
Free cholesterol in medium HDL	Lipoprotein subclasses	0.003 (0.002, 0.003)	3.94E-26	1.6	112.7
Total lipids in medium HDL	Lipoprotein subclasses	0.032 (0.027, 0.036)	5.66E-45	2.7	200.8
Concentration of medium HDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	8.74E-39	2.3	171.7
Phospholipids in medium HDL	Lipoprotein subclasses	0.016 (0.014, 0.018)	9.76E-57	3.5	256.5
Triglycerides in medium HDL	Lipoprotein subclasses	0.000 (0.000, 0.001)	4.33E-03	0.1	8.1
Cholesterol in medium LDL	Lipoprotein subclasses	0.056 (0.052, 0.060)	3.38E-144	8.7	684.6
Cholesterol esters in medium LDL	Lipoprotein subclasses	0.044 (0.041, 0.048)	2.22E-136	8.3	645.3
Free cholesterol in medium LDL	Lipoprotein subclasses	0.010 (0.009, 0.011)	5.39E-124	7.5	583.5
Total lipids in medium LDL	Lipoprotein subclasses	0.070 (0.065, 0.076)	1.97E-127	7.7	600.6
Concentration of medium LDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	5.27E-136	8.3	643.5
Phospholipids in medium LDL	Lipoprotein subclasses	0.014 (0.012, 0.015)	2.64E-124	7.6	585.1
Triglycerides in medium LDL	Lipoprotein subclasses	0.003 (0.003, 0.004)	1.27E-49	3	222.7
Cholesterol in medium VLDL	Lipoprotein subclasses	0.009 (0.007, 0.011)	5.56E-21	1.2	88.9
Cholesterol esters in medium VLDL	Lipoprotein subclasses	0.007 (0.006, 0.008)	1.18E-47	2.9	213.4
Free cholesterol in medium VLDL	Lipoprotein subclasses	0.003 (0.002, 0.004)	4.92E-12	0.7	47.9
Total lipids in medium VLDL	Lipoprotein subclasses	0.029 (0.023, 0.036)	4.09E-17	1	71.1
Concentration of medium VLDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	1.32E-10	0.6	41.4
Phospholipids in medium VLDL	Lipoprotein subclasses	0.005 (0.004, 0.007)	2.66E-15	0.9	62.8
Triglycerides in medium VLDL	Lipoprotein subclasses	0.008 (0.004, 0.012)	4.51E-05	0.2	16.7
Cholesterol in small HDL	Lipoprotein subclasses	0.017 (0.015, 0.018)	2.84E-111	6.8	520.5
Cholesterol esters in small HDL	Lipoprotein subclasses	0.013 (0.012, 0.015)	7.14E-87	5.3	401.2
Free cholesterol in small HDL	Lipoprotein subclasses	0.002 (0.002, 0.003)	2.96E-36	2.2	159.9
Total lipids in small HDL	Lipoprotein subclasses	0.041 (0.038, 0.044)	3.71E-142	8.6	674.4
Concentration of small HDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	1.01E-145	8.8	692.3
Phospholipids in small HDL	Lipoprotein subclasses	0.024 (0.022, 0.027)	1.63E-112	6.9	526.6
Triglycerides in small HDL	Lipoprotein subclasses	0.002 (0.001, 0.002)	1.28E-18	1.1	78
Cholesterol in small LDL	Lipoprotein subclasses	0.035 (0.032, 0.038)	4.63E-152	9.2	724.4
Cholesterol esters in small LDL	Lipoprotein subclasses	0.028 (0.026, 0.030)	1.15E-144	8.8	687
Free cholesterol in small LDL	Lipoprotein subclasses	0.006 (0.006, 0.007)	2.82E-123	7.5	579.9
Total lipids in small LDL	Lipoprotein subclasses	0.044 (0.041, 0.048)	9.21E-134	8.1	632.2
Concentration of small LDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	3.94E-140	8.5	664.2
Phospholipids in small LDL	Lipoprotein subclasses	0.009 (0.008, 0.010)	4.82E-119	7.2	558.9
Triglycerides in small LDL	Lipoprotein subclasses	0.001 (0.001, 0.002)	4.57E-21	1.2	89.3

Cholesterol in small VLDL	Lipoprotein subclasses	0.016 (0.015, 0.018)	3.32E-67	4.1	306.4
Cholesterol esters in small VLDL	Lipoprotein subclasses	0.012 (0.010, 0.013)	8.05E-76	4.6	347.7
Free cholesterol in small VLDL	Lipoprotein subclasses	0.004 (0.004, 0.005)	4.92E-33	2	144.8
Total lipids in small VLDL	Lipoprotein subclasses	0.021 (0.016, 0.026)	2.63E-16	0.9	67.4
Concentration of small VLDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	3.37E-28	1.7	122.3
Phospholipids in small VLDL	Lipoprotein subclasses	0.006 (0.005, 0.007)	2.26E-27	1.6	118.5
Triglycerides in small VLDL	Lipoprotein subclasses	0.007 (0.005, 0.009)	3.91E-10	0.5	39.3
Cholesterol in very large HDL	Lipoprotein subclasses	0.028 (0.026, 0.031)	2.05E-125	7.6	590.6
Cholesterol esters in very large HDL	Lipoprotein subclasses	0.020 (0.019, 0.022)	3.01E-127	7.7	599.7
Free cholesterol in very large HDL	Lipoprotein subclasses	0.008 (0.007, 0.009)	4.75E-120	7.3	563.9
Total lipids in very large HDL	Lipoprotein subclasses	0.058 (0.053, 0.063)	6.58E-105	6.4	489.2
Concentration of very large HDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	7.28E-146	8.8	693
Phospholipids in very large HDL	Lipoprotein subclasses	0.030 (0.027, 0.032)	2.23E-96	5.9	447.4
Triglycerides in very large HDL	Lipoprotein subclasses	0.002 (0.002, 0.002)	2.70E-63	3.9	287.7
Cholesterol in very large VLDL	Lipoprotein subclasses	0.001 (0.000, 0.001)	1.07E-04	0.2	15
Cholesterol esters in very large VLDL	Lipoprotein subclasses	0.000 (0.000, 0.000)	7.13E-06	0.3	20.2
Free cholesterol in very large VLDL	Lipoprotein subclasses	0.000 (0.000, 0.000)	5.95E-04	0.2	11.8
Total lipids in very large VLDL	Lipoprotein subclasses	0.003 (0.002, 0.005)	5.41E-07	0.4	25.2
Concentration of very large VLDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	3.43E-07	0.4	26
Phospholipids in very large VLDL	Lipoprotein subclasses	0.001 (0.000, 0.001)	1.85E-06	0.3	22.8
Triglycerides in very large VLDL	Lipoprotein subclasses	0.002 (0.001, 0.003)	2.77E-08	0.4	30.9
Cholesterol in very small VLDL	Lipoprotein subclasses	0.020 (0.019, 0.022)	8.30E-114	6.9	533
Cholesterol esters in very small VLDL	Lipoprotein subclasses	0.014 (0.013, 0.015)	3.78E-115	7	539.7
Free cholesterol in very small VLDL	Lipoprotein subclasses	0.006 (0.005, 0.007)	2.12E-90	5.5	418.3
Total lipids in very small VLDL	Lipoprotein subclasses	0.033 (0.030, 0.037)	2.76E-71	4.4	325.9
Concentration of very small VLDL particles	Lipoprotein subclasses	0.000 (0.000, 0.000)	2.66E-73	4.5	335.6
Phospholipids in very small VLDL	Lipoprotein subclasses	0.011 (0.010, 0.012)	2.64E-70	4.3	321.2
Triglycerides in very small VLDL	Lipoprotein subclasses	0.003 (0.002, 0.004)	1.01E-11	0.6	46.5
Cholesterol in chylomicrons and extremely large VLDL	Lipoprotein subclasses	0.000 (-0.000, 0.000)	3.85E-01	0	0.8
Cholesterol esters in chylomicrons and extremely large VLDL	Lipoprotein subclasses	-0.000 (-0.000, 0.000)	7.82E-01	0	0.1
Free cholesterol in chylomicrons and extremely large VLDL	Lipoprotein subclasses	0.000 (-0.000, 0.000)	6.70E-02	0	3.4
Total lipids in chylomicrons and extremely large VLDL	Lipoprotein subclasses	0.000 (-0.000, 0.001)	3.56E-01	0	0.9
Concentration of chylomicrons and extremely large VLDL particles	Lipoprotein subclasses	0.000 (-0.000, 0.000)	1.42E-01	0	2.2
Phospholipids in chylomicrons and extremely large VLDL	Lipoprotein subclasses	0.000 (-0.000, 0.000)	2.17E-01	0	1.5

[a] SNPs for isoleucine and leucine taken from: https://www.nature.com/articles/s41588-020-00751-5; remainder of SNPs taken from a recent GWAS of metabolon metabolites (unpublished).
[b] Estimates from linear regression interpreted as difference in metabolite (scaled/imputed metabolites were log-transformed and presented in SD units as per manuscript methods) per SD increase in genetic risk score. MS-derived metabolomics measured using plasma taken during pregnancy around 26-28 weeks' gestation in the BiB cohort (see methods).
N = 1,326 is the number of women in BiB dataset 2 with Metabolon metabolomics data and GWAS data.
Abbreviations: HMDB, The Human Metabolome Database; SNP, single nucleotide polymorphism; GRS, genetic risk score; CI, confidence interval.

Correlations between metaboilic traits (N = 59)



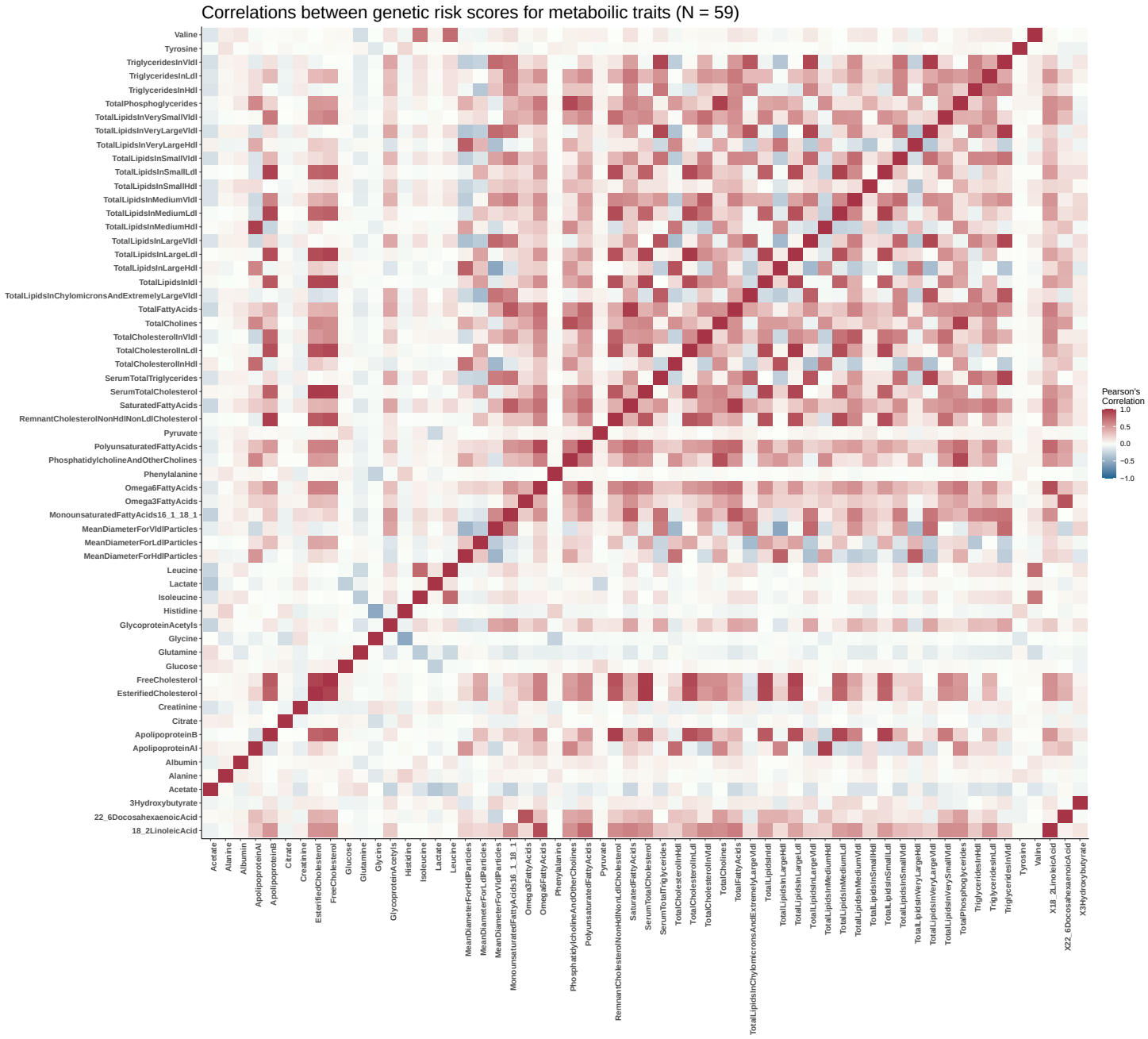
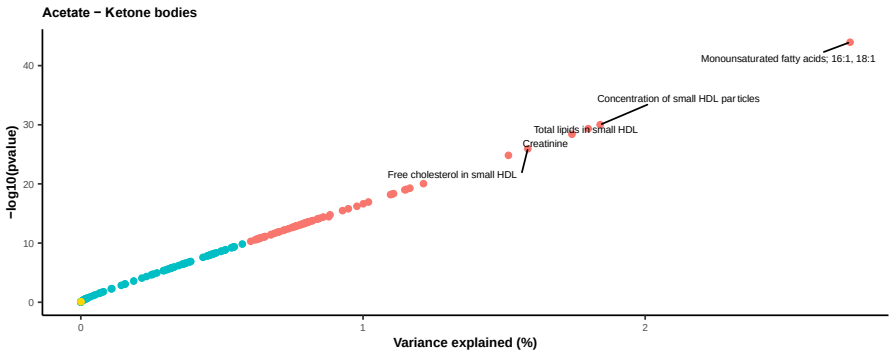
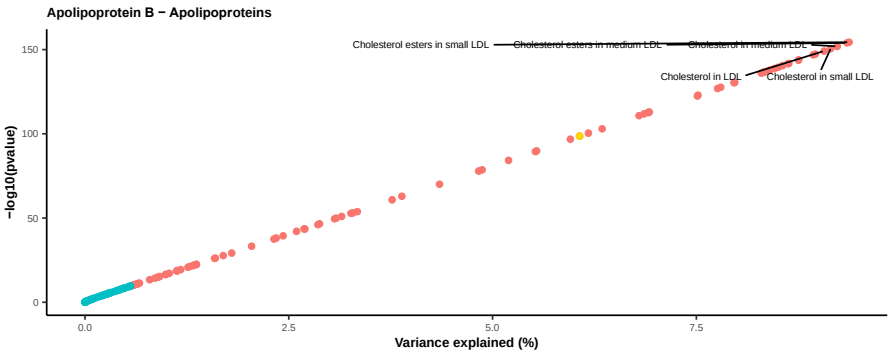
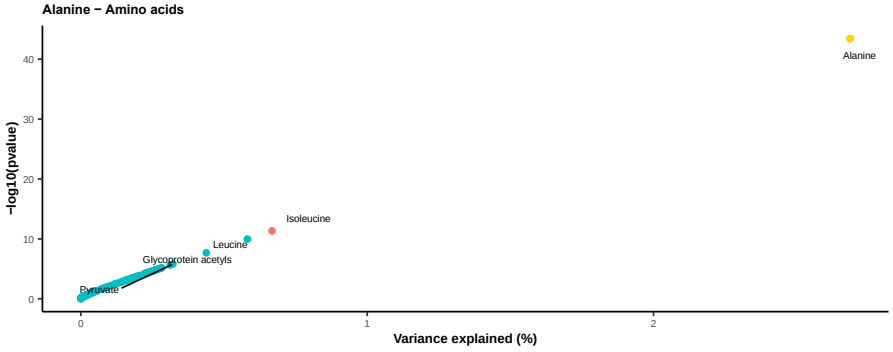
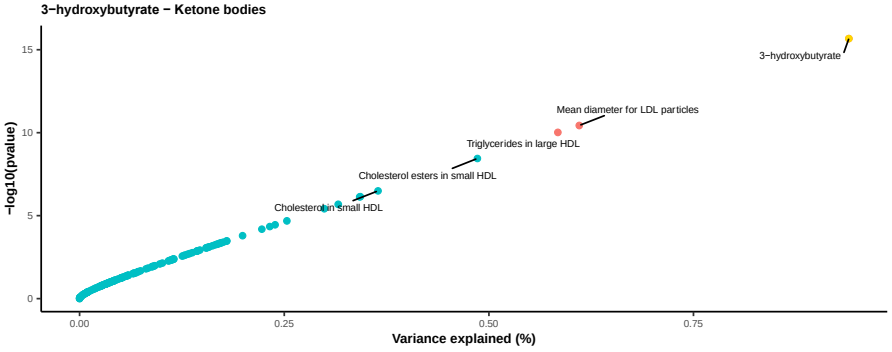


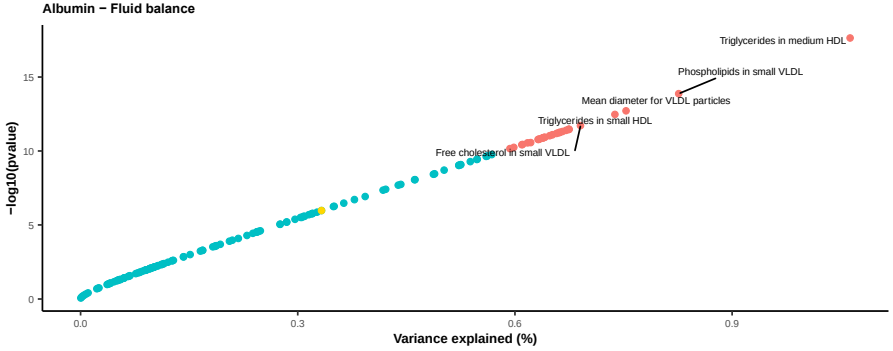
Figure S6.6. Showing the correlation structure of the NMR traits (top) and the GRS's for NMR traits (bottom).

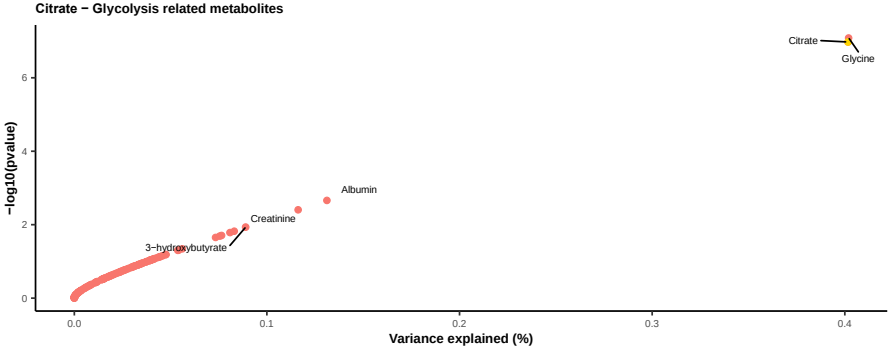

Acetate − Ketone bodies
Monounsaturated fatty acids; 16:1, 18:1
Concentration of small HDL particles
Total lipids in small HDL
Creatinine
Free cholesterol in small HDL
−log10(pvalue)
Variance explained (%)

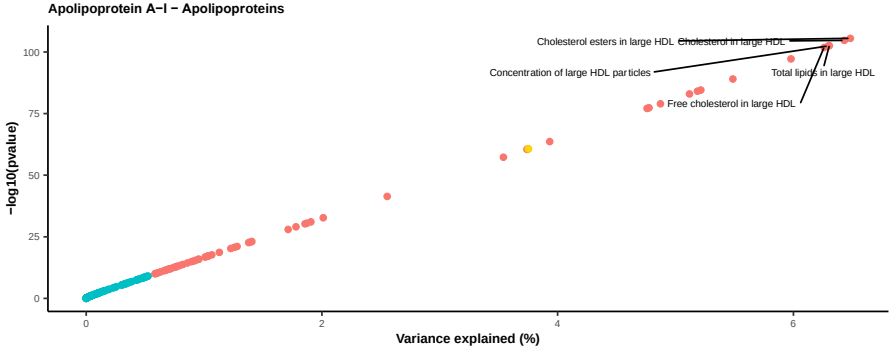

Apolipoprotein B − Apolipoproteins
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol in LDL
Cholesterol in small LDL
−log10(pvalue)
Variance explained (%)

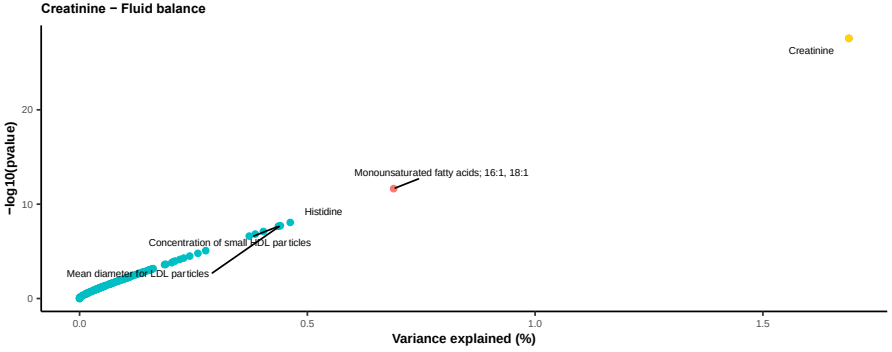

Alanine − Amino acids
Alanine
Isoleucine
Leucine
Glycoprotein acetyls
Pyruvate
−log10(pvalue)
Variance explained (%)

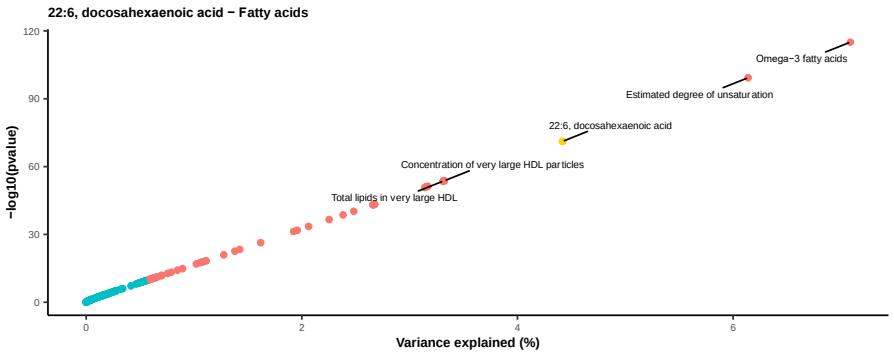

3−hydroxybutyrate − Ketone bodies
3−hydroxybutyrate
Mean diameter for LDL particles
Triglycerides in large HDL
Cholesterol esters in small HDL
Cholesterol in small HDL
−log10(pvalue)
Variance explained (%)

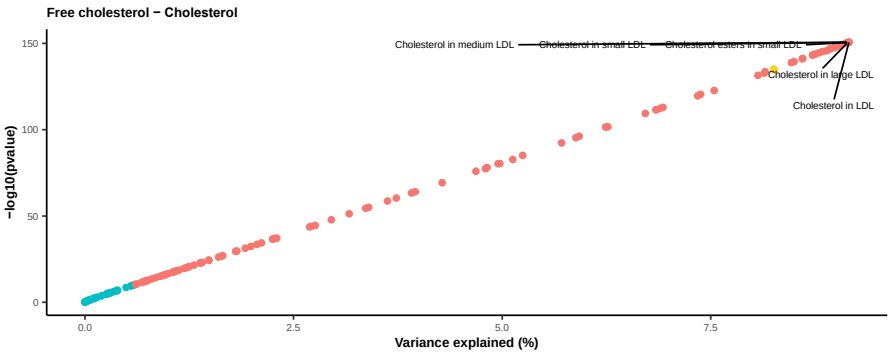

Albumin − Fluid balance
Triglycerides in medium HDL
Phospholipids in small VLDL
Mean diameter for VLDL particles
Triglycerides in small HDL
Free cholesterol in small VLDL
−log10(pvalue)
Variance explained (%)

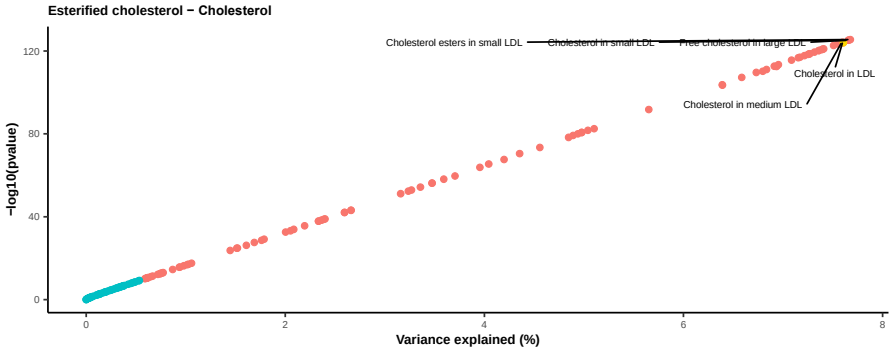

Citrate − Glycolysis related metabolites
Citrate
Glycine
Albumin
Creatinine
3−hydroxybutyrate
−log10(pvalue)
Variance explained (%)

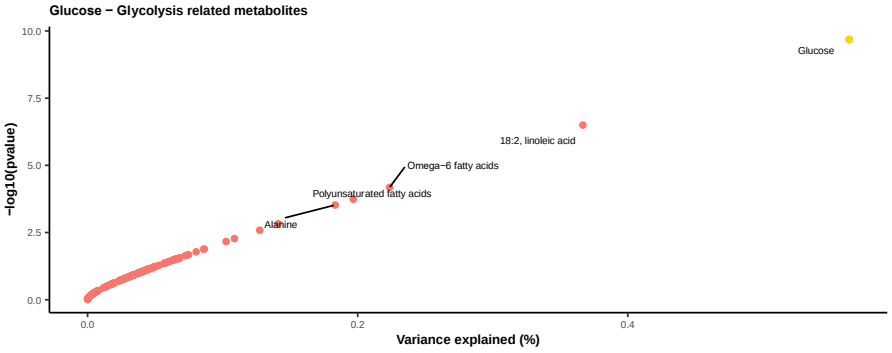

Apolipoprotein A−I − Apolipoproteins
Cholesterol esters in large HDL
Cholesterol in large HDL
Concentration of large HDL particles
Total lipids in large HDL
Free cholesterol in large HDL
−log10(pvalue)
Variance explained (%)

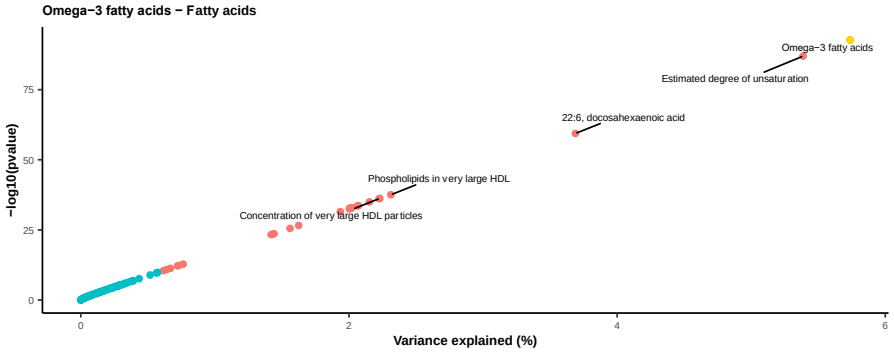

Creatinine − Fluid balance
Creatinine
Monounsaturated fatty acids; 16:1, 18:1
Histidine
Concentration of small HDL particles
Mean diameter for LDL particles
−log10(pvalue)
Variance explained (%)

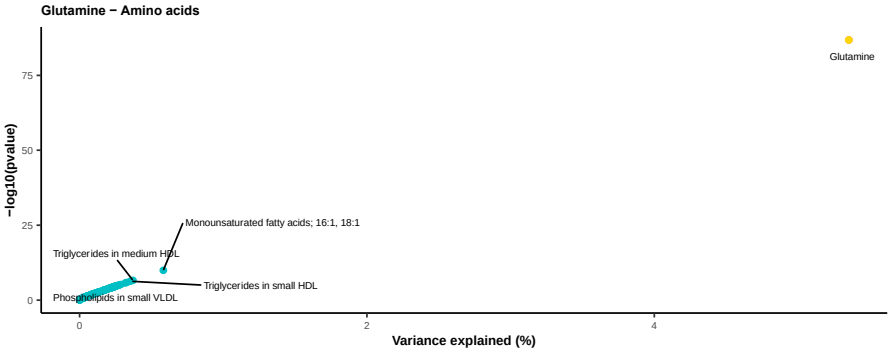

22:6, docosahexaenoic acid ~ Fatty acids
Omega−3 fatty acids
Estimated degree of unsaturation
22:6, docosahexaenoic acid
Concentration of very large HDL particles
Total lipids in very large HDL
−log10(pvalue)
Variance explained (%)

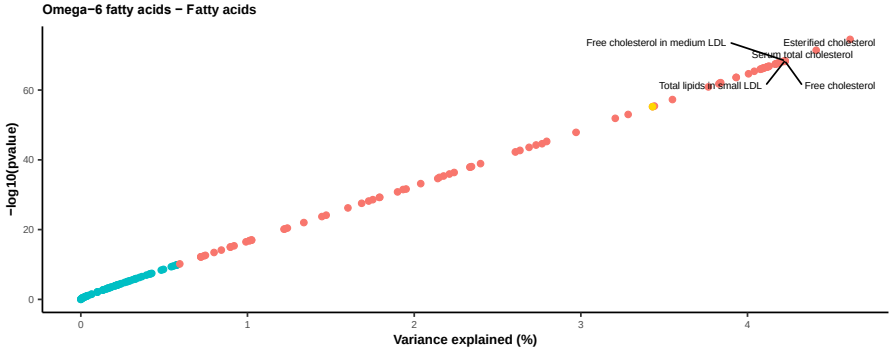

Free cholesterol ~ Cholesterol
Cholesterol in medium LDL
Cholesterol in small LDL
Cholesterol esters in small LDL
Cholesterol in large LDL
Cholesterol in LDL
−log10(pvalue)
Variance explained (%)

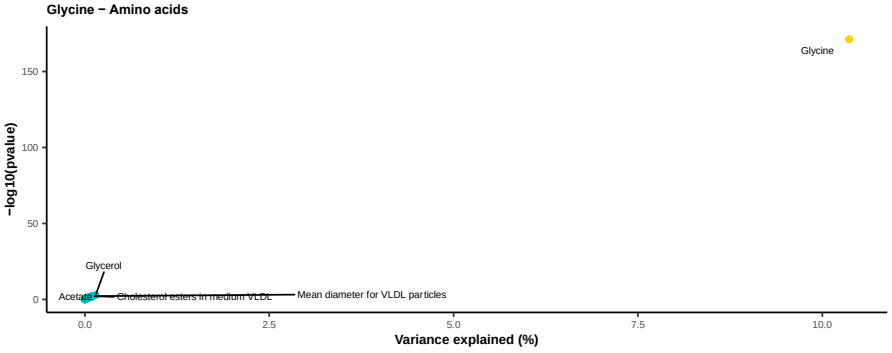

Esterified cholesterol ~ Cholesterol
Cholesterol esters in small LDL
Cholesterol in small LDL
Free cholesterol in large LDL
Cholesterol in LDL
Cholesterol in medium LDL
−log10(pvalue)
Variance explained (%)

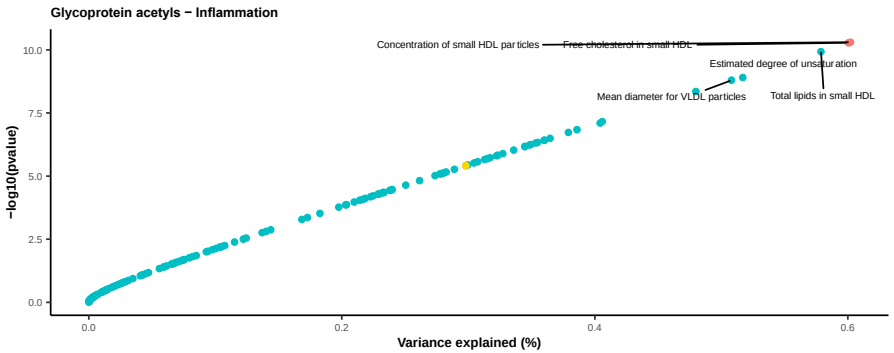

Glucose ~ Glycolysis related metabolites
Glucose
18:2, linoleic acid
Omega−6 fatty acids
Polyunsaturated fatty acids
Alanine
−log10(pvalue)
Variance explained (%)

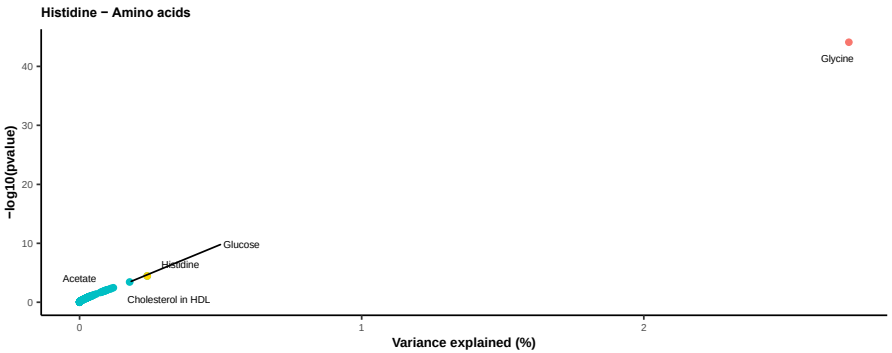

Omega−3 fatty acids ~ Fatty acids
Omega−3 fatty acids
Estimated degree of unsaturation
22:6, docosahexaenoic acid
Phospholipids in very large HDL
Concentration of very large HDL particles
−log10(pvalue)
Variance explained (%)

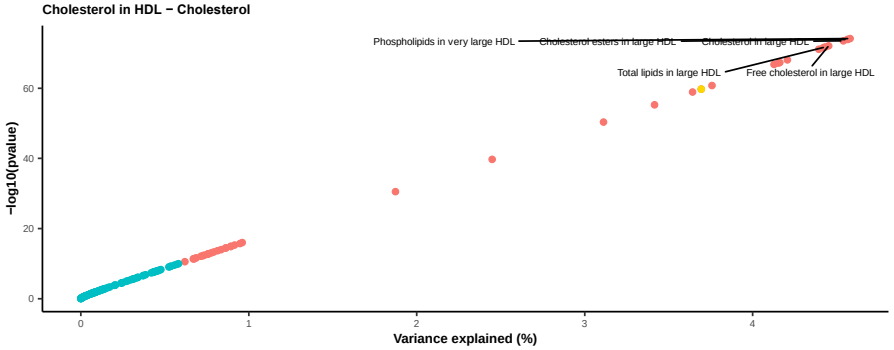

Glutamine ~ Amino acids
Glutamine
Monounsaturated fatty acids; 16:1, 18:1
Triglycerides in medium HDL
Triglycerides in small HDL
Phospholipids in small VLDL
−log10(pvalue)
Variance explained (%)

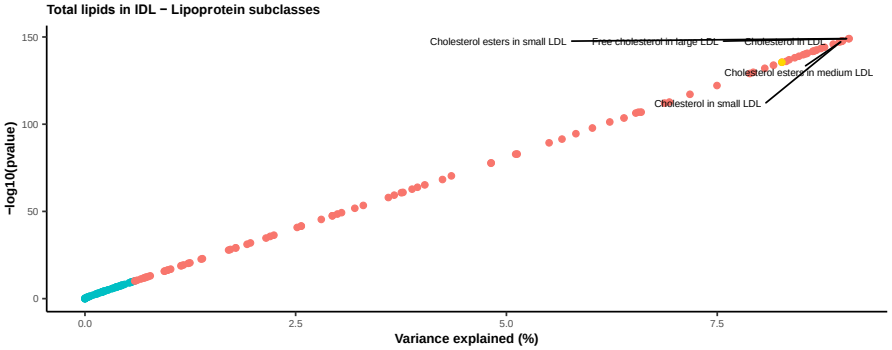

Omega−6 fatty acids ~ Fatty acids
Free cholesterol in medium LDL
Esterified cholesterol
Serum total cholesterol
Total lipids in small LDL
Free cholesterol
−log10(pvalue)
Variance explained (%)

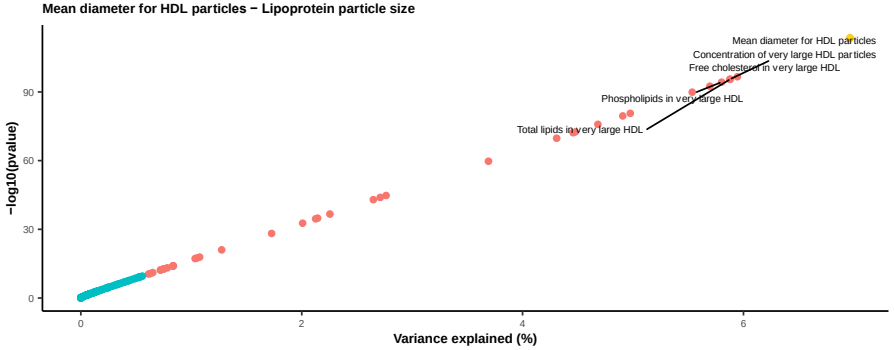

Glycine ~ Amino acids
Glycine
Glycerol
Acetate
Cholesterol esters in medium VLDL
Mean diameter for VLDL particles
−log10(pvalue)
Variance explained (%)

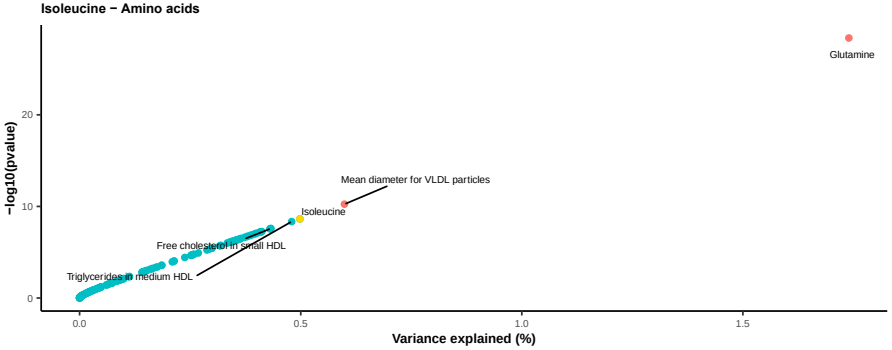

Glycoprotein acetyls − Inflammation
Concentration of small HDL particles
Free cholesterol in small HDL
Estimated degree of unsaturation
Mean diameter for VLDL particles
Total lipids in small HDL
−log10(pvalue)
Variance explained (%)

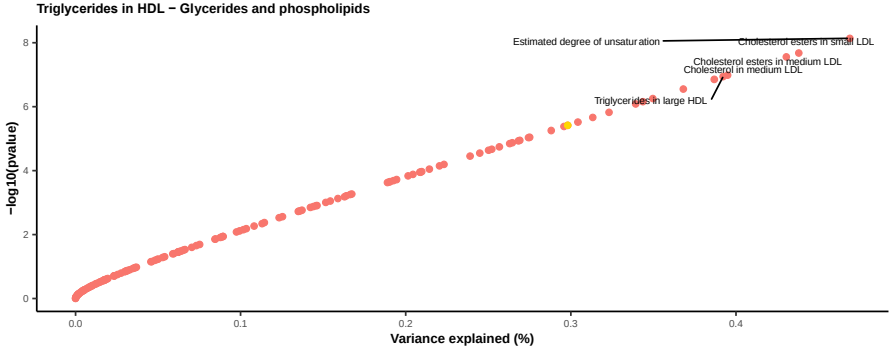

Histidine − Amino acids
Glycine
Glucose
Histidine
Acetate
Cholesterol in HDL
−log10(pvalue)
Variance explained (%)

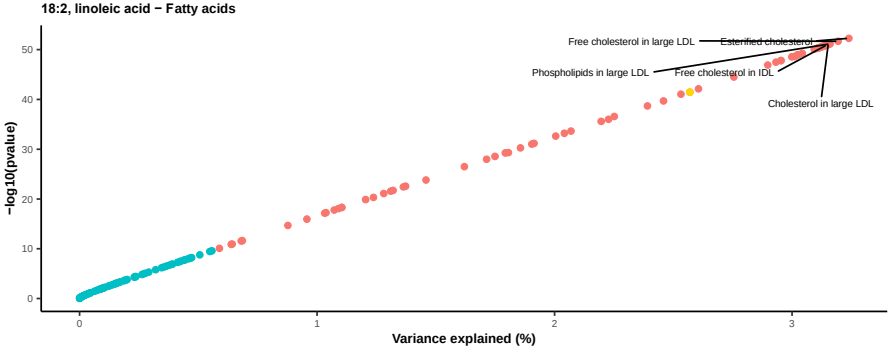

Cholesterol in HDL − Cholesterol
Phospholipids in very large HDL
Cholesterol esters in large HDL
Cholesterol in large HDL
Total lipids in large HDL
Free cholesterol in large HDL
−log10(pvalue)
Variance explained (%)

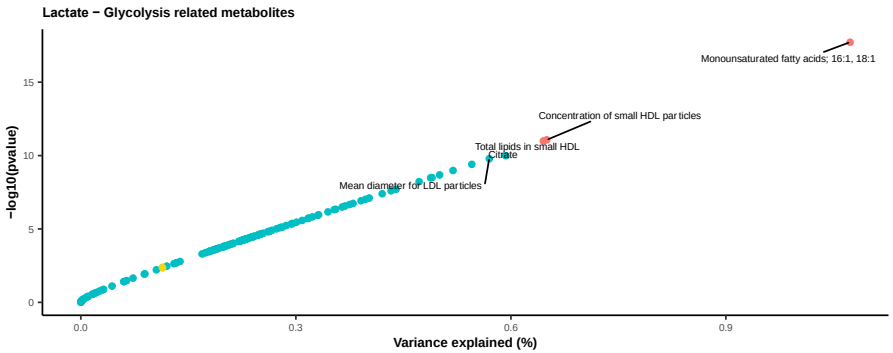

Total lipids in IDL − Lipoprotein subclasses
Cholesterol esters in small LDL
Free cholesterol in large LDL
Cholesterol in LDL
Cholesterol esters in medium LDL
Cholesterol in small LDL
−log10(pvalue)
Variance explained (%)

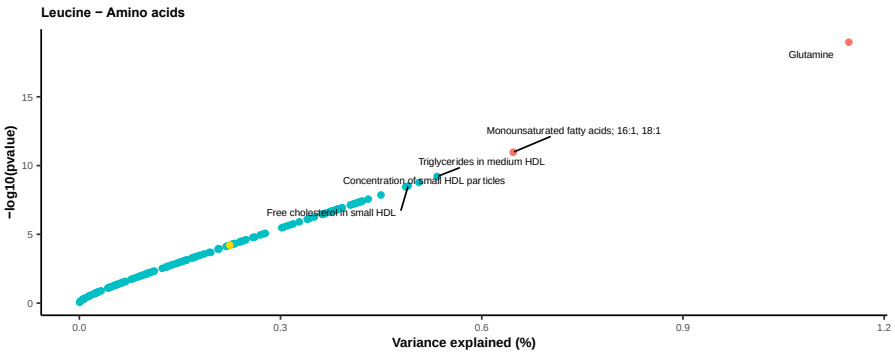

Mean diameter for HDL particles − Lipoprotein particle size
Mean diameter for HDL particles
Concentration of very large HDL particles
Free cholesterol in very large HDL
Phospholipids in very large HDL
Total lipids in very large HDL
−log10(pvalue)
Variance explained (%)

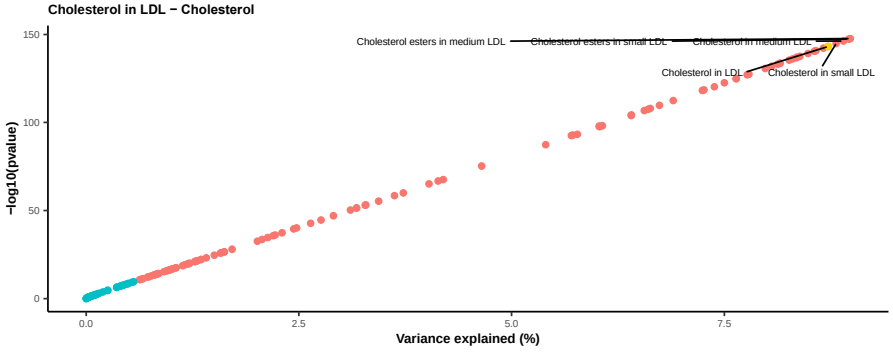

Isoleucine − Amino acids
Glutamine
Mean diameter for VLDL particles
Isoleucine
Free cholesterol in small HDL
Triglycerides in medium HDL
−log10(pvalue)
Variance explained (%)

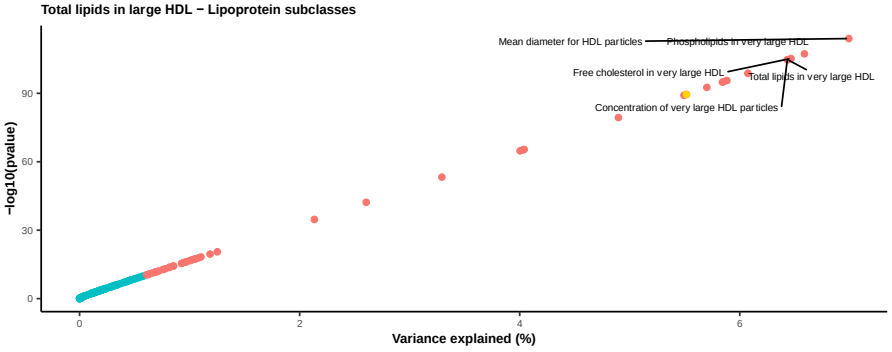

Triglycerides in HDL − Glycerides and phospholipids
Estimated degree of unsaturation
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Triglycerides in large HDL
−log10(pvalue)
Variance explained (%)

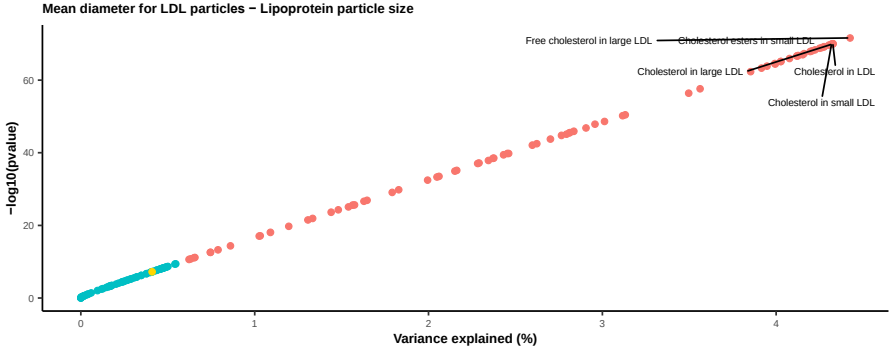

18:2, linoleic acid − Fatty acids
Free cholesterol in large LDL
Esterified cholesterol
Phospholipids in large LDL
Free cholesterol in IDL
Cholesterol in large LDL
−log10(pvalue)
Variance explained (%)

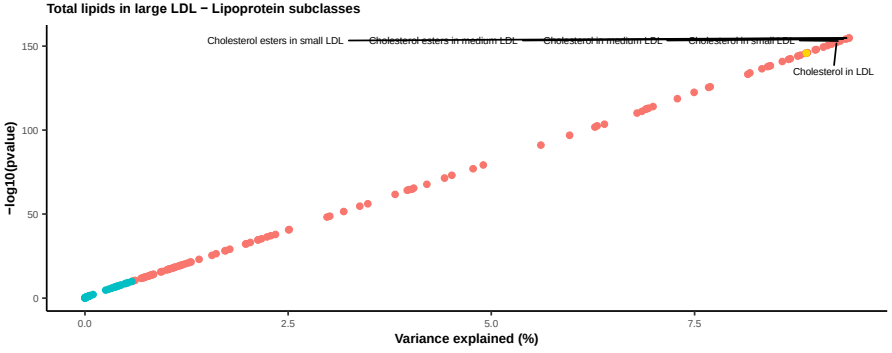

Lactate − Glycolysis related metabolites
Monounsaturated fatty acids; 16:1, 18:1
Concentration of small HDL particles
Total lipids in small HDL
Citrate
Mean diameter for LDL particles
−log10(pvalue)
Variance explained (%)

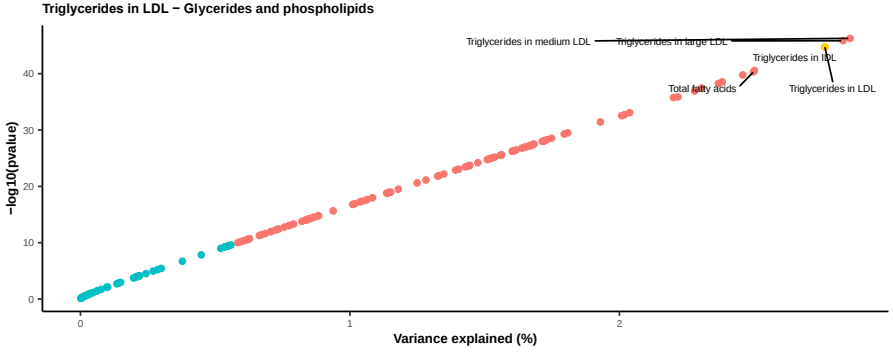

Leucine − Amino acids
Glutamine
Monounsaturated fatty acids; 16:1, 18:1
Triglycerides in medium HDL
Concentration of small HDL particles
Free cholesterol in small HDL
−log10(pvalue)
Variance explained (%)

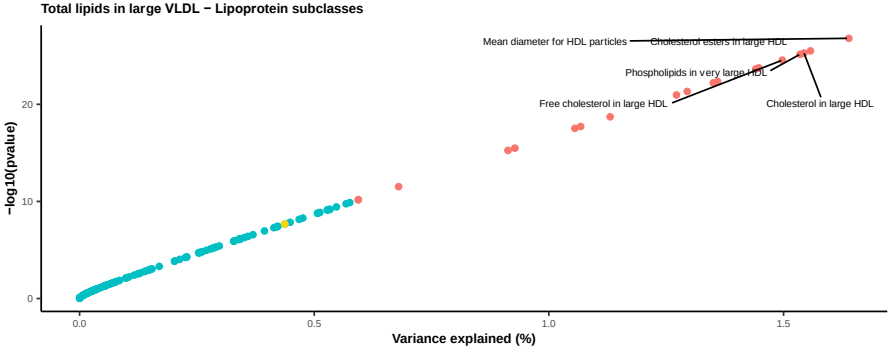

Cholesterol in LDL − Cholesterol
Cholesterol esters in medium LDL
Cholesterol esters in small LDL
Cholesterol in medium LDL
Cholesterol in LDL
Cholesterol in small LDL
−log10(pvalue)
Variance explained (%)

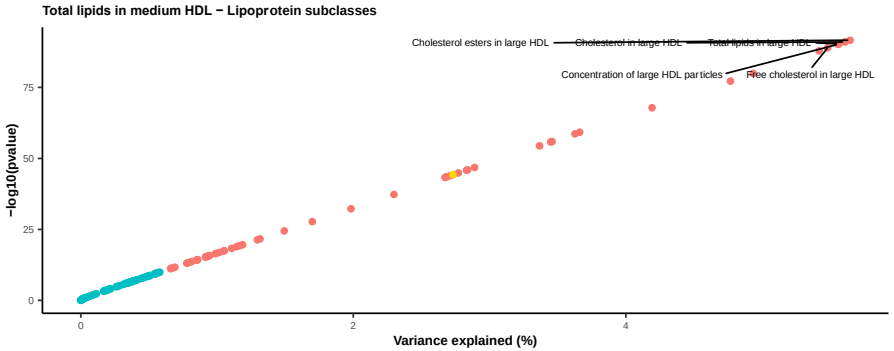

Total lipids in large HDL − Lipoprotein subclasses
Mean diameter for HDL particles
Phospholipids in very large HDL
Free cholesterol in very large HDL
Total lipids in very large HDL
Concentration of very large HDL particles
−log10(pvalue)
Variance explained (%)

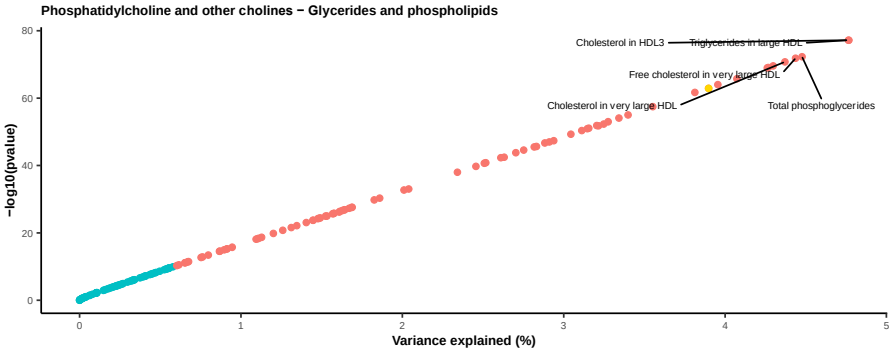

Mean diameter for LDL particles − Lipoprotein particle size
Free cholesterol in large LDL
Cholesterol esters in small LDL
Cholesterol in large LDL
Cholesterol in LDL
Cholesterol in small LDL
−log10(pvalue)
Variance explained (%)

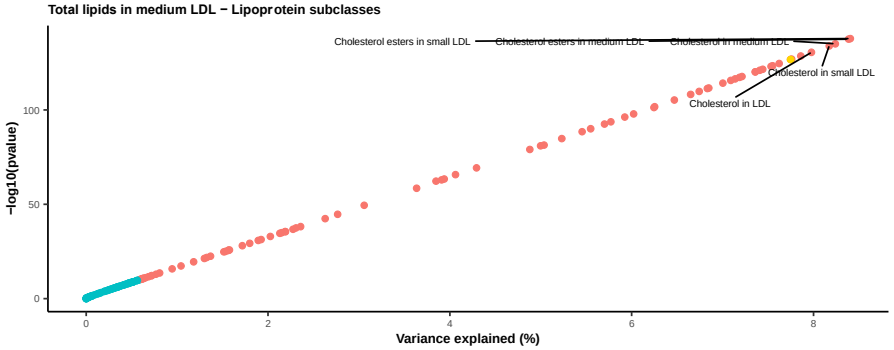

Total lipids in large LDL − Lipoprotein subclasses
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol in small LDL
Cholesterol in LDL
−log10(pvalue)
Variance explained (%)

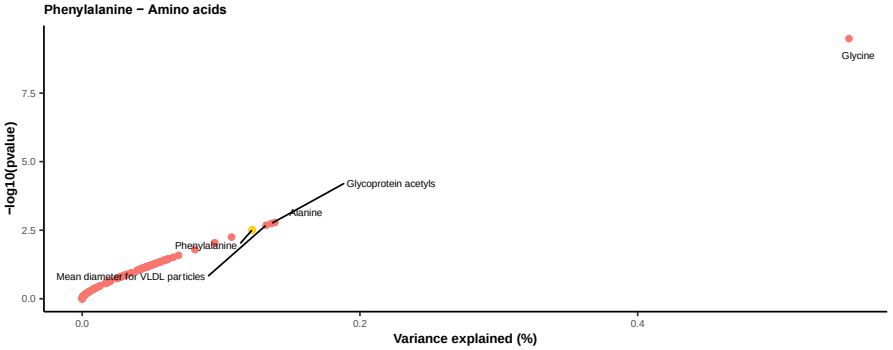

Triglycerides in LDL − Glycerides and phospholipids
Triglycerides in medium LDL
Triglycerides in large LDL
Triglycerides in IDL
Total fatty acids
Triglycerides in LDL
−log10(pvalue)
Variance explained (%)

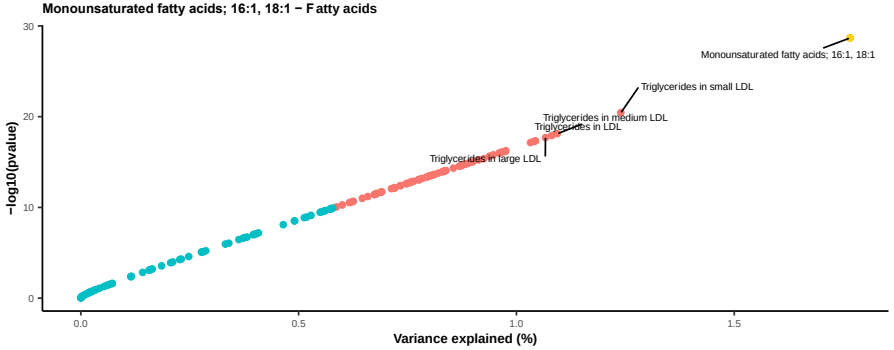

Total lipids in large VLDL − Lipoprotein subclasses
Mean diameter for HDL particles
Cholesterol esters in large HDL
Phospholipids in very large HDL
Free cholesterol in large HDL
Cholesterol in large HDL
−log10(pvalue)
Variance explained (%)

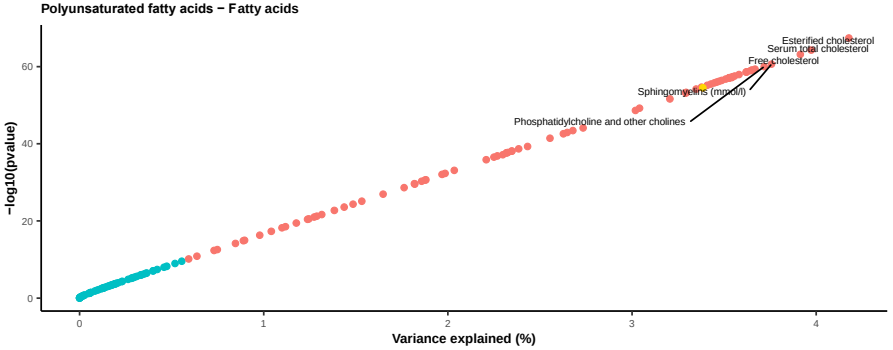

Total lipids in medium HDL − Lipoprotein subclasses
Cholesterol esters in large HDL
Cholesterol in large HDL
Total lipids in large HDL
Concentration of large HDL particles
Free cholesterol in large HDL
−log10(pvalue)
Variance explained (%)

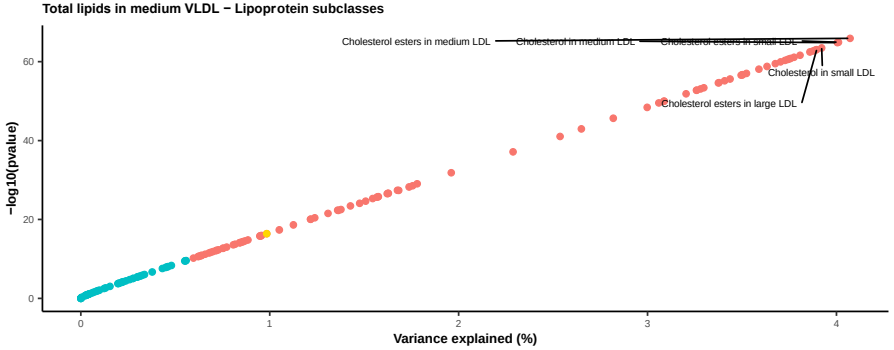

Phosphatidylcholine and other cholines − Glycerides and phospholipids
Cholesterol in HDL3
Triglycerides in large HDL
Free cholesterol in very large HDL
Cholesterol in very large HDL
Total phosphoglycerides
−log10(pvalue)
Variance explained (%)

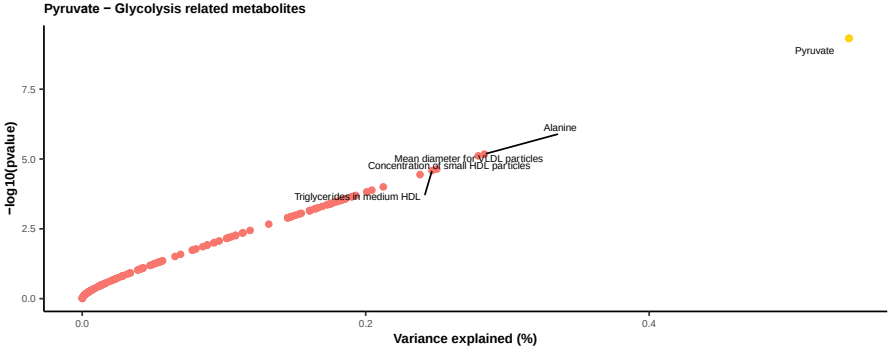

Total lipids in medium LDL − Lipoprotein subclasses
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol in small LDL
Cholesterol in LDL
−log10(pvalue)
Variance explained (%)

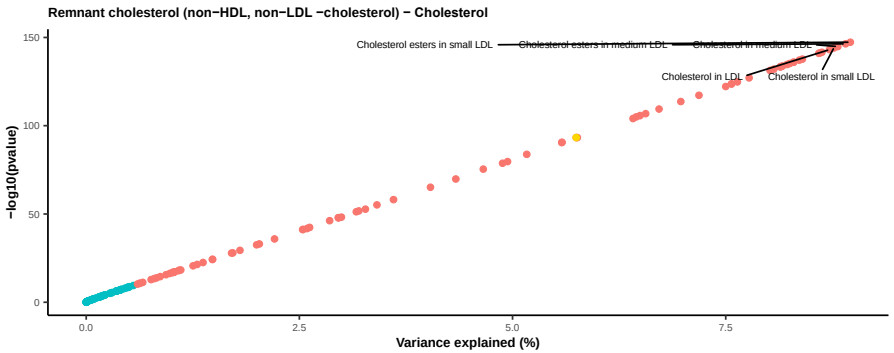

Phenylalanine − Amino acids
Glycine
Glycoprotein acetyls
Alanine
Phenylalanine
Mean diameter for VLDL particles
−log10(pvalue)
Variance explained (%)

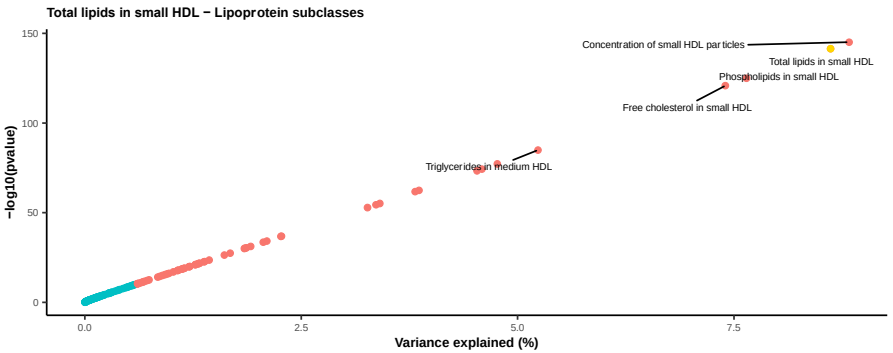

Monounsaturated fatty acids; 16:1, 18:1 − Fatty acids
Monounsaturated fatty acids; 16:1, 18:1
Triglycerides in small LDL
Triglycerides in medium LDL
Triglycerides in LDL
Triglycerides in large LDL
−log10(pvalue)
Variance explained (%)

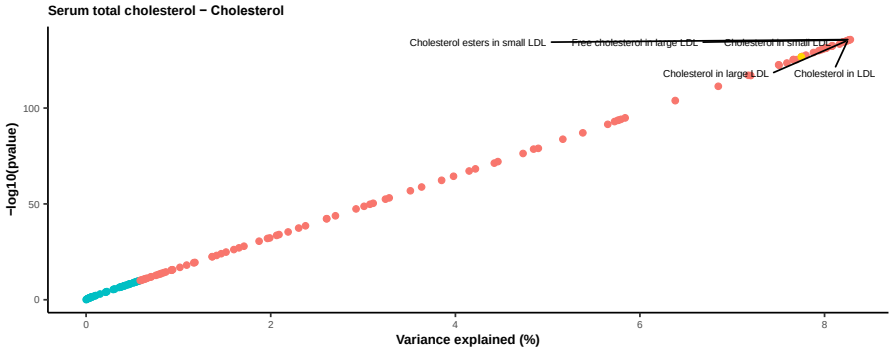

Polyunsaturated fatty acids − Fatty acids
Esterified cholesterol
Serum total cholesterol
Free cholesterol
Sphingomyelins (mmol/l)
Phosphatidylcholine and other cholines
−log10(pvalue)
Variance explained (%)

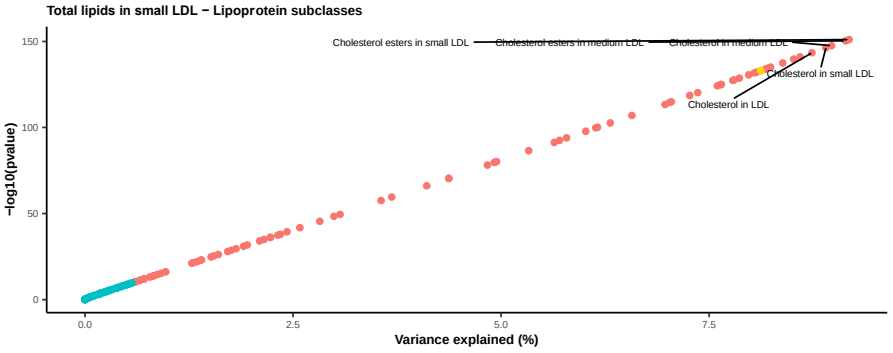

Total lipids in medium VLDL − Lipoprotein subclasses
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol esters in small LDL
Cholesterol in small LDL
Cholesterol esters in large LDL
−log10(pvalue)
Variance explained (%)

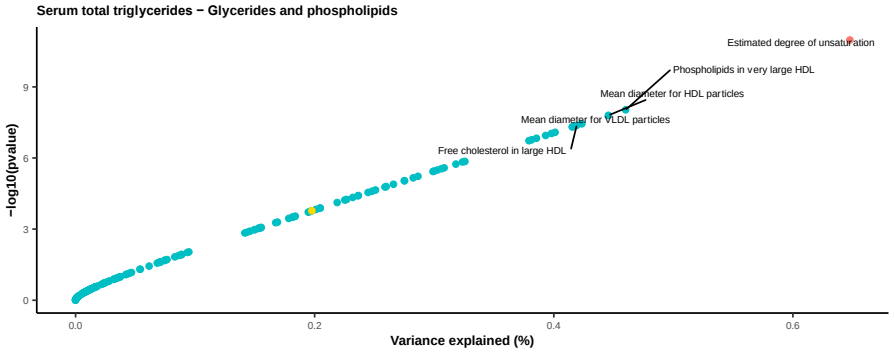

Pyruvate − Glycolysis related metabolites
Pyruvate
Alanine
Mean diameter for VLDL particles
Concentration of small HDL particles
Triglycerides in medium HDL
−log10(pvalue)
Variance explained (%)

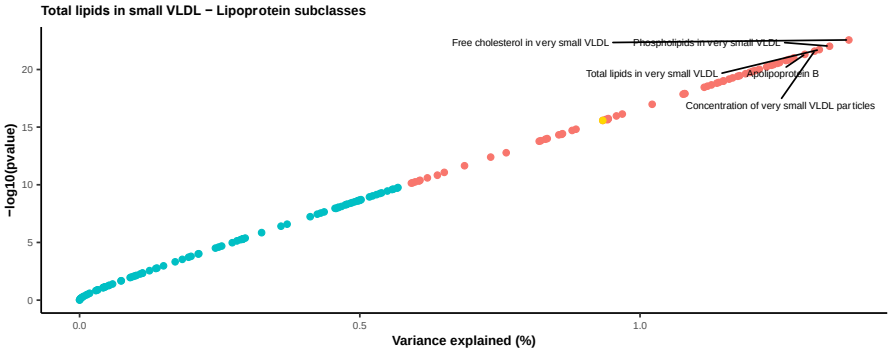

Remnant cholesterol (non−HDL, non−LDL −cholesterol) − Cholesterol
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol in LDL
Cholesterol in small LDL
−log10(pvalue)
Variance explained (%)

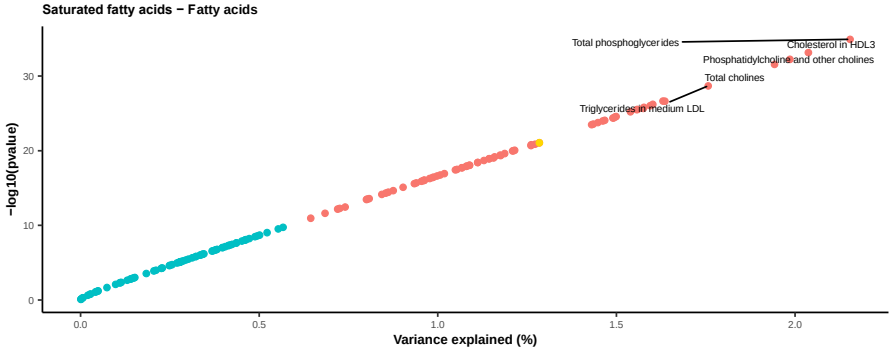

Total lipids in small HDL − Lipoprotein subclasses
Concentration of small HDL particles
Total lipids in small HDL
Phospholipids in small HDL
Free cholesterol in small HDL
Triglycerides in medium HDL
−log10(pvalue)
Variance explained (%)

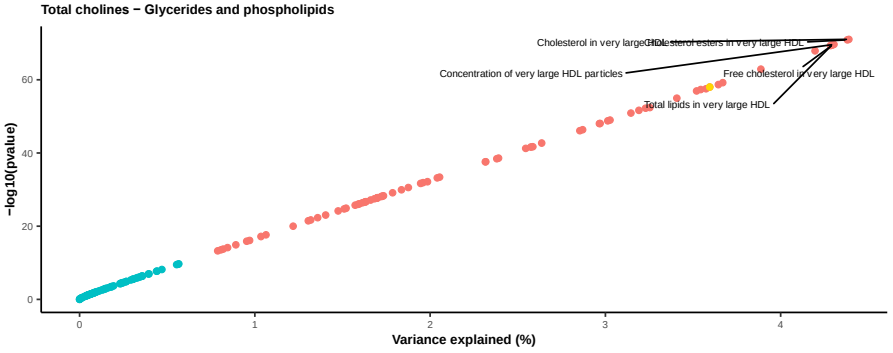

Serum total cholesterol − Cholesterol
Cholesterol esters in small LDL
Free cholesterol in large LDL
Cholesterol in small LDL
Cholesterol in large LDL
Cholesterol in LDL
−log10(pvalue)
Variance explained (%)

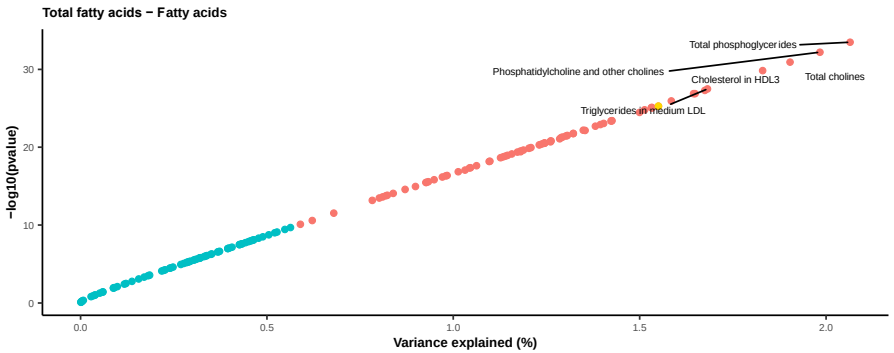

Total lipids in small LDL − Lipoprotein subclasses
Cholesterol esters in small LDL
Cholesterol esters in medium LDL
Cholesterol in medium LDL
Cholesterol in small LDL
Cholesterol in LDL
−log10(pvalue)
Variance explained (%)

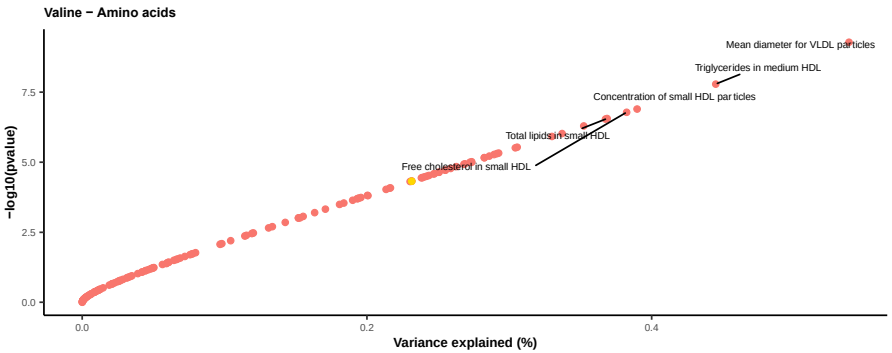

Serum total triglycerides − Glycerides and phospholipids
Estimated degree of unsaturation
Phospholipids in very large HDL
Mean diameter for HDL particles
Mean diameter for VLDL particles
Free cholesterol in large HDL
−log10(pvalue)
Variance explained (%)

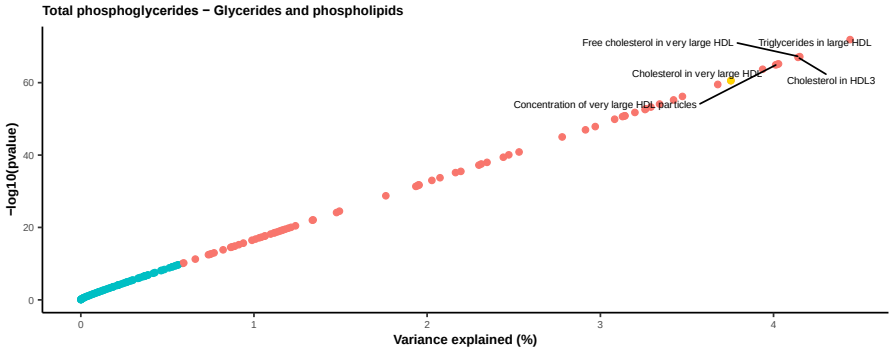

Total lipids in small VLDL − Lipoprotein subclasses
Free cholesterol in very small VLDL
Phospholipids in very small VLDL
Total lipids in very small VLDL
Apolipoprotein B
Concentration of very small VLDL particles
−log10(pvalue)
Variance explained (%)

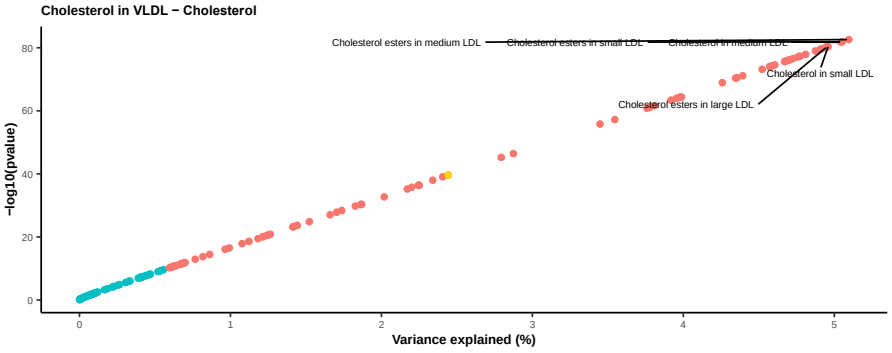

Saturated fatty acids − Fatty acids
Total phosphoglycerides
Cholesterol in HDL3
Phosphatidylcholine and other cholines
Total cholines
Triglycerides in medium LDL
−log10(pvalue)
Variance explained (%)

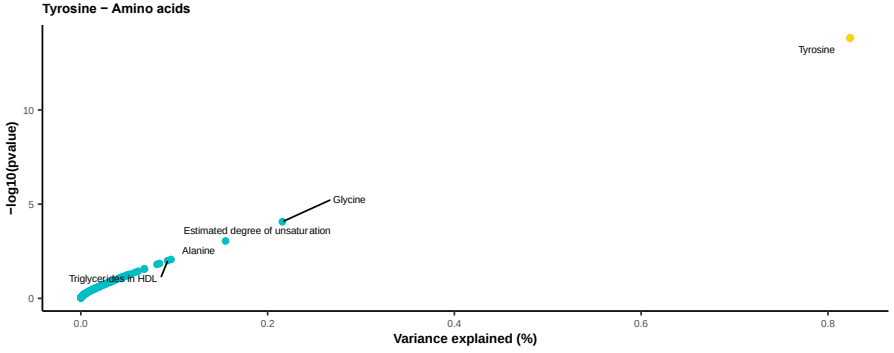

Total cholines − Glycerides and phospholipids
Cholesterol in very large HDL
Cholesterol esters in very large HDL
Concentration of very large HDL particles
Free cholesterol in very large HDL
Total lipids in very large HDL
−log10(pvalue)
Variance explained (%)

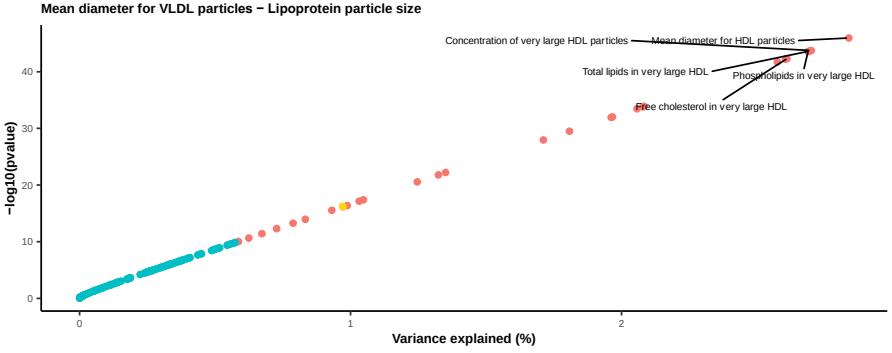

Total fatty acids ~ Fatty acids
Total phosphoglycerides
Phosphatidylcholine and other cholines
Total cholines
Cholesterol in HDL3
Triglycerides in medium LDL
−log10(pvalue)
Variance explained (%)

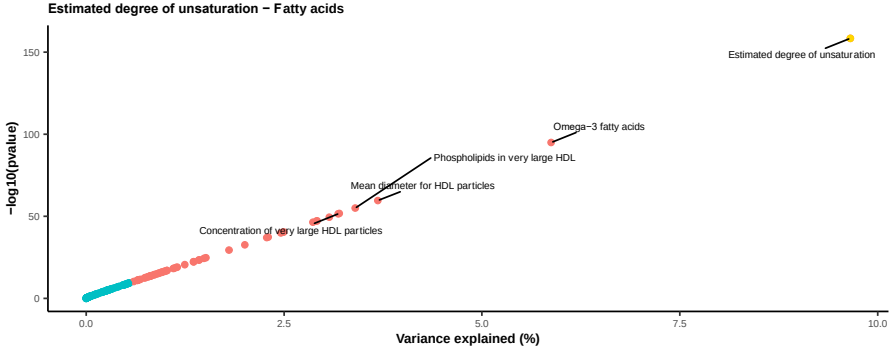

Valine ~ Amino acids
Mean diameter for VLDL particles
Triglycerides in medium HDL
Concentration of small HDL particles
Total lipids in small HDL
Free cholesterol in small HDL
−log10(pvalue)
Variance explained (%)

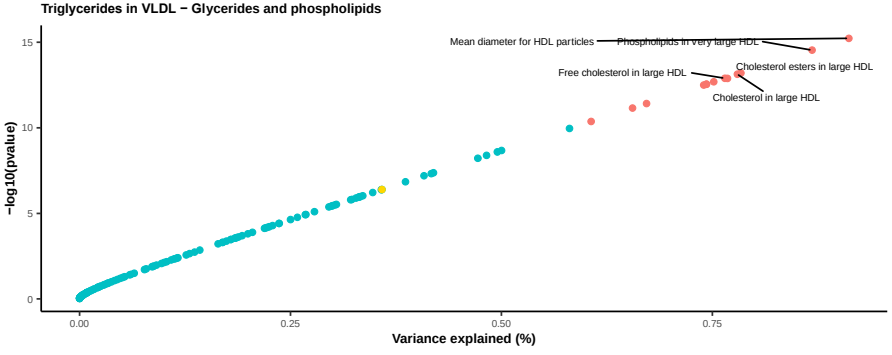

Total phosphoglycerides ~ Glycerides and phospholipids
Free cholesterol in very large HDL
Triglycerides in large HDL
Cholesterol in very large HDL
Cholesterol in HDL3
Concentration of very large HDL particles
−log10(pvalue)
Variance explained (%)

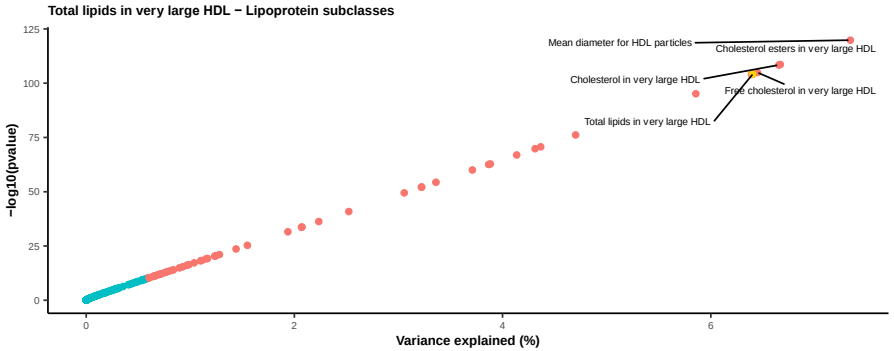

Cholesterol in VLDL ~ Cholesterol
Cholesterol esters in medium LDL
Cholesterol esters in small LDL
Cholesterol in medium LDL
Cholesterol in small LDL
Cholesterol esters in large LDL
−log10(pvalue)
Variance explained (%)

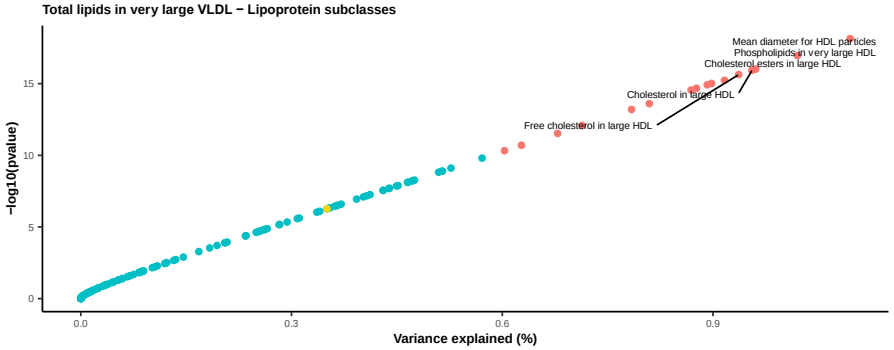

Tyrosine ~ Amino acids
Tyrosine
Glycine
Estimated degree of unsaturation
Alanine
Triglycerides in HDL
−log10(pvalue)
Variance explained (%)

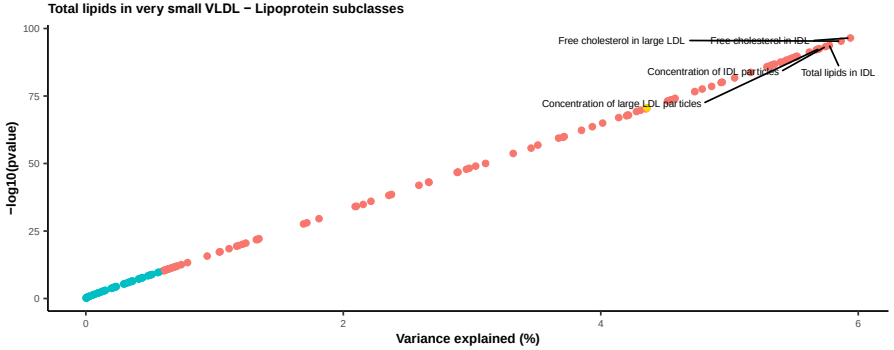

Mean diameter for VLDL particles ~ Lipoprotein particle size
Concentration of very large HDL particles
Mean diameter for HDL particles
Total lipids in very large HDL
Phospholipids in very large HDL
Free cholesterol in very large HDL
−log10(pvalue)
Variance explained (%)


Estimated degree of unsaturation ~ Fatty acids
Estimated degree of unsaturation
Omega−3 fatty acids
Phospholipids in very large HDL
Mean diameter for HDL particles
Concentration of very large HDL particles
−log10(pvalue)
Variance explained (%)


Triglycerides in VLDL ~ Glycerides and phospholipids
Mean diameter for HDL particles
Phospholipids in very large HDL
Free cholesterol in large HDL
Cholesterol esters in large HDL
Cholesterol in large HDL
−log10(pvalue)
Variance explained (%)


Total lipids in very large HDL − Lipoprotein subclasses
Mean diameter for HDL particles
Cholesterol esters in very large HDL
Cholesterol in very large HDL
Free cholesterol in very large HDL
Total lipids in very large HDL
−log10(pvalue)
Variance explained (%)


Total lipids in very large VLDL − Lipoprotein subclasses
Mean diameter for HDL particles
Phospholipids in very large HDL
Cholesterol esters in large HDL
Cholesterol in large HDL
Free cholesterol in large HDL
−log10(pvalue)
Variance explained (%)


Total lipids in very small VLDL − Lipoprotein subclasses
Free cholesterol in large LDL
Free cholesterol in IDL
Concentration of IDL particles
Total lipids in IDL
Concentration of large LDL particles
−log10(pvalue)
Variance explained (%)

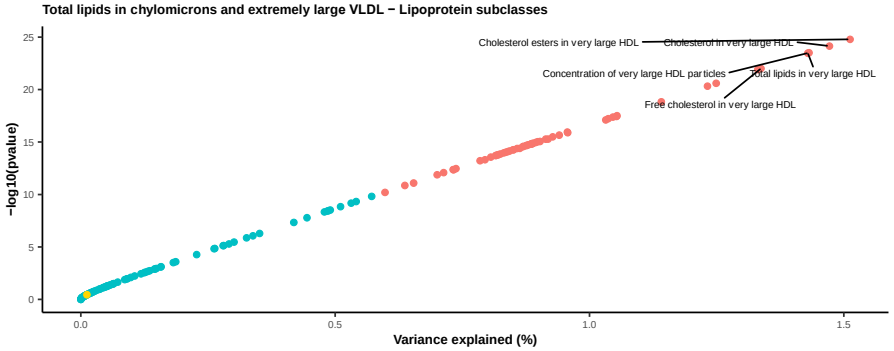
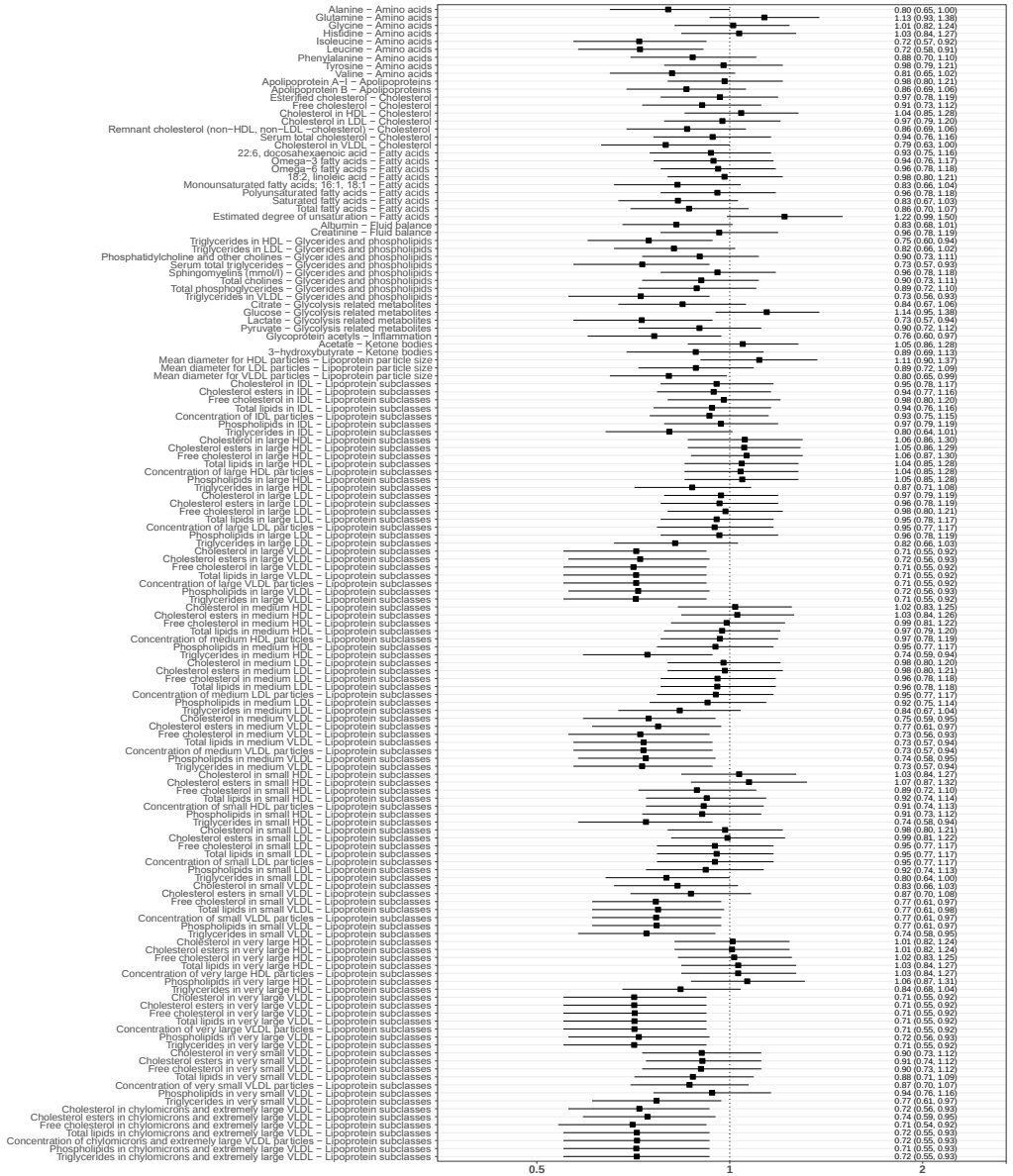

Total lipids in chylomicrons and extremely large VLDL − Lipoprotein subclasses
Cholesterol esters in very large HDL
Cholesterol in very large HDL
Concentration of very large HDL particles
Total lipids in very large HDL
Free cholesterol in very large HDL
−log10(pvalue)
Variance explained (%)

Figure S6.7. Scatter plots of the variance explained by the weighted genetic risk score for 60 metabolic traits included in MR analyses. There were 145 traits included in total, but 60 are shown here to match the truncated figures of key traits presented in the main text for clarity. Results are from linear regression analyses of GRS against all metabolic traits (exposure: GRS, outcome: metabolic trait) in BiB. The x-axes are R^2 expressed as a percentage and the y-axes are the -log10 P-value. The 5 metabolic traits with the lowest p-values in each scatter plot are labelled. Those with a -log10 P-value >10 are filled in red, with the remainder filled grey. The metabolic trait I am trying to instrument with the GRS is filled with gold. A highly specific GRS for a given metabolite would produce a scatter plot with the gold-filled point in the top right corner (high variance explained and strongly associated) with the remainder of the metabolites lower down towards the left corner.

Exploring directional consistency between phenotype (conventional multivariable regression) and genotype (Mendelian randomization) associations with metabolic traits

A: Main multivariable regression results



B: Mendelian randomization results

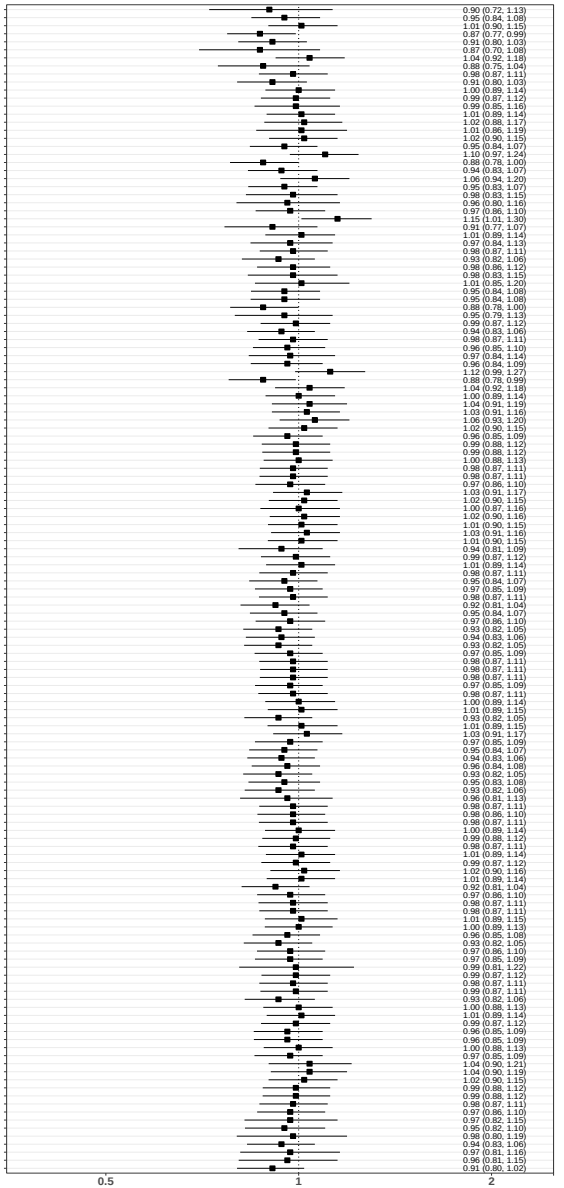
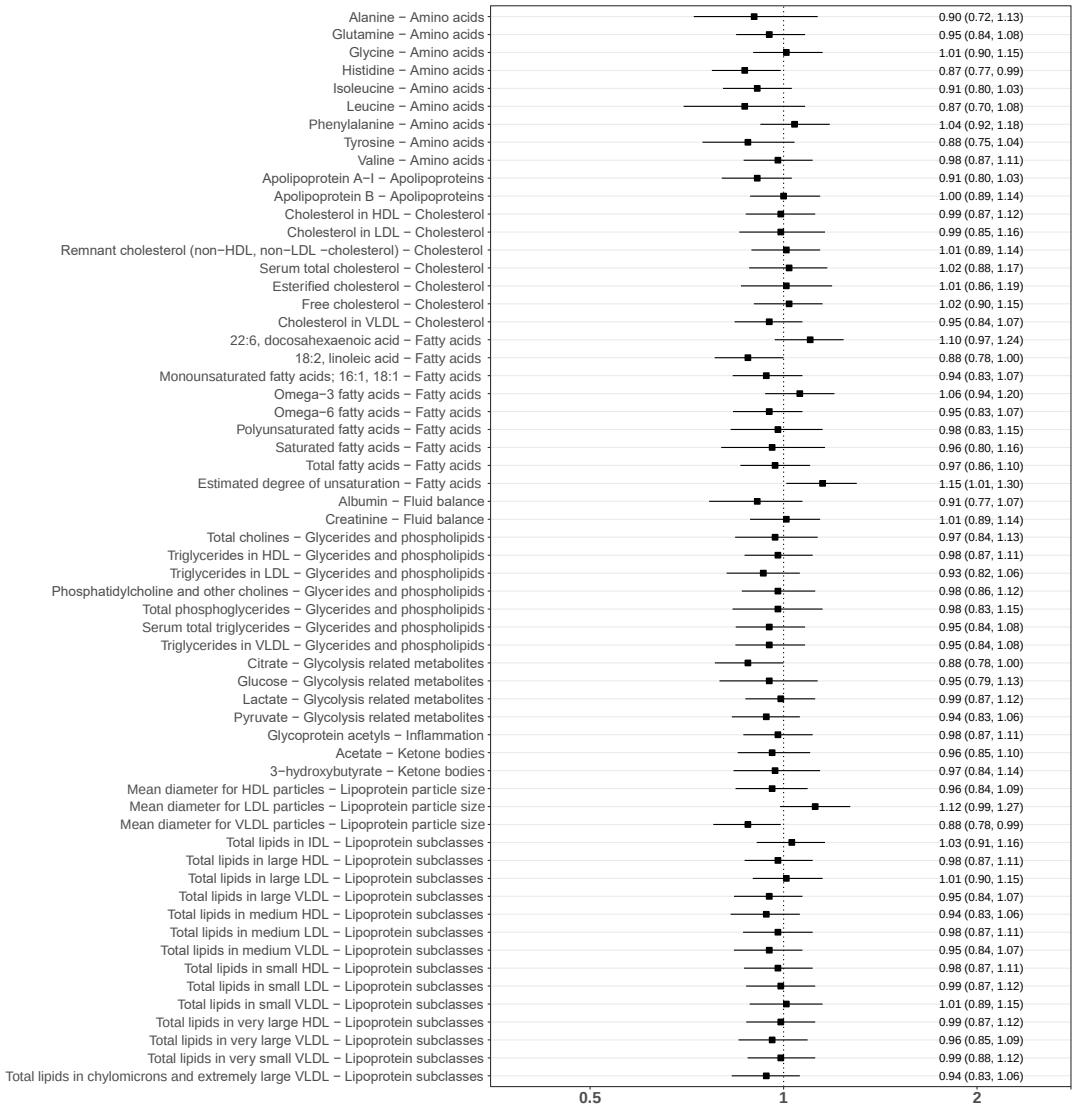


Figure S6.8. Showing results comparing the main confounder adjusted associations of all maternal metabolic traits with offspring CHDs (Panel A: N = 8,551 & N CHD cases = 96) to the Mendelian randomisation analyses of maternal genetic risk scores and offspring CHDs (Panel B: N = 38,662 & N CHD cases = 319). N.B. results from each analysis are presented on different scales; I am not attempting to quantify estimates in the MR analyses, the aim is to compare the direction of effect. The confounder adjusted associations are as above in Figure SX. The MR analyses are adjusted for the top 10 genetic principal components and genetic batches in MoBa. The results were pooled using random effects meta-analyses. Abbreviations: BiB, Born in Bradford; CHD, congenital heart disease; GRS, genetic risk score; MR, Mendelian randomisation; OR, odds ratio; CI, confidence interval.

Exploring directional consistency between main MR results and MR results excluding the BiB cohort

A: Main MR results



B: MR results excluding BiB

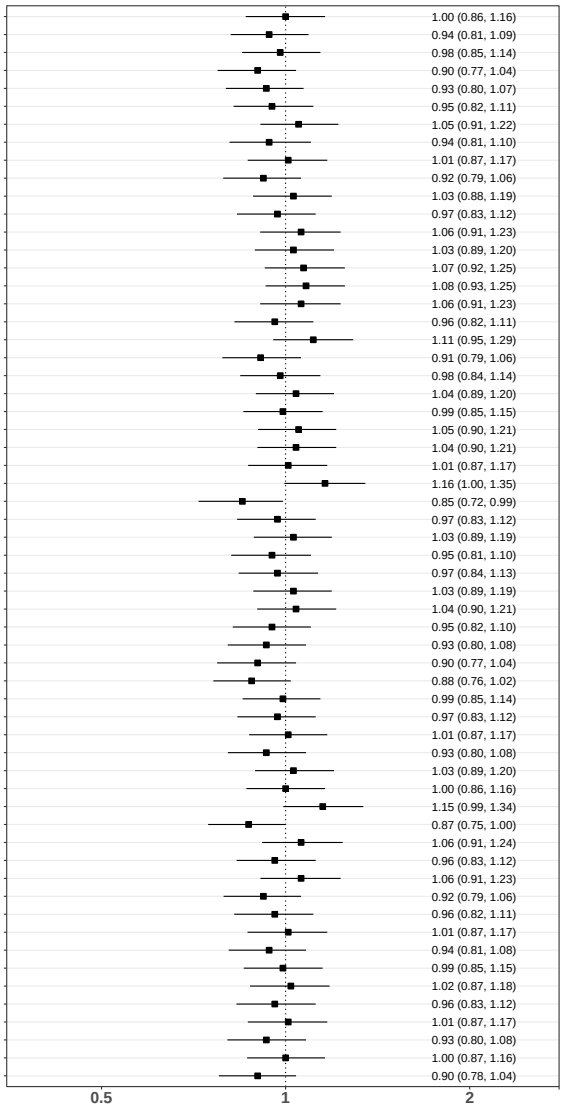
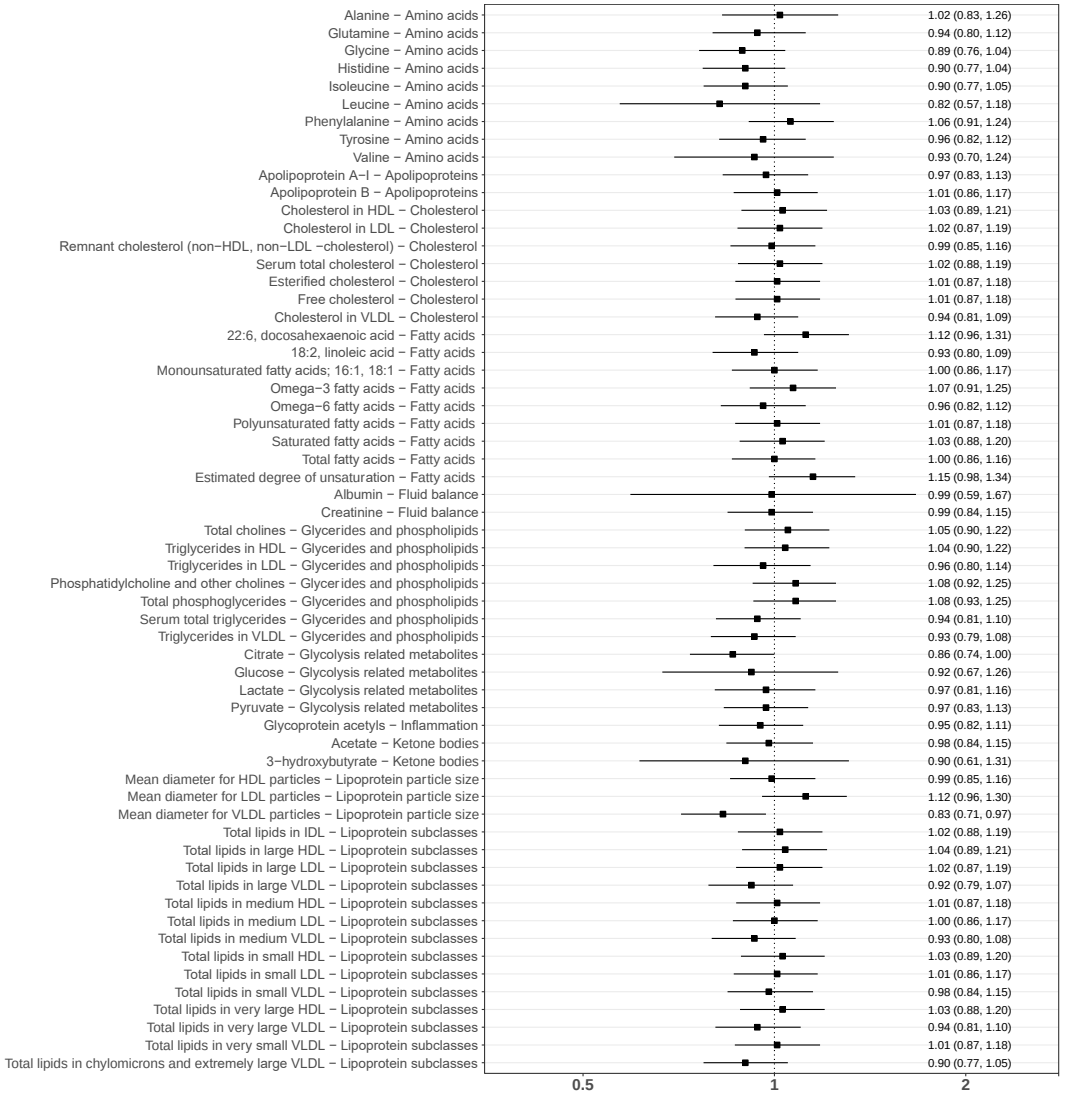


Figure S6.9. Exploring consistency of MR analyses when excluding BiB. Results on the left were pooled using random effects meta analyses and results on the right (for ALSPAC and MoBa) were pooled using fixed effect meta analyses.

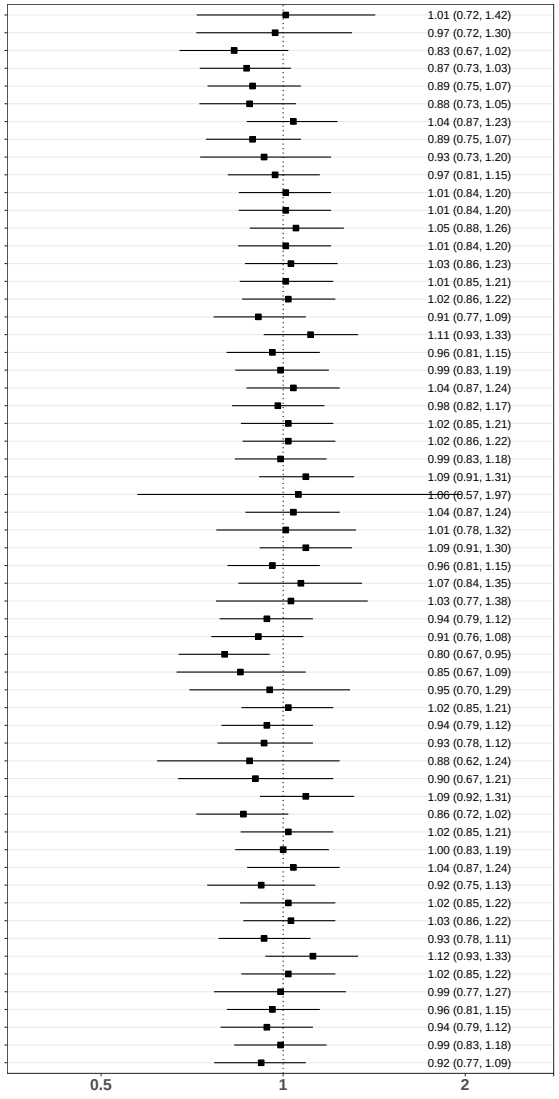
Comparing MR results in those with offspring genotype data and MR results adjusted for offspring genotype

A: MR results without offspring genotype adjusment



OR (95% CI) of CHD per SD change in GRS

B: MR results adjusting for offspring genotype



OR (95% CI) of CHD per SD change in GRS

Figure S6.10. Pooled MR analyses in all participants with mother and offspring genotype data. Left results were adjusted for the top 10 genetic principal components and genetic batches in MoBa. Results on the right were additionally adjusted for offspring genotype. The results were pooled using random effects meta-analyses.