# Epidemiology of Prenatal Alcohol Use and Fetal Alcohol Spectrum Disorder

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Thesis submitted for the degree of Doctor of Philosophy

School of Medicine, Cardiff University

2018

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# Acknowledgements

I would like to thank my fantastic supervisors Professor Shantini Paranjothy, Dr Lisa Hurt and Professor Alison Kemp for their expert advice, time and thoughtful feedback.

I am also extremely grateful to Dr Raja Mukherjee for welcoming me into his clinic in the early stages of my PhD and for sharing his valuable insight and enthusiasm for FASD research with me. Thank you also to Dr Rhian Daniel for sharing her expertise on directed acyclic graphs and for her input on the causal diagram chapter; Dr Andrea Higgins for contributing to the development and validation of the FASD case ascertainment algorithm; Dr Caryl Wilson-Nagrani for advising on her lip-philtrum guide; Dr Daniel Farewell and Dr Giles Greene for their help with the statistical aspects of this thesis; Dr Helena Kemp for her feedback on the biomarker review; Mala Mann for her help with the systematic review search strategy and Julia Hodgson for administrative support.

I am grateful to all the families from the ALSPAC cohort, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. Thank you to Cardiff University School of Medicine for funding this work and to fellow students and friends in the Division of Population Medicine, with special mention to Lauren, Laura and Harriett.

This thesis is a product of the enduring support and encouragement of family and friends. Special thanks to Chris, Toni, Mum, Dad, Auntie Mar, Clive, Nan, Granch and all of the 'Palmers'.

# Summary

Prenatal alcohol exposure (PAE) can lead to fetal alcohol spectrum disorder (FASD). FASD refers to a range of lifelong conditions caused by PAE, characterised by a distinctive facial phenotype, growth deficiencies and/or neurobehavioural impairments. This thesis presents four studies that I conducted to address knowledge gaps relevant to the epidemiology of PAE and FASD.

First, objective measures of PAE are essential for identifying children at risk of adverse outcomes. Biomarkers have been advocated for use in universal PAE screening programs but their validity had not been comprehensively evaluated. I conducted a systematic review and found that biomarker test performance varied widely across studies. The quality of published studies was low, resulting in insufficient evidence to support the use of objective measures of PAE in practice.

Second, the prevalence of FASD in the UK was unknown. Active case ascertainment studies have not been possible due to funding and ethical issues. To overcome these issues, I developed an algorithm to estimate FASD prevalence using existing data from a population-based birth cohort in England (ALSPAC). Up to 17% of children met criteria for FASD, indicating that it is a significant public health concern.

Third, although PAE is the sole necessary cause of FASD, it is not always sufficient. Understanding risk factors for FASD is important for informing prevention strategies. However, existing studies have mostly been limited to discussion of association, rather than causation. I produced a causal diagram to depict hypothesised causal pathways to FASD. I used this diagram to guide analyses in a FASD risk factor study, reported below.

Finally, I investigated FASD risk factors using multivariable logistic regression within the ALSPAC cohort. Prenatal stress, smoking and mental health problems increased the odds of FASD. Social support and folic acid supplementation were protective. These results indicate novel potential targets for FASD intervention.

# Abbreviations

| | |
|---|---|
| **ADH** | Alcohol dehydrogenase |
| **ADHD** | Attention deficit hyperactivity disorder |
| **AH** | Dr Andrea Higgins (Educational psychologist) |
| **ALDH** | Aldehyde dehydrogenase |
| **ALSPAC** | Avon Longitudinal Study of Parents and Children |
| **ALT** | Alanine aminotransferase |
| **AK** | Professor Alison Kemp (PhD co-supervisor and community paediatrician) |
| **aOR** | Adjusted odds ratio |
| **APAs** | Acetaldehyde-protein adducts |
| **APPG** | All Party Parliamentary Group |
| **ARBD** | Alcohol-related birth defects |
| **ARND** | Alcohol-related neurodevelopmental disorder |
| **AUDIT** | Alcohol Use Disorder Identification Test |
| **BAC** | Blood alcohol concentration |
| **BMI** | Body mass index |
| **BSID** | Bayley Scales of Infant Development |
| **CAGE** | Alcohol use questionnaire with four items: cut down, annoyed, guilt, eye-opener |
| **CARIS** | Congenital Anomaly Register and Information Service |
| **CDC** | Centers for Disease Control and Prevention |
| **CDT** | Carbohydrate-deficient transferrin |
| **CI** | Confidence interval |
| **CMO** | Chief Medical Officer |
| **CNS** | Central Nervous System |
| **DAG** | Directed acyclic graph |
| **DANVA** | Diagnostic Analysis of Nonverbal Accuracy scale |
| **DSM-5** | Diagnostic and Statistical Manual of Mental Disorders (5th Edition) |
| **EtG** | Ethyl glucuronide |
| **EtS** | Ethyl sulphate |
| **FAEE** | Fatty acid ethyl ester |
| **FAS** | Fetal alcohol syndrome |
| **FASD** | Fetal alcohol spectrum disorder |
| **FN** | False negative |
| **FP** | False positive |
| **GGT** | Gamma-glutamyltransferase |
| **GIN** | Guidelines International Network |
| **Hb-Ach** | Haemoglobin acetaldehyde adducts |
| **HPA** | Hypothalamic-pituitary-adrenal |
| **HSCIC** | Health and Social Care Information Centre |
| **HSROC** | Hierarchical summary receiver operating characteristic |
| **ICD-10** | International Statistical Classification of Diseases and Related Health Problems (10th Edition) |
| **IOM** | Institute of Medicine |
| **IQ** | Intelligence quotient |
| **JH** | Ms Julia Hodgson (Professional Support Services) |
| **LH** | Dr Lisa Hurt (PhD co-supervisor) |
| **LR+** | Positive likelihood ratio |
| **LR-** | Negative likelihood ratio |

| | |
|---|---|
| **MAR** | Missing at random |
| **MCAR** | Missing completely at random |
| **MCV** | Mean corpuscular volume |
| **MICE** | Multiple imputation by chained equations |
| **MNAR** | Missing not at random |
| **NET** | Alcohol use questionnaire with three items: normal drinker, eye-opener, tolerance |
| **ND-PAE** | Neurodevelopmental Disorder-Prenatal Alcohol Exposure |
| **NHS** | National Health Service |
| **NICE** | National Institute for Health and Care Excellence |
| **NPV** | Negative predictive value |
| **ODD/CD** | Oppositional/conduct disorder |
| **OFC** | Occipital frontal circumference |
| **OR** | Odds ratio |
| **PAE** | Prenatal alcohol exposure |
| **PAF** | Population attributable fraction |
| **PEDW** | Patient Episode Database Wales |
| **PEth** | Phosphatidylethanol |
| **pFAS** | Partial fetal alcohol syndrome |
| **PPV** | Positive predictive value |
| **PRISMA** | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| **PROSPERO** | Prospective register of systematic reviews |
| **QUADAS-2** | Quality Assessment of Diagnostic Accuracy Studies |
| **RevMan** | Review Manager |
| **RM** | Dr Raja Mukherjee (Consultant psychiatrist and lead clinician from the UK National Clinic for Fetal |
| | Alcohol Spectrum Disorders) |
| **ROC** | Receiver operating characteristic |
| **SCDC** | Social Communication Disorders Checklist |
| **SD** | Standard deviation |
| **SDQ** | Strengths and Difficulties Questionnaire |
| **SEN** | Special educational needs |
| **SES** | Socioeconomic status |
| **SMAST** | Short Michigan Alcohol Screening Test |
| **SNPs** | Single nucleotide polymorphisms |
| **SP** | Professor Shantini Paranjothy (Primary PhD supervisor) |
| **STARD** | Standards for the Reporting of Diagnostic Accuracy Studies |
| **T-ACE** | Alcohol use questionnaire with four items: tolerance, annoyed, cut-down, eye-opener |
| **TLFB** | Timeline follow-back procedure |
| **TN** | True negative |
| **TP** | True positive |
| **TWEAK** | Alcohol use questionnaire with five items: tolerance, worried, eye-opener, amnesia and cut-down |
| **WISC-III** | Wechsler Intelligence Scale for Children (3rd revision) |
| **WOLD** | Weschler Objective Language Dimensions |

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# Chapter 1. Introduction

## 1 Overview

This thesis aims to describe the epidemiology of prenatal alcohol exposure (PAE) and fetal alcohol spectrum disorder (FASD). In this introductory chapter, I describe the existing evidence on PAE and FASD, and the knowledge gaps that I will address in this thesis. The main aims and research questions of this thesis are presented at the end of this chapter.

## 2 Background

### 2.1 Alcohol use in pregnancy: existing evidence and knowledge gaps

#### 2.1.1 Introduction to alcohol in pregnancy

Alcohol is a teratogen. It is associated with a range of adverse perinatal and long-term outcomes including spontaneous abortion, preterm delivery, cognitive and behavioural impairment.[1-6] During pregnancy, alcohol consumed by the mother passes freely through the placenta and within one hour the level of alcohol within the fetal bloodstream approximates that of the mother.[7] The fetus cannot effectively process alcohol and the intrauterine environment creates a recycling loop for ethanol, whereby the ethanol excreted by the fetus re-enters the fetal bloodstream as it swallows the amniotic fluid. The fetus therefore experiences prolonged exposure to ethanol and the primary route for elimination of this substance is through the metabolic capacity of the mother.[8,9] Evidence from animal studies suggests that alcohol disrupts fetal development through a variety of

mechanisms including cell damage and death, disrupted growth factor signalling, altered gene expression and hypoxia.[10]

### 2.1.2 Prevalence of alcohol use in pregnancy

The UK has the fourth highest prevalence of PAE in the world, according to a systematic review and meta-analysis, published in 2017. This review produced a pooled prevalence estimate of 41% for any PAE, although there was significant heterogeneity between studies.[11] Recent prospective studies produce higher estimates, suggesting that 75% to 79% of women in the UK drink while pregnant.[12,13] The prevalence and quantity of prenatal alcohol use is highest in the first trimester.[12,13] While most women significantly reduce their intake or abstain after the first trimester, 34% to 63% continue to drink alcohol in the second trimester and 49% in the third trimester.[12,13] Up to 33% of women report binge drinking at some point during pregnancy, most commonly in the first trimester[5,12] and the prevalence of chronic/heavy PAE (daily drinking or consuming more than 8 units per week) ranges from 1% to 3% in the second and third trimesters.[12-18]

### 2.1.3 Outcomes associated with alcohol use in pregnancy

Heavy episodic and chronic prenatal alcohol consumption are the most likely to lead to adverse outcomes, including FASD (described below).[19] Reviews of the effects of low to moderate PAE on developmental outcomes are inconclusive[20-22] and debate is ongoing as to whether it is possible to identify a safe limit for drinking in pregnancy.[23] A meta-analysis, published in 2017,[24] explored the impact of drinking up to 32g of alcohol per week during pregnancy (equivalent to the upper limit of previous UK antenatal guidelines[25]), compared to abstinence, on a

range of pregnancy and childhood outcomes. The authors found an 8% increase in the odds of a child being small for gestational age and some evidence for an increased odds of preterm birth following light PAE. However, evidence was sparse for most outcomes and confidence intervals for the preterm birth estimate were inconclusive.[24] Studies of the effects of low to moderate PAE on neurocognitive and behavioural outcomes have produced results that range from evidence of harm,[22,26-28] to null findings,[20,29-34] to evidence of benefit.[14,35,36] Studies of the effects of low to moderate PAE on growth trajectories and birth outcomes have also produced mixed results.[13,37-42] Discrepancies in findings are likely due to measurement error of the exposure and outcome (in some samples children may have been too young for comprehensive assessment of neurodevelopmental outcomes),[43] and residual confounding due to the socioeconomic patterning of prenatal alcohol use (discussed further in Chapter 5).

### 2.1.4 Guidance on alcohol use in pregnancy

These inconsistencies in the evidence base are reflected in guidelines for drinking in pregnancy. Most countries in America, Asia, Europe and Australasia endorse a clear abstinence message.[44] Since 1981, the USA Surgeon General's statement has advised pregnant women, or those planning a pregnancy, not to drink alcohol.[45] UK guidelines for drinking in pregnancy have been unclear. Up until 2016 the Department of Health, National Institute for Health and Care Excellence (NICE), NHS Choices and Royal College of Obstetricians and Gynaecologists recommended that, while it is safest not to drink while pregnant, there is no known risk of harm at low levels and suggested that if women choose to drink they should not exceed

one to two units once or twice per week.[25,46,47] In 2016, the UK Chief Medical Officer (CMO) issued new guidance stating that women should avoid alcohol throughout pregnancy.[48] This guidance has since been adopted by NHS Choices and the Royal College of Obstetricians and Gynaecologists.[49,50] NICE have issued a statement in support of the CMO recommendations, but are yet to update their Antenatal Care Guideline (last checked 26.02.2018).[25,51]

### 2.1.5 Public awareness and attitudes towards PAE guidance

The Infant Feeding Survey 2010 reported that 29% of women did not receive any information about PAE from health professionals. Women who did receive advice reported being given mixed messages, with some being told to abstain and some to limit the amount they drank.[52] Given the inconclusive evidence about the risks of low to moderate levels of PAE, opinions about PAE guidance are also divided. Some groups endorse a 'no alcohol no risk' perspective and suggest that guidance that takes a precautionary approach by advising abstention is warranted.[24,53] Others warn against the 'policing of pregnancy,' state that giving up alcohol may be a 'pointless sacrifice,' and argue that women should be free to make an educated choice about whether they drink while pregnant or trying to conceive.[53-55]

In the context of the inconsistencies in UK guidance, mixed advice from healthcare professionals and differences in public opinion, 71% of individuals said that they found PAE guidance confusing, when asked in a 2015 study.[56] In contrast, the report from the public consultation on the updated CMO guidance found that 80% of respondents thought that the abstinence message was clear and helped to resolve some of the confusion around the previous guidelines.[48] Clarification of the

guidance is only part of the picture. Studies have found that public awareness of the PAE guidance is low (in 2015, 40% of individuals reported that they did not know what the government guidance was)[56] and adherence to guidance on prenatal lifestyle factors is also low.[57-59] Therefore, the impact of the change in guidance on public awareness and alcohol use in pregnancy remains to be seen.

### 2.1.6 Conclusions and implications for this thesis

In summary, existing epidemiological studies suggest that PAE is a significant public health concern in the UK. However, there is wide variation in results between studies. A major limitation of the existing evidence base is the reliance on self-report methods for ascertaining PAE. Self-report methods are likely to underestimate true PAE for reasons including social desirability bias, fear of persecution, and an inability to accurately recall and quantify drinking behaviour.[60-64] The measurement error introduced by self-reported PAE creates uncertainty in prevalence estimates and complicates attempts to investigate the adverse outcomes associated with PAE, as described in the next section. Objective measures are needed to strengthen epidemiological research into PAE. For clinicians, objective measures of PAE could support FASD diagnosis and guide efforts to prevent alcohol-related harm.[65-70] Biomarkers of prenatal alcohol use have been proposed as an objective alternative to self-report, but have not been comprehensively evaluated. To address this research gap, I present a systematic review of the validity of objective measures of prenatal alcohol use in Chapter 2 of this thesis.

## 2.2 Fetal alcohol spectrum disorder (FASD): existing evidence and knowledge gaps

FASD is an umbrella term that describes a range of consequences of prenatal exposure to alcohol including a distinctive facial phenotype, growth deficiencies and neurobehavioural impairments that persist across the lifespan.[71] Figure 1 provides an overview of the main subtypes that are recognised within the FASD continuum, according to the core features of FASD. Differences between diagnostic frameworks for FASD are described in more detail in Chapter 3. Generally, conditions within the FASD spectrum are said to include fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS) and alcohol-related neurodevelopmental disorder (ARND). Some frameworks suggest an additional category, known as alcohol-related birth defects (ARBD), but this subtype is not widely accepted.[72]

FAS is the most recognisable form of FASD and is characterised by a triad of facial anomalies (short palpebral fissure length, thin upper lip and smooth philtrum), growth impairment and central nervous system (CNS) dysfunction. Figure 2 shows the facial phenotype of FAS. An estimated one in 10 children with FASD will have FAS and therefore it represents a relatively rare subtype within the spectrum.[11] Due to the specificity of the facial phenotype to alcohol exposure, FAS can be diagnosed without confirmed PAE.[75] In other words, when information about PAE is not available, the facial phenotype can be used as a proxy indicator of exposure. Partial FAS (pFAS) is used to describe individuals who have two of the features of the FAS facial phenotype and CNS dysfunction. ARBDs refer to congenital anomalies with confirmed PAE. Finally, ARND is the most common subtype of FASD,

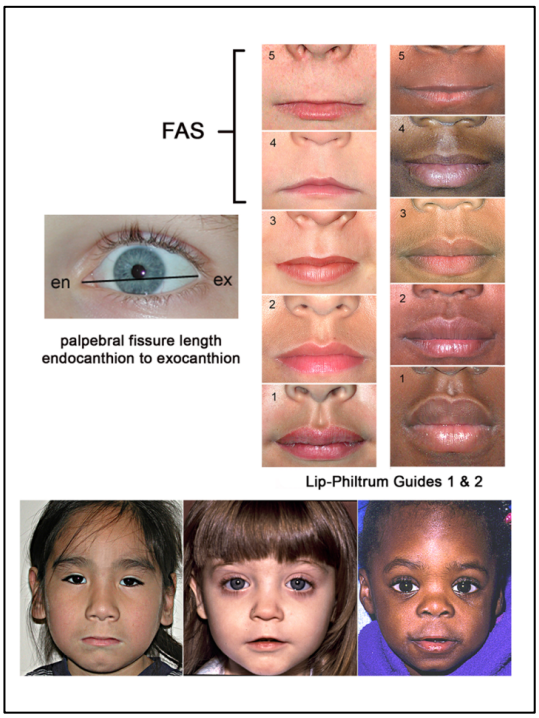
but it is the most difficult to diagnose. ARND is the non-dysmorphic subtype of FASD and diagnosis requires confirmation of PAE and significant CNS impairment.[72,73] Often evidence of PAE is missing or inaccurate and CNS dysfunction is not apparent until later in childhood.[43] Therefore, FASD is thought to be significantly underdiagnosed. One study that carried out assessments in a high risk sample of fostered and adopted children found that 80% of children with FASD had not been previously diagnosed.[76] For this reason, FASD has been referred to by some as an invisible disability.[77]

*Figure 1: Summary of FASD subtypes and core features*

| | **Fetal alcohol syndrome (FAS)** | **Partial fetal alcohol syndrome (pFAS)** | **Alcohol-related neurodevelopmental disorder (ARND)** | **Alcohol-related birth defects (ARBD)[a]** |
|---|---|---|---|---|
| **Prenatal alcohol exposure (PAE)** | Confirmed or unconfirmed | Confirmed | Confirmed | Confirmed |
| **Central nervous system (CNS) impairment** | Yes | Yes | Yes | Not required |
| **Facial anomalies** | Yes (3 features) | Yes (2 features) | Not required | Yes (2 features) |
| **Growth deficiency** | Yes | Not required | Not required | Not required |
| **Congenital structural defects** | Not required | Not required | Not required | Yes |

[a] ARBD is included in the Institute of Medicine guidelines but is not recognised as a subtype within most FASD frameworks.

*Figure 2: The three diagnostic facial features of FAS include: 1) short palpebral fissure length, 2) a smooth philtrum (Rank 4 or 5 on the Lip-Philtrum Guide), and 3) a thin upper lip (Rank 4 or 5 on the Lip-Philtrum Guide). Lip-Philtrum Guides 1 and 2 are used to rank upper lip thinness and philtrum smoothness. The philtrum is the vertical groove between the nose and upper lip. The guides reflect the full range of lip and philtrum shapes with Rank 3 representing the population mean. Ranks 4 and 5 reflect the thin lip and smooth philtrum that characterize the FAS facial phenotype. Guide 1 is used for Caucasians and all other races with lips like Caucasians. Guide 2 is used for African Americans and all other races with lips as full as African Americans. Examples of the FAS facial phenotype across three races: Native American, Caucasian, and African American. Copyright 2015, Susan Astley PhD, University of Washington*




### 2.2.1 Prevalence of FASD

Notwithstanding difficulties in detection, research suggests that FASD is one of the most common causes of preventable developmental disability worldwide.[78] Studies from the USA, Croatia, France, Poland and Italy suggest that 1% to 10% of children in the general population have FASD.[79-87] The prevalence of FASD is higher among children in care, where the pooled prevalence is 17%.[88] Rural communities in South Africa, where binge pattern PAE is common, have the highest known prevalence of FASD in the world (up to 28%).[80,89] FASD is associated with over 400 comorbid conditions[90] and a significantly increased risk of adverse outcomes in later life including mental health conditions, lack of independent living and involvement

with the criminal justice system.[91,92] Early identification is important for improving outcomes among affected individuals and for preventing future alcohol-exposed pregnancies, but is complicated by difficulties in case ascertainment.[65,69,93]

Despite known high levels of prenatal alcohol use in the UK, there are no reliable estimates of the prevalence of FASD.[94] In the absence of empirical studies, one study, published in 2017, estimated that 3.3% (95% CI 2.0% - 4.9%) of children in the general population of the UK may have FASD based on a calculation that considered the prevalence of PAE and the assumption that one in 13 children with PAE will develop FASD.[86]

Existing UK studies have been limited to FAS rather than the full spectrum of FASD and have methodological limitations, including the use of surveillance methods. Surveillance methods use existing records, such as birth defects registers and hospital admissions data, and benefit from being relatively inexpensive and easy to implement.[95] However, studies that use surveillance methods are likely to underestimate FASD because most subtypes are not readily detectable at birth and case ascertainment is often performed by individuals who are not FASD specialists.[10,95] This is borne out in data from the Scottish Paediatric Surveillance Unit, which showed that between 2010 and 2015 FAS was reported to affect 0.19 per 1,000 children.[96] In Wales, the Congenital Anomaly Register and Information Service (CARIS) reported that FAS occurred in 0.78 per 10,000 live births between 1998 and 2015.[97] Hospital admissions data are also of limited use for ascertaining prevalence, since FASD is not often a reason for hospitalisation and under-recognition of FASD means that it is less likely to be coded as a co-existing

condition in routine admissions data.[10,98] The Health and Social Care Information Centre (HSCIC) in England reported 0.17 per 10,000 admissions in which FAS was recorded as a primary or secondary diagnosis in 2012 to 2013.[99,100] Morleo and colleagues reported that the rate of hospital admissions for FAS was 0.08 per 10,000 population in England between 2002 and 2008.[98] Three other UK studies found no cases of FAS, using a range of methodologies including health visitor screening and medical record review (total number of participants/records = 12,741).[101-103] Gregory and colleagues identified 72 potential cases of FASD during a retrospective audit of children who visited a UK community paediatric clinic between 2010 and 2013. However, it was not possible to calculate the prevalence due to an uncertain denominator.[104]

Other methodological difficulties with ascertaining prevalence include the fact that health professionals in the UK report a lack of knowledge and confidence in identifying FASD.[105] Information on PAE is often missing or inaccurate and this further complicates FASD identification. Perceived stigma and the lack of a clearly defined care pathway have been cited as additional reasons for not investigating potential cases of FASD in the UK.[96,105,106] The International Statistical Classification of Diseases and Related Health Problems (ICD-10) only includes a code for FAS (Q86.0 Fetal alcohol syndrome [dysmorphic]) and not for FASD.[107] This is likely to further contribute to the lack of routine reporting for the full spectrum of FASD cases. A meta-analysis published in 2017 reported a FAS prevalence of 37 per 10,000 in the European region.[11] Therefore, the FAS prevalence estimates described above are significantly lower than what would be expected, given the high levels of PAE in the UK and based on comparisons with international studies.

In summary, several factors point to the likelihood that the prevalence of FAS and FASD is significantly underestimated in the UK. Active case ascertainment studies (where researchers recruit and actively assess participants in the general population, most commonly in the form of in-school prevalence studies) have produced higher prevalence estimates than surveillance methods and have been advocated as the preferred approach for FASD prevalence studies.[95] In 2015/16, the All Party Parliamentary Group (APPG) on FASD and British Medical Association expressed an urgent need for a population-based prevalence study in the UK to guide prevention efforts and policy for alcohol use in pregnancy.[10,108] However, to date, proposals for active case ascertainment studies of FASD in the UK have not been successful.[108] To address this research gap, I developed and validated FASD case ascertainment algorithms (Chapter 3) to enable investigation of the epidemiology of FASD using existing data from a population-based birth cohort in England (the Avon Longitudinal Study of Parents and Children; ALSPAC). In Chapter 4, I describe a study in which I applied these case ascertainment algorithms to estimate the prevalence of FASD in the ALSPAC cohort.

### 2.2.2 Risk factors for FASD

Alcohol is the sole necessary cause of FASD, however it is not always sufficient. Following any PAE an estimated one in 13 children will develop FASD and one in 67 will develop FAS.[11,86] Differences in maternal alcohol metabolism, maternal physical characteristics such as weight, and co-occurring exposures are thought to influence the risk of FASD.[109-111] However, much of the FASD literature has centred around discussion of association, rather than causation.[109,111] While information about

association is important for describing who is at risk of FASD, understanding the causal factors that influence the teratogenicity of PAE is essential for identifying potential opportunities for intervention and prevention. In Chapter 5, I present a causal diagram of risk factors for FASD, based on a systematic literature search and narrative synthesis. In Chapter 6, I use this causal diagram to inform multivariable analyses of risk factors for FASD using data from the ALSPAC cohort.

# 3 Thesis aims and research questions

## 3.1 Aims

i. To assess the validity of the biological tests that are available to obtain an objective measure of prenatal alcohol exposure (PAE).
ii. To describe the epidemiology of fetal alcohol spectrum disorder (FASD) within a population-based birth cohort in England (ALSPAC).

## 3.2 Research questions

i. What is the diagnostic accuracy of objective measures of prenatal alcohol use?
ii. What is the prevalence of FASD within the ALSPAC cohort?
iii. What are the risk factors for FASD within the ALSPAC cohort?

# 4 Thesis synopsis

This introductory chapter has described the context and research questions for this thesis.

**Chapter 2** presents a systematic review of the validity of objective measures of prenatal alcohol use.

**Chapter 3** describes the development and validation of case ascertainment algorithms for FASD.

**Chapter 4** applies the FASD case ascertainment algorithms that I developed in Chapter 3 to produce prevalence estimates for FASD using data from a population based birth cohort in England (ALSPAC).

**Chapter 5** presents a narrative literature review and causal diagram (directed acyclic graph; DAG) of risk factors for FASD.

**Chapter 6** applies the FASD case ascertainment algorithms from Chapter 3 and uses the causal diagram from Chapter 5 to inform a multivariable analysis of potentially modifiable causal risk factors for FASD using ALSPAC data.

**Chapter 7** provides a summary of the main results, strengths and limitations, implications and conclusions of this thesis.

# Chapter 2. Validity of objective measurement of alcohol use in pregnancy: a systematic review

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The results from this chapter were published in *Pediatrics* (McQuire C, Paranjothy S, Hurt L, Mann M, Farewell D, Kemp A. Objective measures of prenatal alcohol exposure: a systematic review. *Pediatrics.* 2016;138(3): doi: 10.1542/peds.2016-0517).

## 1 Overview

This chapter describes a systematic review of the validity of biomarkers of prenatal alcohol exposure (PAE). First, I describe existing approaches to PAE screening and introduce biomarkers of PAE. I then present the systematic review methods, results, discussion and conclusions. Finally, I describe the implications of this review for the remaining chapters in this thesis.

## 2 Background

### 2.1 Current practice in screening for prenatal alcohol use

Routine antenatal screening is available for all pregnant women in the UK. Screening aims to identify a range of conditions including haematological disorders, infections, fetal anomalies, gestational diabetes, Down Syndrome and genetic disorders.[25,112,113] Screening is also available for lifestyle-related behaviours such as cigarette smoking. The harms associated with tobacco use in pregnancy are well known and midwives can use self-report measures in conjunction with carbon monoxide testing to refer women to National Health Service (NHS) smoking

cessation services.[114] While antenatal screening for physical health conditions and tobacco use is well established in the UK, there is no standardised process to assess prenatal alcohol use, despite the known risks to the fetus. The Welsh Government recommends that midwives discuss alcohol use with pregnant women at the first antenatal appointment and NICE state that women should be advised about the risks associated with prenatal alcohol consumption.[25,115] However, there is a lack of guidance on how to assess prenatal alcohol use and a recent UK survey reported that 40% of midwives do not routinely ask about PAE.[116] Poor screening for alcohol use in antenatal care has been identified as a significant barrier to support.[64]

Self-report measures are the most common method for assessing maternal alcohol use.[117] A range of brief screening questionnaires have been used to assess hazardous drinking among pregnant women including: TWEAK[a], T-ACE[b], CAGE[c], NET[d], the Alcohol Use Disorder Identification Test (AUDIT), AUDIT-consumption (AUDIT-C) and the Short Michigan Alcohol Screening Test (SMAST).[118-120] These measures may be useful for identifying particularly high risk pregnancies. However, most prenatal alcohol use is occasional and low level[12,13,52] and therefore, measures of hazardous drinking may not be applicable for the majority of individuals in the general antenatal population. The timeline follow-back procedure (TLFB), another self-report method, captures the daily frequency and quantity of alcohol consumption for particular drinks within a specified time period and thus documents the full spectrum of alcohol use.[121] The TLFB and an abbreviated

[a] TWEAK has five items: tolerance, worried, eye-opener, amnesia and cut-down
[b] T-ACE has four items: tolerance, annoyed, cut-down, eye-opener
[c] CAGE has four items: cut down, annoyed, guilt, eye-opener
[d] NET has three items: normal drinker, eye-opener, tolerance

version, known as the retrospective diary,[63] have been shown to elicit higher estimates of maternal drinking than those reported using other measures.[63,122,123] Some consider TLFB based measures to be the gold standard in maternal alcohol assessment.[117] However, no studies have been conducted to evaluate the diagnostic accuracy of the TLFB with a sample of pregnant women.

Although self-report measures are widely used to estimate prenatal alcohol use, they are likely to underestimate true consumption and, therefore, represent an imperfect reference standard. A systematic review and meta-analysis found that the estimated prevalence of PAE was four times higher in studies that tested for biomarkers of ethanol metabolism, compared to those that used self-report.[124] The underreporting of PAE in self-report measures could be due to factors including a fear of persecution, social stigma, difficulties in accurately quantifying alcohol use and the fact that mothers may simply not remember their drinking habits during the antenatal period.[60,125-127]

## 2.2 Biomarkers: objective measures of prenatal alcohol use

Due to the limitations of self-report methods, biomarkers have received increasing attention as an objective way to establish gestational alcohol use.[117,126,128-133] Biomarkers are "indicators or signalling events in biological systems."[134(p. 488)] Objective measurement has been defined as a procedure that prevents individuals from misrepresenting themselves in terms of the characteristics that are being measured by the test.[135]

Biomarkers of alcohol metabolism can be classified into two categories: direct and indirect. Direct markers of alcohol use include the measurement of ethanol itself in

breath and urine. However, ethanol can only be detected for a very short period with these methods (typically less than one day), and therefore these direct tests are difficult to implement in research and practice.[117,130] Other direct markers, such as ethanol metabolites, can be detected for longer periods of time and therefore may have better clinical utility. Examples of such biomarkers include fatty acid ethyl esters (FAEEs). FAEEs do not freely cross the placenta and, therefore, FAEEs in meconium (the first stool of the neonate) can be considered to be direct biomarkers of fetal alcohol exposure.[134] Other direct markers of recent alcohol consumption include ethyl glucuronide (EtG) and ethyl sulphate (EtS). EtG can be measured in urine for around five days after use and EtS for around one and a half days.[136,137] Finally, phosphatidylethanol (PEth) is a direct ethanol metabolite that can be detected in blood for approximately three weeks following alcohol intake.[117,138]

Indirect markers are those that signal alcohol-induced pathology following prolonged exposure. Examples include the liver enzyme gamma-glutamyltransferase (GGT); mean corpuscular volume (MCV), a measure of red blood cell volume; and carbohydrate-deficient transferrin (CDT), a form of iron-transporting transferrin. All are elevated following sustained heavy alcohol use and have been used most commonly to evaluate alcohol use among those with alcohol misuse disorders.[117,130,139] Table 1 provides a summary of objective measures of PAE.

*Table 1 (continued overleaf): Overview of objective measures of prenatal alcohol use*

| | Biomarker | Matrix | Detection window[a] | Lowest detectable level of alcohol use |
|---|---|---|---|---|
| **Direct biomarkers** | Ethanol | Blood, breath, urine | < 1 day | Low[b] |
| | Ethyl sulphate (EtS) | Urine | 30 hours | Low/moderate |
| | | Blood | < 1 day | |
| | | Placenta | Unknown | |
| | | Fetal tissue | Unknown | |
| | | Meconium | Reflects 2nd and 3rd trimester exposure | |
| | Ethyl glucuronide (EtG) | Urine | < 5 days | Low/moderate |
| | | Hair | Maternal hair: months to years depending on hair length[c]<br>Neonatal hair: captures third trimester exposure and typically sheds 3 months after birth | Moderate |
| | | Blood | < 1 day | |
| | | Meconium | Reflects 2nd and 3rd trimester exposure. | |
| | | Placenta | Unknown | |
| | | Fetal tissue | Unknown | |
| | Fatty acid ethyl esters (FAEEs) | Meconium | Reflects 2nd and 3rd trimester exposure. | Moderate |
| | | Hair | Maternal hair: months to years depending on hair length<br>Neonatal hair: captures third trimester exposure and typically sheds 3 months after birth | |
| | | Blood | 1 day | |
| | Haemoglobin acetaldehyde adducts (Hb-Ach) | Blood | ≤ 4 weeks | Moderate |
| | Phosphatidylethanol (PEth) | Blood | ≤ 3 weeks[d] | Moderate |

| | Biomarker | Matrix | Detection window[a] | Lowest detectable level of alcohol use |
|---|---|---|---|---|
| **Indirect biomarkers** | Alanine aminotransferase (ALT) | Blood | ≤ 3 weeks | High |
| | Acetaldehyde-protein adducts (APAs) | Blood | < 4 weeks | High |
| | Aspartate aminotransferase (AST) | Blood | < 3 weeks | High |
| | Carbohydrate deficient transferrin (CDT) | Blood | < 4 weeks | High |
| | Gamma glutamyltransferase (GGT) | Blood | ≤ 3 - 4 weeks | High |
| | Mean corpuscular volume (MCV) | Blood | ≤ 17 weeks | High |
| | Ratio of 5-hydroxytryptophol/5-hydroxyindolylacetic acid (5-HTOL/5-HIAA) | Urine | < 1 day | Moderate/high |

Notes: Biomarkers in this table were identified from Joya et al. (2012)[130] and from the search for the current review.
[a] Detection window refers to the period in which the biomarker can be detected following alcohol consumption.
[b] Definitions vary between studies. This review classifies light drinking as equivalent to < 3 drinks per week, moderate drinking 3 to 7 drinks per week, and heavy drinking > 7 drinks per week or a binge pattern of ≥ 4 drinks per occasion.
[c] Hair grows at approximately 1 cm per month. The section closest to the root represents the most recent period of exposure.
[d] In maternal blood; duration in neonatal blood unknown.

# 3 Study rationale and aim

Recently, some groups have advocated the introduction of universal biomarker screening programmes for PAE.[127,140] For example, meconium testing is recommended within the Canadian FASD National Screening Tool Kit.[141] However, the evidence for the diagnostic accuracy of biomarker testing has not been comprehensively evaluated. In this context, the aim of this chapter was to present a systematic review of the diagnostic accuracy of objective measures of PAE.

# 4 Method

I followed the Cochrane Collaboration guidelines for systematic reviews of diagnostic test accuracy,[142] the Standards for the Reporting of Diagnostic Accuracy Studies (STARD),[143] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[144] The study protocol was published in the PROSPERO international prospective register of systematic reviews (www.crd.york.ac.uk; record number CRD42014015420).

## 4.1 Search strategy

I searched 13 electronic databases, including sources of grey literature, from January 1990 to August 2015 for original articles using combinations of terms related to *objective measures* and *diagnostic accuracy* and *prenatal alcohol exposure*. Searches were limited to publications from January 1990 onwards to increase precision. A scoping exercise of existing non-systematic reviews revealed no relevant articles prior to 1990.[126,130,134,137,145] Databases are listed in Figure 4 and the full search strategy for Medline is available in Appendix 1. The Medline search

string was translated for use in all other databases. I searched supplementary sources for articles published between November 2012 and October 2015 and contacted authors to request further information about missing or conflicting data.

## 4.2 Inclusion and exclusion criteria

Eligible studies were those that investigated the diagnostic accuracy of any objective measure of PAE in comparison to any reference standard, among pregnant and/or postpartum women and/or neonates. The Cochrane Collaboration defines a gold standard reference test as the method, procedure, or measurement that is widely accepted as being the best available and against which new developments should be compared.[146] Due to the inherent limitations of self-report and because there is no consistently applied method for measuring alcohol use in pregnancy, no one reference test could be considered the gold standard for the purposes of this review.[147]

Eligible study designs included randomised screening studies and diagnostic 'cohort-type', and 'case-control type' studies. Diagnostic accuracy studies are typically cross-sectional in design. Participants either have the target condition or not (i.e. prenatal alcohol consumption) at the point of inclusion and they receive the reference test to verify their status, which is compared to the result of the index test(s). However, these studies can be further classified as diagnostic 'cohort type', 'case-control type' or 'nested case-control type'. Cohort type studies recruit participants based on a single set of inclusion criteria that are not based on whether the target condition is present or absent (e.g. all women attending a healthcare centre in the first trimester of their pregnancy), whereas case-control

type studies use different selection criteria based on whether the target condition is present or absent (e.g. the study selects one group of women who are known to have consumed alcohol while pregnant and one group of abstainers). Diagnostic nested case-control studies are those in which cases and controls are drawn from a well-defined cohort and then a subset of cases and controls are selected for analysis.[148-150]

I excluded conference abstracts, studies with missing outcome data, and studies of non-human animals. Non-English language publications were excluded due to a lack of funding for translation costs.

## 4.3 Study selection

After removing duplicates, I screened the search records against the pre-determined inclusion criteria and excluded ineligible studies based on the title or abstract. I obtained full text versions to determine the inclusion of potentially relevant studies. A random selection of 10% of these studies were independently assessed for eligibility by two members of the supervisory team (LH and SP). The level of agreement for inclusion decisions was 100%.

## 4.4 Data extraction

I extracted all data into a standardised electronic form, which I designed based on the Guidelines International Network (GIN) template for diagnostic studies and the Standards for Reporting of Diagnostic Accuracy (STARD).[143,151] SP or LH independently repeated data extraction to ensure accuracy. The extracted information included details of the study design, participants, index and reference test characteristics and diagnostic accuracy outcomes. Alcohol data were classified

according to the United States National Institute on Alcohol Abuse and Alcoholism criteria for the general population in which one standard drink is equivalent to 0.6 oz or 14 g of ethanol and light drinking is equivalent to < 3 drinks per week, moderate drinking 3 to 7 drinks per week, and heavy drinking > 7 drinks per week or a binge pattern of ≥ 4 drinks per occasion.[152]

## 4.5 Quality assessment

I used a modified version of the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2) to assess the methodological quality of included diagnostic accuracy studies.[150] QUADAS-2 was tailored to address specific areas of relevance for the review, following guidance from the Cochrane Collaboration.[153,154] The methodological quality evaluation assessed risk of bias in nine domains: participant selection, reference standard, detection window, partial verification, differential verification, incorporation bias, uninterpretable results, withdrawals and selective outcome reporting (see Appendix 2 for full quality coding criteria).

## 4.6 Analysis and data synthesis

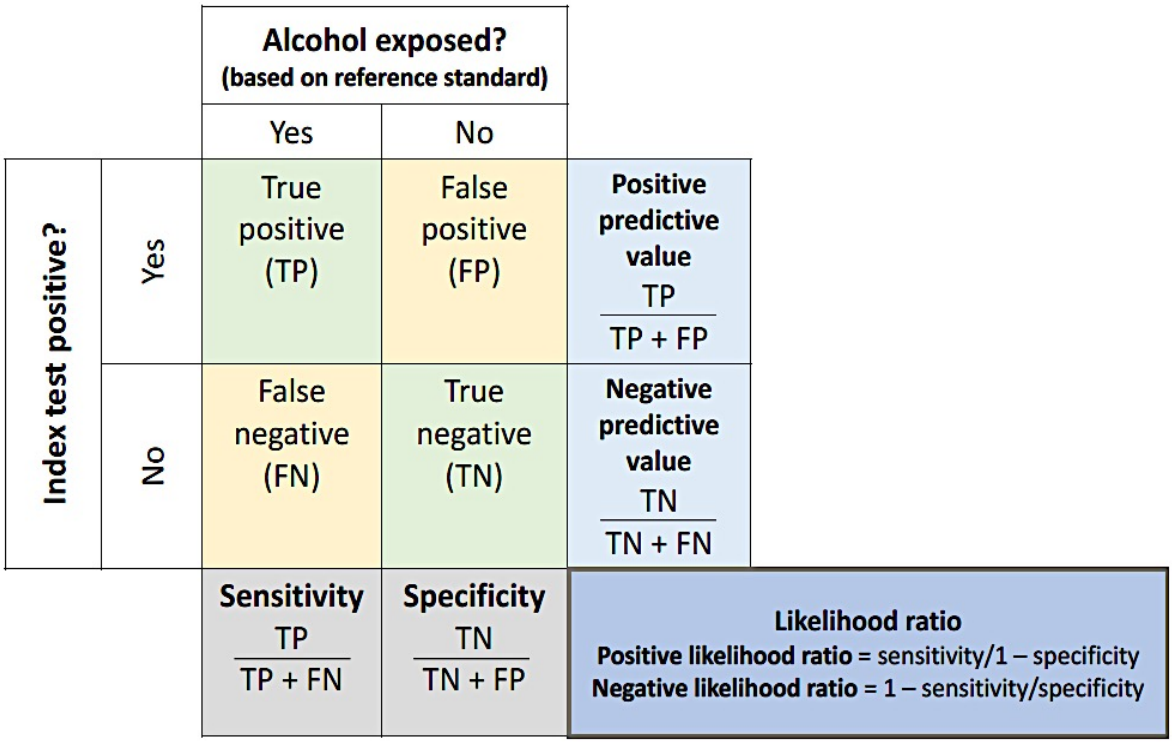
The Rutter and Gatsonis hierarchical summary receiver operating characteristic (HSROC) model is recommended for meta-analyses of diagnostic accuracy data. However, a minimum of four studies per test is required for this method.[155,156] Moses-Littenberg SROC curves can be generated in Review Manager (RevMan)[157] software with fewer than four studies, but are not recommended for use, except for in exploratory analyses, such as investigations of heterogeneity.[156] Due to the diverse nature of the data, which included a variety of measures and assay methods across multiple matrices, none of the index test categories had a sufficient

number of studies to facilitate meta-analysis. Therefore, I conducted a narrative synthesis of the data.

The key diagnostic accuracy outcomes were sensitivity, specificity, predictive values, and likelihood ratios. Figure 3 presents a schematic summary of the key diagnostic accuracy outcomes. Sensitivity and specificity are used widely and capture the intrinsic accuracy of a test, as they are not influenced by the prevalence of the target condition.[158] Sensitivity and specificity are conditional on the 'disease status' of the individual (i.e. whether the target condition, in this case PAE, is present or absent). Sensitivity is the proportion of true positives that are correctly identified by the test and specificity is the proportion of true negatives that are correctly identified by the test. In absolute terms, a test with a sensitivity of 80% will detect 80 out of every 100 individuals with the target condition but will miss 20. A test with a specificity of 90% will correctly identify 90 out of every 100 individuals without the target condition but 10 will be wrongly identified as having the condition.[156,159]

NICE have previously recommended that sensitivity and specificity values of 80% (with a lower 95% confidence interval limit of greater than 70%) indicate acceptable diagnostic test accuracy.[160] However, it is important to note that the relative importance of sensitivity and specificity values are likely to vary according to the condition being assessed. There is no research to suggest what level of diagnostic accuracy is acceptable for assessing PAE, or the relative importance of false positive and false negative errors.

*Figure 3: Schematic overview of key diagnostic accuracy statistics*




While sensitivity and specificity have been considered fundamental metrics within diagnostic test accuracy research, some argue that positive and negative predictive values provide greater clinical utility.[158,161] Positive and negative predictive values are conditional on the test outcome and capture the probability that a test will provide the correct result. In clinical practice, it is often the case that only the index test result, rather than the true presence of the target condition, is known. Therefore, it is important to evaluate how many individuals with positive test results actually have the condition of interest.[161,162] Positive predictive value (PPV) refers to the proportion of individuals with a positive test result who are correctly identified as having the target condition. Negative predictive value (NPV) refers to the proportion of individuals with a negative test result who are correctly identified as not having the target condition. In absolute terms, for a test with a positive predictive value of 80%, 80 out of every 100 individuals with a positive test result

will have the target condition, but 20 will not. For a test with a negative predictive value of 90%, 90 out of every 100 individuals with a negative test result will not have the target condition, but 10 will. Unlike sensitivity and specificity, positive and negative predictive values are dependent on prevalence and therefore, it may not be possible to generalise findings across settings, where prevalence differs.[158,159,162]

Finally, likelihood ratios express how many times more or less likely a participant with the target condition is to have a particular test result than those without the target condition.[163] The particular advantage of likelihood ratios is that, like predictive values, they give the probability of the target condition given certain test results. Furthermore, they are not dependent on a fixed prevalence of the target condition and can be adapted to accommodate differences in baseline prevalence across settings. Thus, they have greater potential for generalisability and clinical application than predictive values.[163]

I used RevMan to collate the diagnostic accuracy data.[157]Where necessary, the RevMan calculator was used to derive diagnostic summary statistics from true positive, true negative, false positive and false negative values and vice-versa. If there were insufficient data to enable the use of the RevMan calculator, R software[164] was used to conduct an exhaustive search of all possible 2 x 2 tables that were consistent with the data supplied in the primary study. I generated confidence intervals using the exact binomial method[165] in RevMan for sensitivity and specificity values and with the MedCalc online calculator[166] for predictive values. Confidence intervals for likelihood ratios were generated using the method described by Koopman.[167]

Many studies reported a range of diagnostic accuracy values according to characteristics such as positivity cut-off and period of measurement. To aid clarity of findings, I report only the highest values of both sensitivity and specificity per study within the summary of results.

# 5 Results

## 5.1 Search results

From 4,278 search records, 12 studies with 1,614 unique participants, were eligible for inclusion. Figure 4 presents a flow diagram of the search and study selection process, and Table 2 presents the characteristics of the included studies.

## 5.2 Characteristics of included studies

Eligible studies included data on participants from the USA (5),[168-172] Korea (2),[173,174] Spain (1),[175] South Africa (1),[176] and Finland (1)[177], and combined data from the USA and Jordan (1),[178] and Canada and Israel (1).[179] Diagnostic accuracy data were available for eight types of biomarker: carbohydrate deficient transferrin (CDT),[168,172,177] ethyl sulphate (EtS),[168,172] ethyl glucuronide (EtG),[168,172,175] fatty acid ethyl esters (FAEEs),[168-171,173,176,178,179] gamma glutamyltransferase (GGT),[168,172,177] haemoglobin acetaldehyde adducts (Hb-Ach),[177] mean corpuscular volume (MCV)[177] and phosphatidylethanol (PEth),[168,172,174] within six matrices: meconium,[168-170,173,176,178,179] placenta,[171] maternal urine,[168,172] maternal blood,[168,172,174,177] maternal hair[172,175] and infant blood.[168] Eight studies investigated tests of moderate to heavy prenatal alcohol consumption[168,171,172,174,176-179] and nine recruited women from high-risk settings, such as substance misuse clinics.[168-172,176-179] Eight of the eligible studies recruited pregnant women who reported

abstinence from alcohol as a comparison group.[168-170,172-174,178,179] Two of these studies included an additional control group of pregnant women from cultures that promote abstinence from alcohol.[178,179] Three of the studies explored test performance for distinguishing between heavy drinkers and women with lower levels of prenatal alcohol consumption[171,176,177] and one study did not report characteristics of the control group.[175]

*Figure 4: Flow diagram of the search and study selection process*

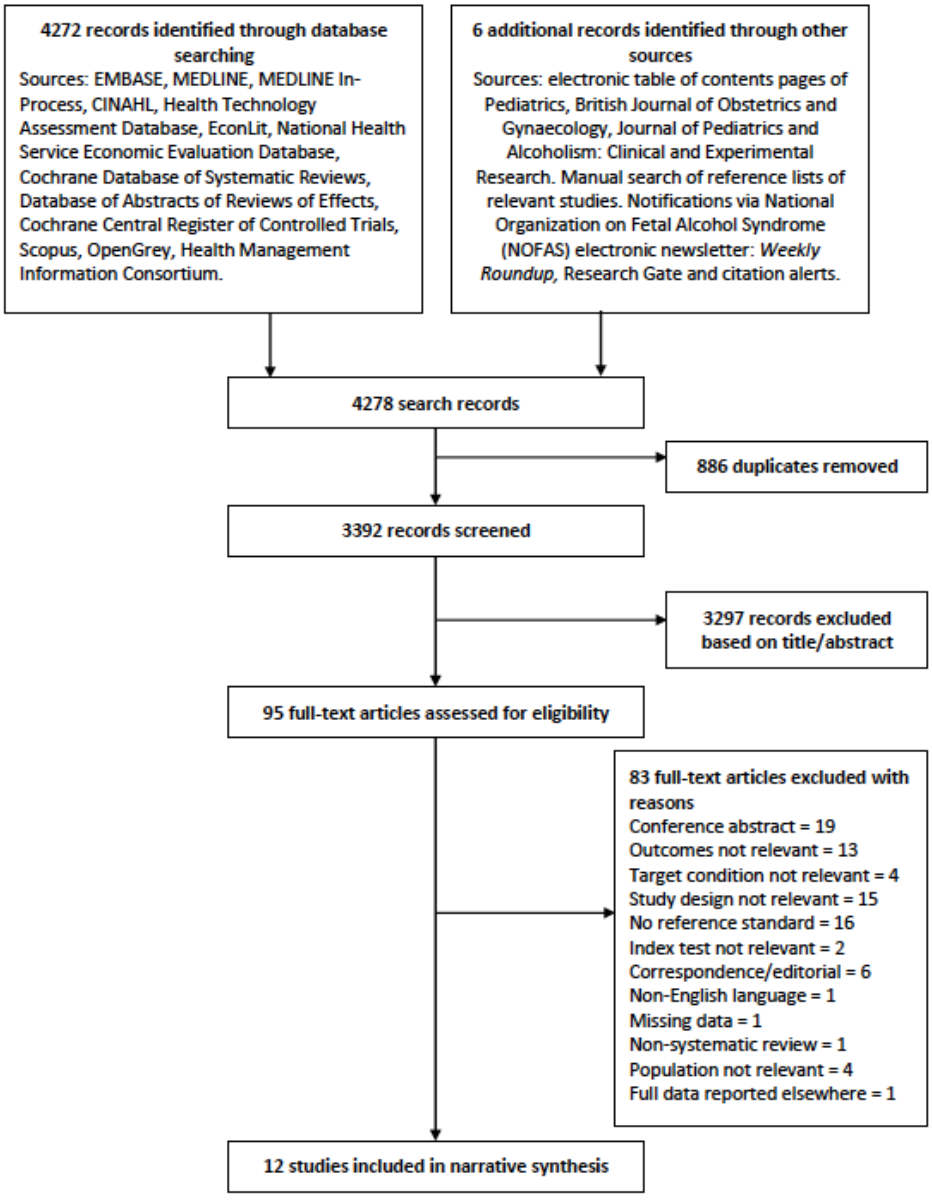


*Table 2 (continued overleaf): Characteristics of included studies of objective measures of prenatal alcohol exposure (PAE).*

| Study | Country | Cases[a] | Controls[a] | High risk setting | PAE level | Study prevalence of PAE | Matrix | Analyte(s) | Period of sample collection | Reference standard |
|---|---|---|---|---|---|---|---|---|---|---|
| **Bakhireva 2014**[168] | USA | 28 | 32 | Substance misuse clinic | Moderate-heavy | 47% | Meconium | FAEE | Postnatal | TLFB and AUDIT |
| | | | | | | | Infant blood | PEth[b] | Postnatal | |
| | | | | | | | Maternal blood | CDT, GGT, PEth[b] | Prenatal | |
| | | | | | | | Maternal urine | EtG, EtS[b] | Prenatal | |
| **Bearer 1999**[169] | USA | 56 | 88 | Substance misuse clinic | Any | 39% | Meconium | FAEE | Postnatal | Maternal postnatal interview |
| **Bearer 2003**[176] | South Africa | 19 | 6 | Dop system region | Heavy | 76% | Meconium | FAEE | Postnatal | TLFB |
| **Bearer 2005**[178] | Jordan & USA | 13[c] | 211[c] | Substance misuse clinic | Heavy | 6%[c] | Meconium | FAEE | Postnatal | Maternal postnatal interview |
| **Chan 2003**[179] | Canada & Israel | 6 | 73 | Women with alcoholism | Heavy | 8% | Meconium | FAEE | Postnatal | Controls: unspecified self-report<br>Cases: confirmed by the referring agency or physician |
| **Gauthier 2015**[171] | USA | 11 | 69 | Premature newborns | Heavy | 14% | Placenta | FAEE | Postnatal | Self-report based on AUDIT |
| **Gutierrez 2015**[172] | USA | 42[d] | 43[d] | Substance misuse clinic | Moderate-heavy | 49% | Maternal blood | CDT, GGT, PEth | Prenatal | TLFB and AUDIT |
| | | | | | | | Maternal urine | EtG, EtS | | |

| Study | Country | Cases[a] | Controls[a] | High risk setting | PAE level | Study prevalence of PAE | Matrix | Analyte(s) | Period of sample collection | Reference standard |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Maternal hair | EtG | | |
| **Joya 2016**[175] | Spain | 30 | 50 | NR | Any | 38% | Maternal hair | EtG | Postnatal | Meconium EtG |
| **Kwak 2014a**[173] | Korea | 54 | 182 | NR | Low-moderate | 23% | Meconium | FAEE | Postnatal | Unspecified self-report |
| **Kwak 2014b**[174] | Korea | 117 | 188 | NR | Low | 38% | Maternal blood | PEth | Prenatal | Unspecified self-report |
| | | 30 | 275 | | Moderate | 10% | | | | |
| | | 8 | 297 | | Heavy | 3% | | | | |
| **Ostrea 2006**[170] | USA | 93 | 31 | Substance misuse clinic | Any | 75% | Meconium | FAEE | Postnatal | Unspecified self-report, MAST, CAGE and TACE |
| **Sarkola 2000**[177] | Finland | 13[e] | 31[e] | Substance misuse clinic | Heavy | 30% | Maternal blood | CDT, GGT, Hb-Ach, MCV | Prenatal | Unspecified self-report |

Abbreviations: AUDIT, Alcohol Use Disorder Identification Test; CAGE, cut down, annoyed, guilt, eye-opener; CDT, carbohydrate deficient transferrin; EtG, ethyl glucuronide; EtS, ethyl sulphate; FAEE, fatty acid ethyl esters GGT, gamma glutamyltransferase; Hb-Ach, haemoglobin acetaldehyde adducts; PEth, phosphatidylethanol; MAST, Michigan Alcohol Screening Test; NA not applicable; NR, not reported; TACE, tolerance, annoyed, cut-down, eye-opener; TLFB, timeline follow-back procedure.

[a] Number of participants included in analysis. Cases are participants with the defined level of PAE within the study and controls are those without PAE as defined by the study.

[b] I excluded data from this study that compared the results of postnatal maternal EtG, EtS, GGT, CDT and PEth in dried infant blood spots with prenatal self-report due to the short detection window of these biomarkers.

[c] The number of cases and controls were not reported in this study. Therefore, the figures presented in the table are those that provided the closest match to the study outcome data based on a simulation of all possible values in R software.

[d] Cases and controls for analysis of hair EtG. Due to missing data, there were 41 cases and 42 controls in the analysis of urine EtG and EtS, 40 cases and 43 controls in the analysis of PEth, and 40 cases and 42 controls in the analysis of GGT and CDT.

[e] Due to missing data, there were 13 cases and 28 controls for GGT analyses due to exclusion of three participants with hepatitis C and elevated alanine aminotransferase.

## 5.3 Methodological quality of included studies

Results of the QUADAS-2 quality assessment are presented in Figure 5 and Figure 6.

*Figure 5: Methodological quality summary for each included study. Ratings indicate risk of bias for each domain. Green symbol = low risk; yellow symbol = unclear risk; red symbol = high risk.*

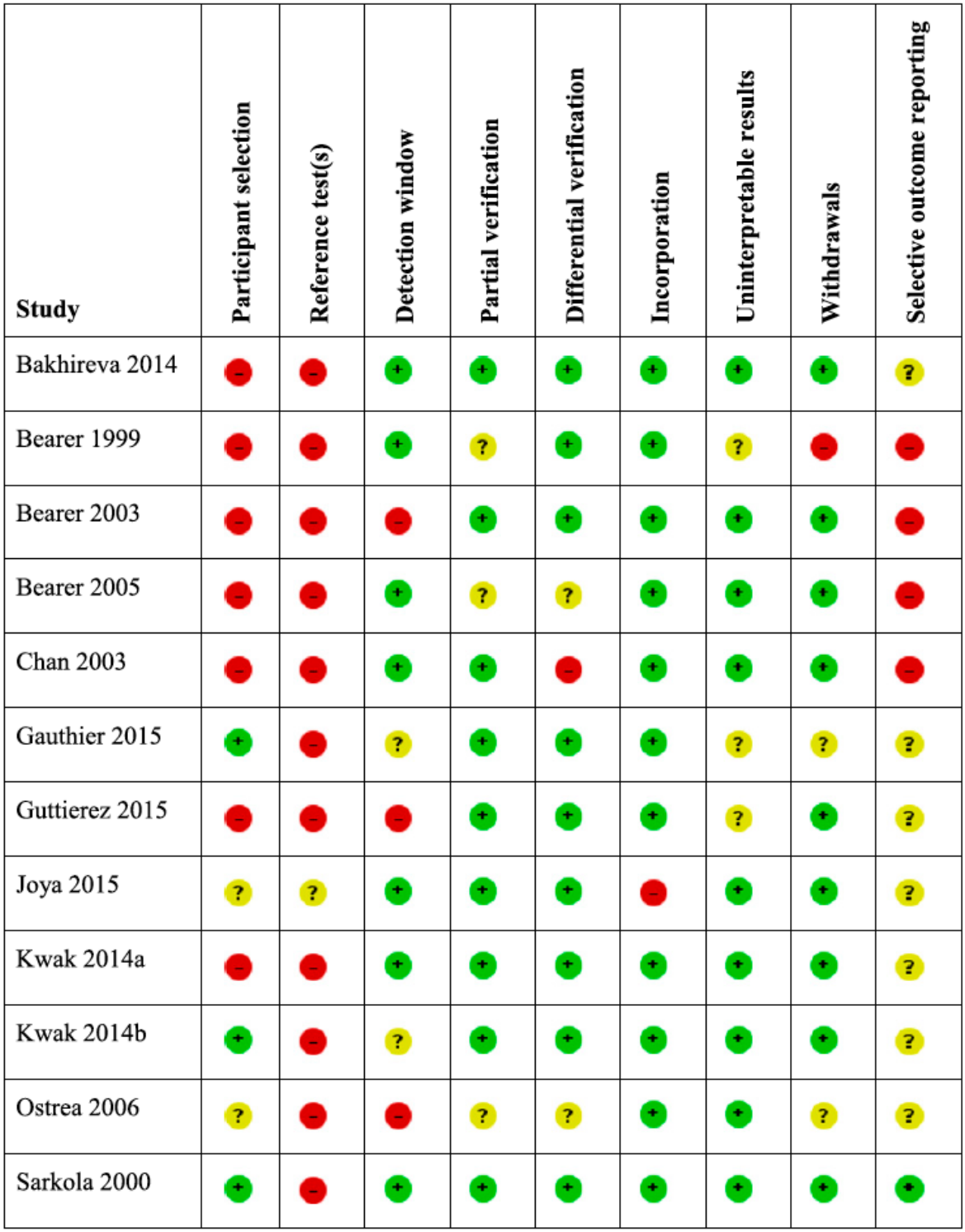
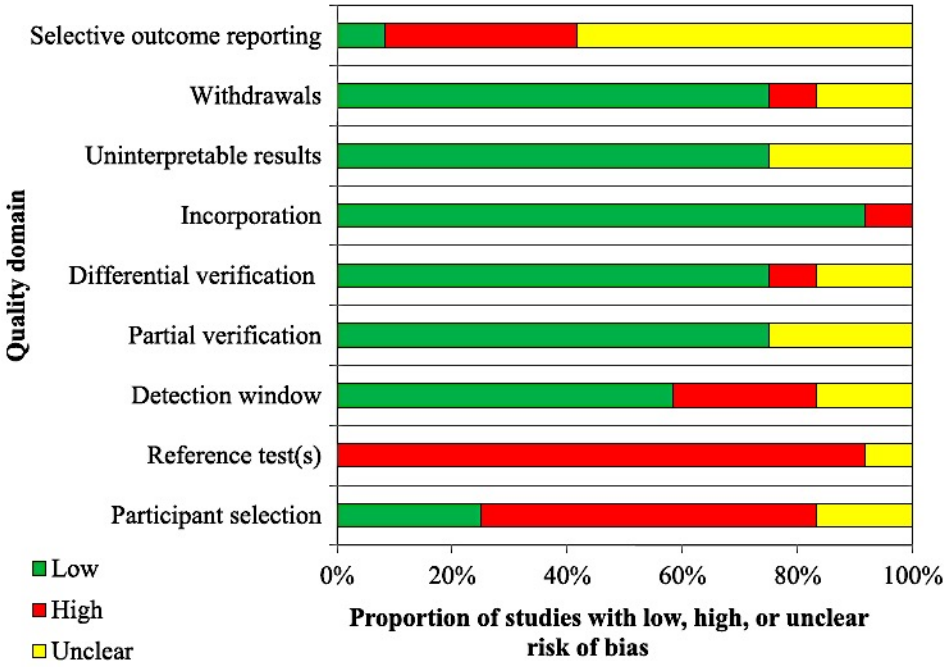


*Figure 6: Methodological quality ratings for each domain represented as a percentage across all included studies.*




All but one of the included studies had a high risk of bias for the reference standard domain due to the use of a self-report reference standard.[168-174,176-179] Self-report is known to be an imperfect reference standard for reasons previously described. The remaining study[175] used meconium EtG as the reference standard. This study was considered to have an unclear risk of bias as the validity of meconium EtG has not been established and there is a lack of agreement about the optimal positivity threshold for this biomarker.[180-182] Seven studies had a high risk of bias in the participant selection domain due to the use of diagnostic case-control designs,[168,169,172,173,176,178,179] which may inflate diagnostic accuracy estimates.[149,183,184] Nine studies had a low risk of bias for uninterpretable results,[168,170,173-179] withdrawals,[168,172-179] differential verification,[168,169,171-177] and partial verification.[168,171-177,179] One study had a high risk of incorporation bias, as

EtG was used to indicate alcohol exposure in both the reference standard and index test.[175]

Seven studies had a low risk of bias for the detection window domain.[168,169,173,175,177-179] Of these studies, two reported data for multiple index tests both with and without an appropriate window of detection.[168,178] To reduce the risk of bias, I excluded data from one study that looked at the agreement between self-reported alcohol use in the first trimester and meconium testing,[178] as meconium does not begin to accumulate until the second and third trimesters.[134] I also excluded data from another study that compared self-reported PAE during the second trimester with postnatal tests of maternal EtG, EtS, GGT, CDT and PEth in dried infant blood spots due to the short detection window of these biomarkers.[168] Of the remaining studies, two had an unclear risk of bias and three had a high risk of bias. Two of these studies[170,176] were deemed to have a high risk of bias as the accuracy of meconium testing was verified against alcohol use across the whole of pregnancy, including the first trimester before meconium is generated. It was not possible to exclude first trimester data from my analysis due to the way results were reported. One study,[172] which collected maternal hair during pregnancy for EtG testing, was also considered to have a high risk of bias as the specimen may have captured alcohol use prior to pregnancy due to the broad detection window of EtG within this matrix.

Finally, selective outcome reporting introduced a high risk of bias in four studies.[169,176,178,179] Of these studies, three measured multiple FAEEs in meconium but only reported the diagnostic accuracy outcomes for a subset of these

FAEEs.[169,176,178] Two studies[171,178] did not provide sufficient data to enable the calculation of missing true positive, true negative, false positive and false negative values. For these studies, I used a R simulation to produce data that replicated the sensitivity, sensitivity and predictive values reported in one of the studies,[171] and to generate values that approximated the published data in another study.[178] For the remaining study of meconium FAEEs,[179] the positive predictive value reported in the study did not match the value suggested by the raw data (see Table 3 for further details). The study authors were unable to provide data to further explore this discrepancy. Finally, I was not able to replicate the published sensitivity and specificity values based on the true positive, true negative, false positive, and false negative values presented in one study of maternal hair testing.[175] Following correspondence with the authors the correct sensitivity and specificity values were derived based on the raw values presented in the paper (see Table 6).

## 5.4 Diagnostic test accuracy results

Tables 3 to 8 present diagnostic accuracy outcomes with 95% confidence intervals.

### 5.4.1 Meconium testing

Meconium testing for FAEEs was the most commonly investigated index test, featuring in seven studies.[168-170,173,176,178,179] The diagnostic accuracy of FAEEs varied widely across studies (see Table 3). A measure of the total concentration of four FAEEs showed the highest levels of diagnostic accuracy overall, but there were a high number of false positives in one study[168] and specificity was inconsistent.[168,179]

### 5.4.2 Placenta testing

One study measured FAEEs in the placenta tissue of a sample of premature deliveries.[171] Sensitivity and specificity values were high, although 30% to 56% of positive test results were false positives (see Table 4).

### 5.4.3 Blood testing

Four studies investigated CDT, GGT, Hb-Ach, MCV and PEth[168,172,174,177] within prenatal samples of maternal blood. Blood biomarkers generally demonstrated high specificity but low sensitivity (see Table 5). Likelihood ratios suggested that the accuracy of PEth testing improved as PAE increased from low to moderate to heavy. However, findings of high levels of sensitivity (100%) and specificity (96%) in one study of heavy PAE[174] were not replicated in two other studies in which sensitivity ranged from 18% to 22%.[168,172]

### 5.4.4 Hair testing

Maternal hair was tested for EtG in two studies,[172,175] with contrasting results (see Table 6). Neither of the studies demonstrated high levels of both sensitivity and specificity.

### 5.4.5 Urine testing

Two studies of maternal urine testing found that measures of EtS and EtG had low sensitivity but high specificity[168,172] (see Table 7).

### 5.4.6 Test batteries

Three studies investigated the diagnostic accuracy of test batteries, which included combinations of different biomarkers across several matrices.[168,172,177] Sensitivity was poor, while specificity was generally good (see Table 8).

*Table 3 (continued overleaf): Diagnostic accuracy outcomes for studies of meconium testing for prenatal alcohol exposure (PAE).*

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ethyl arachidonate** | Bearer 2005[178(a,b)] | 3rd | GC/FID | 306 ng/g | 88 (55-98) | 63 (56-70) | 9 (6-21) | 99 (95-100) | 2.3 (1.7-3.1) | 0.2 (0.1-0.9) |
| | Ostrea 2006[170] | Any | GC/MS | 902 ng/g | 18 (11-28) | 97 (83-100) | 94 (73-100) | 28 (20-38) | 5.7 (0.8-40.9) | 0.8 (0.8-1.0) |
| **Ethyl docosahexanoate** | Ostrea 2006[170] | Any | GC/MS | 1000 ng/g | 4 (1-11) | 100 (89-100) | 100 (40-100) | 26 (18-35) | ∞ (0.1-∞) | 1.0 (0.9-1.0) |
| **Ethyl laurate** | Ostrea 2006[170] | Any | GC/MS | 50 ng/g | 19 (12-29) | 81 (63-93) | 75 (53-90) | 25 (17-35) | 1.0 (0.4-2.3) | 1.0 (0.8-1.2) |
| **Ethyl linoleate** | Bearer 2005[178(a,b)] | 2nd | GC/FID | 383 ng/g | 89 (64-100) | 58 (51-65) | 9 (6-20) | 99 (95-100) | 2.2 (1.8-2.7) | 0.1 (0.0-0.9) |
| | Bearer 1999[169(b)] | 3rd | GC/FID | 1 pmol/g | 68 (54-80) | 51 (40-62) | 47 (36-58) | 71 (60-82) | 1.4 (1.1-1.8) | 0.6 (0.4-1.0) |
| | Ostrea 2006[170] | Any | GC/MS | 250 ng/g | 27 (18-37) | 97 (83-100) | 96 (80-100) | 31 (22-41) | 8.3 (1.2-59.0) | 0.8 (0.7-0.9) |
| **Ethyl linolenate** | Ostrea 2006[170] | Any | GC/MS | 100 ng/g | 3 (1-9) | 100 (89-100) | 100 (29-100) | 26 (18-34) | ∞ (0.1-∞) | 1.0 (0.9-1.0) |
| **Ethyl myristate** | Ostrea 2006[170] | Any | GC/MS | 50 ng/g | 68 (57-77) | 29 (14-48) | 74 (63-83) | 23 (11-39) | 1.0 (0.7-1.2) | 1.1 (0.6-2.1) |

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ethyl oleate** | Bearer 2005[178(a,b)] | 3rd | GC/FID | 445 ng/g | 80 (46-95) | 58 (51-65) | 5 (5-18) | 99 (93-100) | 1.8 (1.3-2.6) | 0.4 (0.2-1.1) |
| | Bearer 2003[176] | Any | GC/MS/MS | 32 ng/g | 84 (60-97) | 83 (36-100) | 94 (71-100) | 63 (24-91) | 5.1 (0.8-30.6) | 0.2 (0.1-0.6) |
| | Bakhireva 2014[168] | Any | GC/MS | 50 ng/g | 86 (67-96) | 25 (11-43) | 50 (35-65) | 67 (35-90) | 1.1 (0.9-1.5) | 0.6 (0.2-1.7) |
| | Ostrea 2006[170] | Any | GC/MS | 50 ng/g | 49 (39-60) | 58 (39-75) | 78 (65-87) | 28(17-40) | 1.2 (0.7-1.9) | 0.9 (0.6-1.2) |
| **Ethyl palmitate** | Ostrea 2006[170] | Any | GC/MS | 50 ng/g | 58 (47-68) | 42 (25-61) | 75 (63-84) | 25 (14-39) | 1.0 (0.7-1.4) | 1.0 (0.6-1.6) |
| **Ethyl stearate** | Ostrea 2006[170] | Any | GC/MS | 100 ng/g | 19 (12-29) | 87 (70-96) | 82 (60-95) | 26 (18-36) | 1.5 (0.5-4.1) | 0.9 (0.8-1.1) |
| **Total concentration of 4 FAEEs[c]** | Bakhireva 2014[168] | Any | GC/MS | 600 ng/g | 100 (88-100) | 13 (4-29) | 50 (36-64) | 100 (40-100) | 1.1 (1.0-1.3) | 0.0 (0.0-2.5) |
| | Chan 2003[179(d)] | Any | GC/FID | 600 ng/g | 100 (54-100) | 98 (93-100) | 63 (24-91) | 100 (95-100) | 73.0 (10.4-511.3) | 0.0 (0.0-1.1) |
| **Total concentration of 6 FAEEs[e]** | Bakhireva 2014[168] | Any | GC/MS | 10,000 ng/g | 86 (67-96) | 13 (4-29) | 46 (32-61) | 50 (16-84) | 1.0 (0.8-1.2) | 1.1 (0.3-4.2) |
| | Bakhireva 2014[168] | Any | GC/MS | 100,000 ng/g | 29 (13-49) | 81 (64-93) | 57 (29-82) | 57 (41-71) | 1.5 (0.6-3.9) | 0.9 (0.7-1.2) |

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Total concentration of 9 FAEEs**[f] | Kwak 2014a[173] | 2nd or 3rd | LC/MS/MS | 20 nmol/g | 4 (0-13) | 98 (95-100) | 41 (5-85) | 78 (72-83) | 2.2 (0.4-13.1) | 1.0 (0.9-1.0) |

Abbreviations: CI, confidence interval; FAEEs, fatty acid ethyl esters; GC/FID, gas chromatography with flame ionization detection; GC/MS, gas chromatography mass spectrometry; GC/MS/MS, gas chromatography tandem mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; sens, sensitivity; spec, specificity.

[a] Confidence intervals are an approximation, due to missing data, based on a simulation of all possible 2 x 2 table in R software assuming N = 224 and 13 individuals with the target condition (cases). The summary statistics reported in this table are those from the original study. The corresponding values suggested by the R simulation were sensitivity 85%, specificity 63%, PPV 12% and NPV 99% for ethyl arachidonate; sensitivity 92%, specificity 58%, PPV 12% and NPV 99% for ethyl linoleate; sensitivity 77%, specificity 58%, PPV 10% and NPV 98% for ethyl oleate.

[b] Bearer 1999 and Bearer 2005 include the same participants.

[c] Ethyl palmitate, ethyl stearate, ethyl oleate, ethyl linoleate.

[d] The PPV presented in this table (63%) for Chan 2003 is the value reported in the primary study. However, my calculations produce a PPV of 87% based on the reported number of participants included in the sensitivity and specificity analyses (79 total, including 6 cases) and reported values of 100% specificity and 98% sensitivity. The authors were unable to provide the original data to further explore this discrepancy.

[e] Ethyl palmitate, ethyl palmitoleate, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl arachidonate.

[f] Ethyl palmitate, ethyl palmitoleate, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl linolenate, ethyl arachidonate, ethyl laurate, ethyl myristate.

*Table 4: Diagnostic accuracy outcomes for one study of placenta testing for prenatal alcohol exposure (PAE)*

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ethyl stearate** | Gauthier 2015[171] | Any | GC/MS | NR | 82 (48-98) | 87 (77-94) | 50 (26-74) | 97 (88-100) | 6.3 (3.2-12.3) | 0.2 (0.1-0.7) |
| **Ethyl linoleate** | Gauthier 2015[171] | Any | GC/MS | NR | 82 (48-98) | 83 (72-91) | 44 (22-66) | 97 (88-100) | 4.7 (2.6-8.4) | 0.2 (0.1-0.8) |
| **Total concentration of 3 FAEEs[a]** | Gauthier 2015[171] | Any | GC/MS | NR | 82 (48-98) | 94 (86-98) | 70 (39-91) | 97 (90-100) | 14.1 (5.2-38.0) | 0.2 (0.1-0.7) |
| **Total concentration of 4 FAEEs[b]** | Gauthier 2015[171] | Any | GC/MS | NR | 82 (48-98) | 93 (84-98) | 64 (35-87) | 97 (89-100) | 11.3 (4.6-27.5) | 0.2 (0.1-0.7) |

Abbreviations: CI, confidence interval; FAEEs, fatty acid ethyl esters; GC/MS, gas chromatography mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; NR, not reported; PPV, positive predictive value; sens, sensitivity; spec, specificity.

[a] Ethyl oleate, ethyl linoleate and ethyl linolenate.

[b] Ethyl oleate, ethyl linoleate, ethyl linolenate and ethyl stearate.

*Table 5 (continued overleaf): Diagnostic accuracy outcomes for studies of blood testing for prenatal alcohol exposure (PAE).*

| Index test analyte | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **%CDT[a]** | Bakhireva 2014[168] | Any | HPLC | 2% | 4 (0-18) | 100 (89-100) | 100 (3-100) | 54 (41-67) | ∞ (0.1-∞) | 1.0 (0.9-1.0) |
| | Gutierrez 2015[172] | Any | HPLC | 2% | 5 (1- 17) | 100 (92-100) | 100 (16-100) | 53 (41-64) | ∞ (0.2-∞) | 1.0 (0.9-1.0) |
| | Sarkola 2000[177] | Any | Immuno-assay | NR | 15 (2-45) | 87 (70-96) | 30 (4-78) | 71 (54-85) | 1.2 (0.2-5.7) | 1.0 (0.7-1.3) |
| **Total CDT** | Sarkola 2000[177] | Any | Immuno-assay | NR | 8 (0-36) | 94 (79-99) | 33 (1-91) | 71 (54-84) | 1.2 (0.1-12.0) | 1.0 (0.8-1.2) |
| **GGT** | Bakhireva 2014[168] | Any | Enzymatic rate method | 40 U/L | 15 (4-33) | 100 (89-100) | 100 (40-100) | 57 (43-70) | ∞ (0.5-∞) | 0.9 (0.7-1.0) |
| | Gutierrez 2015[172] | Any | Enzymatic rate method | 40 U/L | 20 (9-36) | 98 (87-100) | 89 (52-100) | 56 (44-68) | 8.4 (1.1-64.2) | 0.8 (0.7-1.0) |
| | Sarkola 2000[177] | Any | Immuno-assay | NR | 31 (9-61) | 79 (59-92) | 40 (12-74) | 71 (52-86) | 1.4 (0.5-4.2) | 0.9 (0.6-1.3) |
| **Hb-Ach** | Sarkola 2000[177] | Any | Immuno-enzymatic method | NR | 0 (0-25) | 97 (83-100) | 0 (0-98) | 70 (54-83) | 0.0 (0.0-33.4) | 1.0 (1.0-1.1) |
| **MCV** | Sarkola 2000[177] | Any | Coulter C7 cell counter | NR | 15 (2-45) | 100 (89-100) | 100 (16-100) | 74 (58-86) | ∞ (0.5-∞) | 0.8 (0.7-1.1) |

| Index test analyte | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **PEth** | Bakhireva 2014[168] | Any | LC/MS/MS | 8 ng/mL | 22 (8-41) | 100 (89-100) | 100 (54-100) | 60 (45-72) | ∞ (0.8-∞) | 0.8 (0.6-1.0) |
| | Gutierrez 2015[172] | Any | LC/MS/MS | 8 ng/mL | 18 (7-33) | 100 (92-100) | 100 (59-100) | 57 (45-68) | ∞ (0.9-∞) | 0.8 (0.7-1.0) |
| | Kwak 2014b[173] | 1st | LC/MS/MS | 4.2 nmol/L[b] | 100 (63-100) | 96 (93-98) | 43 (20-67) | 100 (99-100) | 27.0 (15.1-48.2) | 0.0 (0.0-0.9) |
| | Kwak 2014b[173] | 1st | LC/MS/MS | 3.8 nmol/L[b] | 67 (47-83) | 96 (93-98) | 67 (47-83) | 96 (93-98) | 18.3 (9.5-35.4) | 0.3 (0.2-0.6) |
| | Kwak 2014b[173] | 1st | LC/MS/MS | 1.2 nmol/L[b] | 41 (32-50) | 95 (91-98) | 85 (72-93) | 72 (66-78) | 8.6 (4.4-16.8) | 0.6 (0.5-0.7) |

Abbreviations: CDT, carbohydrate deficient transferrin; CI, confidence interval; GGT, gamma glutamyltransferase; Hb-Ach, haemoglobin acetaldehyde adducts; HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography tandem mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MCV, mean corpuscular volume; NPV, negative predictive value; NR, not reported; PEth, phosphatidylethanol; PPV, positive predictive value; sens, sensitivity; spec, specificity.

[a] Percentage of CDT in total transferrin.

[b] A threshold of 4.2 nmol/L was specified for the detection of heavy PAE, 3.8 nmol/L for moderate PAE and 1.2 nmol/L for low PAE.

*Table 6: Diagnostic accuracy outcomes for studies of hair testing for prenatal alcohol exposure (PAE)*

| Index test analyte | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **EtG** | Gutierrez 2015[172] | Any | LC/MS/MS | 8 pg/mg | 19 (9-34) | 86 (72-95) | 57 (29-82) | 52 (40-64) | 1.4 (0.5-3.6) | 0.9 (0.8-1.1) |
| | Joya 2016[175(a)] | 2nd or 3rd | UPLC | 11 pg/mg | 87 (69-96) | 56 (41-70) | 54 (39-69) | 88 (71-96) | 2.0 (1.4-2.8) | 0.2 (0.1-0.6) |

Abbreviations: CI, confidence interval; EtG, ethyl glucuronide; LC/MS/MS, liquid chromatography tandem mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; UPLC, ultra-high performance liquid chromatography-tandem mass spectrometry; sens, sensitivity; spec, specificity.

[a] The diagnostic accuracy outcomes presented in the table are for 2nd and 3rd trimester alcohol exposure and were derived from the raw values presented in the original paper, rather than the published sensitivity and specificity values. I am grateful to the authors for clarifying the data. Corresponding values for 3rd trimester alcohol exposure were sensitivity 77% and specificity 72%.

*Table 7: Diagnostic accuracy outcomes for studies of maternal urine testing for prenatal alcohol exposure (PAE)*

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold | Sens % (95% CI) | Spec % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **EtG** | Bakhireva 2014[168] | Any | LC/MS/MS | 25 ng/mL | 15 (4-33) | 97 (84-100) | 81 (28-99) | 57 (42-70) | 4.6 (0.5-38.5) | 0.9 (0.8-1.0) |
| | Gutierrez 2015[172] | Any | LC/MS/MS | 39 ng/mL | 5 (1-17) | 98 (87-100) | 67 (9-99) | 51 (40-63) | 2.0 (0.2-21.7) | 1.0 (0.9-1.1) |
| **EtS** | Bakhireva 2014[168] | Any | LC/MS/MS | 7 ng/mL | 15 (4-33) | 100 (89-100) | 100 (40-100) | 57 (43-70) | ∞ (0.5-∞) | 0.9 (0.7-1.0) |
| | Gutierrez 2015[172] | Any | LC/MS/MS | 7 ng/mL | 7 (2-20) | 98 (87-100) | 75 (19-99) | 52 (40-63) | 3.1 (0.3-28.3) | 0.9 (0.9-1.0) |

Abbreviations: CI, confidence interval; EtG, ethyl glucuronide; EtS, ethyl sulphate; LC/MS/MS liquid chromatography tandem mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; sens, sensitivity; spec, specificity.

*Table 8 (continued overleaf): Diagnostic accuracy outcomes for studies of objective test batteries of maternal biomarkers for prenatal alcohol exposure (PAE).*

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold[a] | Sens % (95% CI) | Spec% (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blood GGT, %CDT and PEth, urine EtG and EtS** | Bakhireva 2014[168] | Any | Enzymatic rate method, HPLC, LC/MS/MS | GGT 40 U/L<br>CDT 2%<br>PEth 8 ng/mL<br>EtG 25ng/mL<br>EtS 7 ng/mL | 32 (16-52) | 97 (84-100) | 90 (56-100) | 62 (47-75) | 10.3 (1.4-76.2) | 0.7 (0.5-0.9) |
| **Hair EtG and urine EtG** | Gutierrez 2015[172] | Any | LC/MS/MS | EtG 8 pg/mg<br>EtG 38.7 ng/mL | 22 (11-38) | 83 (69-93) | 56 (30-80) | 52 (40-65) | 1.3 (0.5-3.2) | 0.9 (0.8-1.2) |
| **Hair EtG and urine EtS** | Gutierrez 2015[172] | Any | LC/MS/MS | EtG 8 pg/mg<br>EtS 7.2 ng/mL | 24 (12-40) | 83 (69-93) | 59 (33-82) | 53 (40-65) | 1.5 (0.6-3.5) | 0.9 (0.7-1.1) |
| **Hair EtG and blood GGT** | Gutierrez 2015[172] | Any | LC/MS/MS, enzymatic rate method | EtG 8 pg/mg<br>GGT 40 U/L | 33 (19-49) | 83 (69-93) | 65 (41-85) | 56 (43-69) | 2.0 (0.9-4.4) | 0.8 (0.6-1.0) |
| **Hair EtG and blood 2% CDT** | Gutierrez 2015[172] | Any | LC/MS/MS, HPLC | EtG 8 pg/mg<br>CDT 2% | 23 (11-38) | 86 (71-95) | 60(32-84) | 54 (41-66) | 1.6 (0.6-4.0) | 0.9 (0.7-1.1) |
| **Hair EtG and blood PEth** | Gutierrez 2015[172] | Any | LC/MS/MS | EtG 8 pg/mg<br>PEth 8 ng/mL | 28 (15-44) | 86 (72-95) | 65 (38-86) | 56 (43-68) | 2.0 (0.8-4.8) | 0.8 (0.7-1.1) |
| **Hair EtG and blood CDT and GGT** | Gutierrez 2015[172] | Any | LC/MS/MS, HPLC, enzymatic rate method | EtG 8 pg/mg<br>CDT 1.7%<br>GGT 40 U/L | 50 (34-66) | 56 (41-72) | 53 (36-69) | 54 (39-70) | 1.2 (0.7-1.9) | 0.9 (0.6-1.3) |

| Index test analyte(s) | Study | Trimester of PAE | Assay | Positivity threshold[a] | Sens % (95% CI) | Spec% (95% CI) | PPV % (95% CI) | NPV % (95% CI) | LR+ (95% CI) | LR- (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hair EtG and blood GGT and PEth** | Gutierrez 2015[172] | Any | LC/MS/MS, enzymatic rate method | EtG 8 pg/mg GGT 40 U/L PEth 8 ng/mL | 38 (23-54) | 83 (69-93) | 68 (45-86) | 58 (45-71) | 2.3 (1.0-4.9) | 0.8 (0.6-1.0) |
| **Hair EtG and blood PEth and % CDT** | Gutierrez 2015[172] | Any | LC/MS/MS, HPLC | EtG 8 pg/mg PEth 8 ng/Ml CDT 1.7% | 43 (27-59) | 60 (43-74) | 50 (32-68) | 52 (37-67) | 1.1 (0.6-1.8) | 1.0 (0.7-1.4) |
| **Blood MCV and GGT** | Sarkola 2000[177] | Any | Coulter C7 cell counter and immunoassay | NR | 38 (14-68) | 79 (59-92) | 45 (17-77) | 73 (54-88) | 1.8 (0.7-4.8) | 0.8 (0.5-1.3) |

Abbreviations: CDT, carbohydrate deficient transferrin; CI, confidence interval; EtG, ethyl glucuronide; EtS, ethyl sulphate; GGT, gamma glutamyltransferase; HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography tandem mass spectrometry; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MCV, mean corpuscular volume; NPV, negative predictive value; PEth, phosphatidylethanol; PPV, positive predictive value; sens, sensitivity; spec, specificity.

[a] A test battery was considered positive if at least one of the results for an individual biomarker exceeded the designated positivity threshold.

# 6 Discussion

This systematic review demonstrated that the reported accuracy of biomarkers of PAE varied widely across studies. Overall, tests of the total concentration of four FAEEs (ethyl palmitate, ethyl stearate, ethyl oleate and ethyl linoleate) showed the highest levels of sensitivity. Sensitivity was 100% in two studies of meconium testing,[168,179] and 82% in one study of placenta testing.[171] However, specificity was inconsistent (13% to 98%). Positive likelihood ratios ranged from 1, suggesting little test utility, to 73. As a guide, positive likelihood ratios greater than 10 may indicate that a test is informative.[159] Confidence intervals were wide due to a small number of cases in these studies, which led to imprecise estimates of diagnostic accuracy. Placenta testing for FAEEs was conducted with a sample of premature newborns.[171] It is important to note that because alcohol is a risk factor for prematurity,[185] the prevalence of PAE is likely to be higher within this sample than in the general population. Accordingly, positive predictive values from this study are likely to be higher than what would be expected within routine antenatal care.[162]

There is no consensus on what level of diagnostic accuracy is acceptable for objective measurement of PAE. Many screening initiatives prioritise sensitivity over specificity and thus permit a high number of false positive results to maximise early detection of asymptomatic conditions.[186] As early diagnosis and intervention are associated with improved outcomes for children with FASD[65] it could be argued that a test with high sensitivity could be favoured over specificity, as it may support appropriate monitoring and follow-up among children with suspected PAE. However, there are ethical considerations. Prenatal alcohol use is an emotive issue

and false positive errors may lead to stigmatisation, unnecessary burden on healthcare resources, and may even be used in legal proceedings against mothers.[113,127,140,186,187] Conversely, false negative errors represent a missed opportunity to provide support to those affected by PAE.[127] Many screening programmes are conducted in a tiered fashion with high sensitivity favoured over specificity in the initial phase. Some authors have suggested that second-tier screening, using methods such as comprehensive maternal interviews and clinical assessment of children with suspected PAE, may be used as a strategy to reduce false positive results.[127] However, these methods also have limitations. Information from maternal interview may be inaccurate, and the cognitive-behavioural profile associated with PAE is diverse and may not be detectable until later in childhood, thus precluding the opportunity for early intervention. Given the implications of both types of test error, various authors have suggested that both high sensitivity and specificity are a prerequisite for the introduction of PAE screening.[127,186,187]

## 6.1 Limitations of the evidence

The methodological quality of studies included in this review was generally poor. There was a high risk of bias due to the use of imperfect self-report reference standards, case-control diagnostic designs, and selective outcome reporting. Therefore, it is unclear whether the low diagnostic accuracy values of the objective measures in this review were due to a true lack of test validity, or were simply an artefact of comparison with an imperfect reference standard. As self-report reference standards are known to underestimate true PAE it is possible that the biomarker index tests explored in this review did correctly detect true PAE, while the self-report reference standards did not. This lack of agreement in classification

would lead to false positive results and reduced specificity. However, it is also possible that the observed false positives were genuine. Incidental exposure to ethanol can occur through an individual's diet, medications, mouthwash and hand sanitizer, although the extent to which incidental exposure influences biomarker tests for PAE has not been fully established.[188-192] It is unlikely that false negative results are due to the use of an imperfect reference standard as mothers are unlikely to report that they drank alcohol while pregnant when they had not.[123] Therefore, the low sensitivity values demonstrated by many studies in this review may be considered the most persuasive evidence against the validity of current objective measures of PAE. Some authors have proposed that apparent false negative errors may occur because self-report measures are better able to detect low levels of alcohol use than many of the objective measures, which typically detect moderate to heavy consumption. This raises the possibility that pregnant women who report drinking modest amounts of alcohol could be detected by the self-report reference standard but not the index test.[124] This explanation is not likely to account for findings in the present review, however, as the majority of included studies investigated moderate to heavy self-reported PAE.

Case-control diagnostic designs may produce overestimates of diagnostic accuracy.[149,184] However, this form of bias is not likely to influence the conclusions of this review as most studies did not show high levels of accuracy despite the use of case-control diagnostic designs. It is, however, important to note that PAE prevalence is fixed by design in many of the studies included in this review due to the use of case-control methods and it is not possible to generalise predictive

values from individual studies to settings with a different prevalence of PAE.[158,159,162]

## 6.2 Limitations of the review

This study is the first systematic review of its kind and provides a rigorous evaluation of the evidence relating to the diagnostic accuracy of a range of objective measures of PAE. The results are broadly consistent with findings from existing non-systematic reviews, which suggest the need for improved objective measures of PAE.[117,130,134] However, due to the diverse nature of the data and a limited number of studies per index test it was not possible to address some of the objectives listed in the original protocol. For example, I was not able to conduct the intended meta-analyses to answer questions about the relative impact of study characteristics on test accuracy. The search strategy was comprehensive and covered a range of published and unpublished sources. It is possible that some studies were missed as a result of excluding non-English language publications. However, this is unlikely to have had a significant impact on the results, since the supplementary search that I conducted included other reviews that did consider publications in any language and these did not reveal any further relevant studies.[124]

As previously noted, participants were mainly recruited from high risk settings, such as substance misuse clinics. Therefore, findings have limited applicability to general population samples. Population-based studies of biomarkers of PAE are needed to inform universal screening strategies and to clarify the epidemiology of PAE and its developmental consequences in the short- and long-term.

## 6.3 Implications for research and practice

Prenatal alcohol use is a challenging and emotive issue. Consequently, tests to detect PAE must be accurate, feasible, and acceptable to the population. These criteria are emphasised in the World Health Organisation and UK National Screening Committee guidelines for screening procedures.[193,194] This review demonstrates that the evidence base for the accuracy of current objective measures of PAE is not yet robust enough to support their use in routine care. Studies of meconium screening for PAE in Scotland[195] and Canada[196] suggest high consent rates under conditions of anonymity (95% to 99%). However, participation rates are significantly lower when screening is not anonymised (78%).[196] More research is required to establish which method is most feasible and acceptable to stakeholders including clinicians, policy makers and families.[140,186,196,197]

Assay methods for the biomarkers included in this review were highly variable. This is likely to account for some of the observed heterogeneity in findings. Future work that aims to standardise procedures may provide a clearer picture of the performance of different biomarkers for PAE. Furthermore, positivity thresholds must be validated with general population samples. The 600 ng/g (2 nmol/g) cut-off for total concentration of four FAEEs was derived from a study that compared abstainers to women with alcoholism and, therefore, may not be suitable for determining PAE in the general population.[179]

With the exception of hair testing, objective measures of PAE have a limited detection period, which does not span the whole of pregnancy (see Table 1). For many women, patterns of alcohol consumption change throughout pregnancy.

Women are most likely to drink in the first trimester and then reduce their intake or abstain in later trimesters.[12] Risk of harm to the developing fetus is highest if a mother drinks heavily throughout pregnancy,[19] however first trimester exposure poses a particular risk of physical abnormalities including dysmorphic facial features.[198] Meconium testing only captures PAE late in the second and third trimesters of pregnancy and, therefore, may fail to identify a large proportion of babies at risk of alcohol-related harm.[134] In addition, currently available biomarkers have insufficient sensitivity to detect low levels of PAE, which is the most prevalent pattern of consumption among pregnant women.[12] An objective test which measures alcohol itself in breath, urine, or blood could detect low level use. However, because alcohol is only present in these matrices for a matter of hours, this form of test is difficult to implement in research or in practice.[117,130] Due to the limitations of current biomarkers, authors have emphasised the need for novel biomarkers that can detect even low levels of alcohol use across the duration of pregnancy.[117]

Given the absence of a gold standard test, research attempting to validate objective measures of PAE may benefit from abandoning the classic diagnostic accuracy paradigm, in which validity is determined by agreement between the index test and reference standard. Instead, future research may benefit from using a clinical validation approach,[199] in which a convergent body of evidence is used to increase confidence in the validity of a measure. Some studies have adopted this method to demonstrate the predictive validity of meconium testing. Prospective studies have reported a significant inverse relationship between levels of FAEEs in meconium and cognitive outcomes up to age 15.[200,201] Such evidence lends support

to the validity of FAEEs as markers of PAE, but require replication. Animal models may also be useful for the development of novel testing procedures for PAE.[117,202,203] However, translation from animal studies to human populations is complicated by differences in alcohol exposure methods, gestation and alcohol metabolism. In summary, validation will rely on an ongoing body of research that produces convergent evidence to suggest that objective measures are meaningfully associated with PAE.[199]

# 7 Conclusions

Tests of the total concentration of FAEEs in meconium and placenta tissue offer some promise as objective measures of PAE but findings are inconsistent, studies are small-scale and require replication. Therefore, I conclude that current evidence is insufficient to support the use of objective measures of PAE in clinical practice. The poor performance of many of the measures evaluated in this review could be due to a true lack of diagnostic validity or a result of bias introduced by sub-optimal study design, most notably the absence of a gold standard for PAE. Further research that investigates test validity, acceptability, and feasibility within large population-based samples is required to inform strategies for population-based screening and epidemiological research.

# 8 Implications for this thesis

One of the reasons for completing this review was to determine whether there were suitable objective measures that I could use to investigate the epidemiology of PAE for this thesis. Since the evidence base does not support the validity of

current objective measures of PAE, I decided not to pursue this avenue further. Instead, I decided to investigate the epidemiology of one of the consequences of PAE; fetal alcohol spectrum disorder (FASD). The remaining chapters in this thesis will describe the development of novel case ascertainment algorithms for FASD (Chapter 3), a study of the prevalence of FASD in a population-based sample in England (Chapter 4), and studies that seek to elucidate causal risk factors for FASD (Chapters 5 and 6).

# Chapter 3. Development and validation of case ascertainment algorithms for FASD

## 1 Overview

In this chapter, I describe how I developed and validated a series of novel case ascertainment algorithms for FASD. First, I provide a summary of current diagnostic frameworks for FASD and describe the chapter aims and objectives (Sections 2 and 3). In Section 4, I describe the development of the case ascertainment algorithms. In Section 5, I describe the algorithm validation stage. I conclude with a general discussion of the strengths and limitations of my approach and the implications of this work for the remaining research in this thesis.

## 2 Background

### 2.1 Rationale for development of a FASD case ascertainment algorithm

As described in Chapter 1, efforts to investigate the epidemiology of FASD in the UK have been compromised by suspected underreporting, due to reliance on surveillance methods. Funding and ethical issues have meant that active case ascertainment studies of FASD in the general population have not been possible.[108] To overcome these challenges, I explored a novel approach, using data from an existing population-based birth cohort. I sought to determine if it was possible to develop and apply an algorithm, based on an existing diagnostic framework for FASD, to the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort data to ascertain FASD cases. If feasible, this would offer an opportunity to investigate FASD epidemiology at the population level. Furthermore, this design minimises the

cost and ethical concerns surrounding an active-case ascertainment study of FASD, as participants have already provided consent, and the anonymised data have been used in a range of research studies, including studies of neurodevelopmental outcomes.[204]

## 2.2 Diagnostic frameworks for FASD

There is no universally accepted diagnostic framework for assessing fetal alcohol spectrum disorder (FASD). Although there is broad consensus on FASD subtypes and the core features (summarised in Chapter 1), diagnostic frameworks differ in the specific criteria and thresholds used to define FASD. Differences also exist in the nomenclature used to describe subcategories within the FASD continuum. Differing criteria lead to variations in case ascertainment and subsequent prevalence estimates.[78,205-207] A brief description of the main frameworks that are currently in use is given below. Appendix 3 provides a full summary and comparison of the different criteria and diagnostic categories for the main FASD classification systems.

The first formal guidelines for FASD diagnosis were introduced by the Institute of Medicine (IOM) in 1996, following a mandate from the U.S. Congress.[208] The IOM criteria formed the basis of all subsequent FASD frameworks, but have been criticised for not providing clear thresholds for what constitutes abnormality in each domain. For example, they did not specify the degree of growth deficiency or cognitive impairment required to meet criteria. Consequently, the original IOM guidelines are not generally used in present-day assessment of FASD.[73]

Hoyme and colleagues produced the first revision of IOM guidelines in 2005 in response to criticism about the lack of clinical utility of the original IOM guidelines. The IOM 2005 criteria retained the original IOM diagnostic categories, but added standardised cut-offs for central nervous system (CNS), growth and facial criteria. The IOM 2005 criteria have been used widely within studies of FASD epidemiology and were revised again in 2016, based on expert consensus.[74,82,83,209]

The FASD 4-Digit Diagnostic Code was developed by Astley and colleagues based on empirical studies at the University of Washington. Versions of the 4-Digit Code were published in 1997, 1999 and 2004. It is used in specialised FASD clinics as part of the Centers for Disease Control and Prevention's (CDC's) funded Washington Diagnostic and Prevention Network, which has been running for over 20 years.[67,75]

The FASD Canadian guidelines for diagnosis were first published in 2005 with the intention of providing a unified approach to FASD identification. Within the Canadian guidelines, a slight adaptation of the 4-Digit Code criteria was mapped on to FASD diagnostic categories, using the IOM nomenclature (with the exception of ARBD, which was removed).[72] The Canadian guidelines were revised in 2016. Notable changes included the introduction of a threshold for the PAE criterion and new terminology for the FASD subtypes (described further in Appendix 3).[210,211]

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for Neurodevelopmental Disorder-Prenatal Alcohol Exposure (ND-PAE)[212] is the newest addition to the range of FASD frameworks that are available. ND-PAE was proposed to describe the full range of developmental disabilities associated with PAE. ND-PAE was placed within the 'conditions for further study' section of

the DSM-5. This means that there was insufficient evidence to include it as a formal mental disorder diagnosis in DSM-5 and it is not intended for use in clinical practice. Nevertheless, the inclusion of ND-PAE in the influential DSM-5 has been heralded as a major step forward in raising the profile of FASD in mainstream diagnosis.[213,214] Furthermore, as DSM is an internationally recognised medical taxonomy, the inclusion of ND-PAE has been viewed by some as progress towards a universal classification of FASD.[215]

Other guidelines include the International Classification of Diseases (ICD-10) and the CDC guidelines (both of which proposed criteria for diagnosing FAS but not for the entire spectrum of FASD, thereby underestimating the true burden of the consequences of PAE).[11,78,107] The criteria of the FASD Study Group of the Research Society on Alcoholism,[216] guidelines by Sokol and Clarren,[217] the FAS Checklist,[218] Smith's Recognizable Patterns of Human Malformations,[219] the Emory Fetal Alcohol Centre Clinical Criteria,[207] and the Two Eyed Seeing Wheel[220] are examples of other guidelines. None of these are widely accepted.[88,221]

### 2.2.1 Justification of the use of the FASD Canadian (2005) guidelines for this study

As described above, there are several diagnostic guidelines available for FASD. The field has undergone much development and, currently, there is no consensus on the optimal framework to use to identify FASD. In the absence of universal criteria for FASD, I had to decide which of the available frameworks was the most appropriate to use to develop an algorithm for FASD case ascertainment, to be applied to data that have already been collected.

I chose to use the FASD Canadian guidelines (2005) as the basis for the FASD case ascertainment algorithms for the following reasons. First, these guidelines are currently the most unified approach to FASD identification (combining the criteria from the FASD 4-Digit Diagnostic Code and IOM revised criteria).

Second, the Canadian 2005 guidelines have been widely adopted across FASD services in Canada,[222] are endorsed by the British Medical Association,[10] the UK FASD Trust,[106] expert consensus guidelines for the identification of attention deficit hyperactivity disorder and associated FASD,[223] and the UK National Clinic for Fetal Alcohol Spectrum Disorder,[224] as the model of best-practice. Evidence suggests that the Canadian guidelines have led to increased consistency and multidisciplinary collaboration within FASD clinics.[222]

Third, the Canadian guidelines have also been used in a research context, having been adopted for a global prevalence study of FASD, supported by the World Health Organisation and National Institute on Alcohol Abuse and Alcoholism.[225] Fourth, compared to the Canadian guidelines, some suggest that the threshold for FASD is too liberal in other guidelines. Although there is no gold standard to determine which of the available FASD frameworks is better for identifying those affected by PAE,[207] studies that have applied the different diagnostic frameworks to the same sample of individuals give an indication of how the choice of framework can influence case ascertainment. These studies found that more individuals were classified as having FASD under the IOM 2005 criteria than the other frameworks.[206,207] For example, 60% of individuals in a study of 1,581 referrals to a FASD clinic in the USA received a FASD diagnosis based on the IOM 2005 criteria,

compared to 38% with the 4-Digit Code, and 25% with the Canadian 2005 guidelines, predominantly due to differences in the facial phenotype and CNS criteria.[207] Compared to other frameworks, the IOM criteria have the most liberal thresholds for head circumference and facial phenotype measures, and they retain ARBD as a diagnostic category. The authors of the IOM guidelines advocate this inclusive approach to FASD diagnosis on the basis that more liberal criteria may lead to opportunities for early detection and intervention for individuals within the broad continuum of FASD.[74] However, critics argue that the relaxed criteria may threaten the validity of the diagnosis.[206] Fifth, the Canadian guidelines (2005) do not specify a threshold for PAE when determining FASD. This approach is the most consistent with current antenatal guidelines, which recommend abstinence from alcohol as the safest option during pregnancy, and with evidence that suggests that there is no known safe level of PAE.[48,226] In contrast, the IOM 2005 criteria offered a qualitative description of the threshold for maternal alcohol exposure, describing it as "a pattern of excessive intake characterized by substantial regular intake or heavy episodic drinking."[(p. 18)73] This description does not specify what quantity of alcohol is considered excessive or heavy and has been called confusing by authors of the Canadian guidelines.[72] In contrast to the Canadian 2005 guidelines, the DSM-5, revised IOM 2016 and Canadian 2016 guidelines all provide a threshold for the alcohol exposure criterion (described further in Appendix 3). However, even the authors of the 2016 revision to the Canadian guidelines, who specify a threshold for their FASD diagnosis, add a note of caution advising that drinking below this limit has not been studied in enough detail to be considered safe; that various factors influence susceptibility to alcohol-related harm; and that the public health

advice should be to avoid alcohol in pregnancy.[211] Therefore, the most appropriate criteria for alcohol exposure domain remains uncertain until there is clearer evidence on the dose-response relationship between PAE and FASD.

The updated Canadian (2016) guidelines have yet to be evaluated.[227] Therefore, I chose to use the Canadian 2005 guidelines for FASD diagnosis as the criteria upon which to develop the FASD case ascertainment algorithms for this study. Figure 7 summarises the diagnostic categories from the Canadian 2005 guidelines, based on the core criteria for FASD. Specific criteria are described in Table 9.

*Figure 7: Summary of core domains and corresponding subtypes of FASD within the Canadian 2005 guidelines for diagnosis*

| | **Fetal alcohol syndrome (FAS)** | **Partial fetal alcohol syndrome (pFAS)** | **Alcohol-related neurodevelopmental disorder (ARND)** |
|---|---|---|---|
| **Prenatal alcohol exposure (PAE)** | Confirmed or unconfirmed | Confirmed | Confirmed |
| **Central nervous system (CNS) impairment** | Yes | Yes | Yes |
| **Facial anomalies** | Yes (3 features) | Yes (2 features) | Not required |
| **Growth deficiency** | Yes | Not required | Not required |

# 3 Aim and objectives

## 3.1 Aim

i. To develop case ascertainment algorithms for FASD and evaluate their validity.

## 3.2 Objectives

i. To use the FASD Canadian guidelines for diagnosis (2005) as a basis for developing algorithms that can be applied to the ALSPAC cohort data for FASD case ascertainment.

ii. To specify a decision-making framework and use this to identify appropriate ALSPAC measures and thresholds for FASD symptomology.

iii. To develop a series of algorithms that systematically vary CNS and PAE criteria to address ambiguity in the FASD Canadian guidelines for diagnosis (2005).

iv. To validate the algorithms by calculating diagnostic accuracy statistics that compare the performance of each algorithm to the FASD classifications assigned by an expert case conference panel.

# 4 Development of case ascertainment algorithms for FASD

## 4.1 Method

### 4.1.1 Data source

ALSPAC is a prospective longitudinal birth cohort study, which recruited 14,541 pregnant women from the Bristol area between 1990 and 1992. Follow-up of participants is ongoing and children from the original cohort are now

approximately 25 years old.[228,229] The ALSPAC dataset includes extensive repeated measures of prenatal exposures, child behavioural and psychological outcomes, growth, facial features and sociodemographic factors, collected through a range of questionnaires, in-clinic assessments and data linkage (including the National Pupil Database and the Pupil Level Annual School Census).

ALSPAC sample characteristics, study phases, methodology and representativeness have been described in previous publications,[228,229] and in detail on the study website (http://www.alspac.bris.ac.uk/welcome/index.shtml). Compared to the general population of mothers with children under the age of one in Britain at the time of recruitment, and those in the Avon area who were not enrolled in the ALSPAC study, participants in the ALSPAC sample were of higher socioeconomic status and were less likely to be of non-White ethnicity.[228,229] Children in the ALSPAC sample were comparable to the UK general population on growth measures, but had higher levels of educational attainment on average.[228,230] These differences present some limitations when attempting to generalise prevalence estimates from the ALSPAC cohort to the general population of the UK (Chapter 4). Representativeness is less of a concern for the validity of the risk factor regression analyses that I present in this thesis (Chapter 6). As Rothman notes, it is not representativeness, but the 'laws of nature' and understanding of causal mechanisms that allows for the generalisation of proposed cause-effect relationships.[231] In Chapter 5, I present hypothesised causal mechanisms for the relationships between a range of exposures and FASD. In Chapter 6, I use multivariable analyses to quantify these relationships.

### 4.1.2 Study approval

Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee (IRB00003312) and the Local Research Ethics Committees.[232] Project approval was granted by the ALSPAC Executive Committee on the 2nd March 2016 (Project B2620).

### 4.1.3 Participants

I included data from all singleton pregnancies in the core ALSPAC sample (i.e. those recruited during pregnancy between 1991 and 1992). I excluded participants who were not alive at one year of age, participants with genetic conditions and those who did not speak English as a primary language. This produced an eligible sample of 13,495 participants.

### 4.1.4 Procedure

I developed a series of FASD case ascertainment algorithms based on the Canadian guidelines for diagnosis (2005).[72] Given the wealth of information available in the ALSPAC dataset, I aimed to develop a case-definition that best reflected real-life practice in FASD diagnosis. Thus, I specified a decision making framework to select variables and thresholds for FASD-relevant outcomes, as described below.

#### *4.1.4.1 Variable selection*

First, I identified potentially relevant variables from the ALSPAC dataset by searching the ALSPAC data dictionary[233] for terms and measures relevant to the pre-specified Canadian FASD criteria. For example, I searched PDF and Excel spreadsheet documentation for key words relating to FASD criteria, such as

"communication," and for known measures such as "Strengths and Difficulties Questionnaire" or "SDQ." This search revealed that there were sufficient data within ALSPAC to derive a FASD classification based on the FASD Canadian guidelines for diagnosis (2005),[72] including repeated prospective measurement of prenatal exposures and child phenotype. Data were obtained for all relevant variables at each available time point from birth up to the age of 15.

I discussed the suitability of the variables that I identified from this search with an expert group that included a consultant psychiatrist from the UK National Clinic for Fetal Alcohol Spectrum Disorder (RM), a community paediatrician (AK) and an educational psychologist (AH). Table 9 provides a summary of the relevant ALSPAC measures that correspond to the FASD Canadian guidelines (2005). In Appendix 4, I provide a more detailed description of the measures, including the age of participants at data collection, procedures, and thresholds. Important factors for differential diagnosis were identified from FASD diagnostic guidelines and were considered in the exclusion criteria and FASD classifications, as described in Appendix 5. Children who were known to have a genetic condition were excluded from the sample.

*Table 9: FASD Canadian guidelines for diagnosis (2005) and corresponding ALSPAC measures. Full details of ALSPAC measures are provided in Appendix 4.*

| FASD Canadian guidelines (2005) criterion | ALSPAC measures |
|---|---|
| **Prenatal alcohol exposure**<br>Confirmation of maternal alcohol use during the index pregnancy based on reliable clinical observation, self-report, reports by a reliable source, medical records documenting positive blood alcohol, alcohol treatment or social/legal/medical problems. No threshold specified. | Maternal self-reported prenatal alcohol consumption |
| **Central nervous system**<br>Impairment in ≥ 3 subdomains including:<br>a. Hard and soft neurologic signs<br>b. Brain structure<br>c. Cognition<br>d. Communication<br>e. Academic achievement<br>f. Memory<br>g. Executive functioning and abstract reasoning<br>h. Attention deficit/hyperactivity<br>i. Adaptive behaviour, social skills, social communication<br><br>A domain is considered "impaired" when on a standardized measure: scores are ≥ 2 SD below the mean or there is a discrepancy of ≥ 1 SD between subdomains. When standardized measurements are not available, a clinical judgment of "significant dysfunction" is made. | a. Hard and soft neurologic signs<br>ALSPAC coordination test, cerebral palsy, seizures |
| | b. Brain structure<br>Occipital frontal circumference |
| | c. Cognition<br>Wechsler Intelligence Scale for Children (WISC-III) |
| | d. Communication<br>Wechsler Objective Language Dimensions, special educational needs for communication, speech/language problems |
| | e. Academic achievement<br>Special educational needs, exam scores at Key Stages 1 and 2 |
| | f. Memory<br>WISC-III forward digit span task, non-word repetition task |
| | g. Executive functioning and abstract reasoning<br>Opposite Worlds task, Counting Span task, Stop Signal task, Backwards Digit Span task |
| | h. Attention deficit/hyperactivity<br>ADHD, Strengths and Difficulties Questionnaire (SDQ) hyperactivity, Sky Search task |
| | i. Adaptive behaviour, social skills, social communication<br>Diagnostic Analysis of Nonverbal Accuracy scale faces subtest, Social Communication Disorders Checklist score, SDQ conduct or peer problems, oppositional defiant disorder, conduct disorder, disruptive behaviour disorder, school emotional/behavioural difficulties, SEN for behavioural/emotional needs, autism. |
| **Growth**<br>≤ 10$^{th}$percentile for pre- and/or postnatal height or weight or weight-to-height ratio | Clinical assessment |
| **Face**<br>Short palpebral fissure length (≤ 3$^{rd}$ percentile),<br>Smooth philtrum<br>Thin upper lip | Three-dimensional (3D) facial scan data |

#### *4.1.4.2 Thresholds for impairment*

##### 4.1.4.2.1 Prenatal alcohol exposure

The FASD Canadian guidelines (2005) do not specify a threshold for the PAE criterion. However, they do state that the amount and frequency of alcohol use should be documented if this information is available.[72] In practice, clinicians consider the PAE profile of individuals who are referred for FASD assessment.[224] I collated data on prenatal alcohol consumption across a range of maternal self-report measures in ALSPAC. These included: dose/frequency measures (number of glasses[e] of alcohol per week or per day in the first trimester, around the time the mother first felt the baby move, and in the third trimester; measured at 18 weeks gestation, 32 weeks gestation and 8 weeks postpartum, respectively); total weekly alcohol consumption (number of drinks per week; measured at 8 and 32 weeks gestation); binge drinking[f] (measured at 18 and 32 weeks gestation); and finally, a measure that captured the amount that a mother drank in the period before pregnancy (dose/frequency) and whether she subsequently changed her drinking behaviour during pregnancy.

##### 4.1.4.2.2 Central nervous system

Appendix 4 provides an overview of the relevant CNS tests that were available in ALSPAC and the thresholds that were chosen to indicate impairment. According to the Canadian FASD guidelines,[72] the CNS criterion is met when there is evidence of

[e] The ALSPAC questionnaire defined a glass of alcohol as equivalent to a pub measure of spirits, ½ pint lager/beer, wine glass of wine etc.

[f] 'Binge drinking' was defined as the consumption of the equivalent of two pints of beer, four glasses of wine or four pub measures of spirits on a single occasion.

impairment in three or more CNS subdomains. In the first instance, I used available norms for standardised tests to determine thresholds for impairment that were established and reliable. For example, I used standard thresholds for the Strengths and Difficulties Questionnaire (SDQ; full information and scoring criteria are provided at http://www.sdqinfo.com). This measure has established reliability and validity based on a general population sample of 10,438 five to 15-year-olds in Britain.[234]

If commonly accepted test norms were not available I used thresholds from the research literature, if they appeared justified. For example, several studies have defined poor motor performance as an ALSPAC coordination test score <5$^{th}$ centile, so I selected this threshold for the case ascertainment algorithms.[235]

If existing norms were not readily available, I established thresholds based on the distribution of participant data within ALSPAC. Thresholds for significant dysfunction were set at ≥ 2 standard deviations below the mean for data that were normally distributed, or ≤ 3$^{rd}$ percentile for data with a skewed distribution. If the test value at the 3$^{rd}$ percentile was not unique (e.g. if it spanned from 3$^{rd}$ - 5$^{th}$ percentile), I selected the next lowest unique value (e.g. 2$^{nd}$ percentile). I assigned different thresholds according to participant sex and age categories, when there was evidence of differential performance. Final decisions about appropriate thresholds for impairment were made in consultation with the expert group (RM, AH, AK).

#### 4.1.4.2.3 Facial phenotype

Facial phenotype data were available from 3D scans, which were carried out when ALSPAC participants were approximately 15 years old. I obtained data from ALSPAC that classified upper lip thinness and philtrum smoothness based on a system developed by Wilson-Nagrani and colleagues.[236] This morphological trait scale ranks philtrum shape on a progressive six-point scale that ranges from 0 (smooth philtrum) to 6 (deep groove extending through vermilion border), and upper lip fullness on a three-point scale (0 thin; 1 medium; 2 thick). The University of Washington lip-philtrum guide is the recommended method for assessing the FAS facial phenotype. Following discussion with Dr Wilson-Nagrani, I reclassified the ALSPAC philtrum data to approximate the recommended classifications from the University of Washington lip scale, such that philtrum scores of 0 - 1 and an upper lip vermilion score of 0 on the Wilson-Nagrani et al. scale were deemed comparable to the recommended University of Washington rank 4/5 lip-philtrum classifications.

I used the University of Washington palpebral fissure length z-score calculator[237] to identify age and sex-specific cut-offs for short palpebral fissure length, defined as a z-score of ≤ 1.96, based on the recommended Scandinavian charts.[238]

#### 4.1.4.2.4 Growth data

I used the LMS Growth add-in for Microsoft Excel,[239] which is based on reference data from a nationally representative sample,[240] to categorise children using the appropriate centiles for their sex and age (from birth to age 9). The LMS Growth add-in did not have reference values for the length of babies born before 35 weeks.

Therefore, I calculated birth length centiles for babies with a gestational age of less than 35 weeks using the Fenton 2013 weight calculator.[241] This measure uses reference data from several large population-based surveys of preterm births (N = 34,639), including a sample from Scotland.[242] Although the FASD Canadian guidelines specify a 10th percentile cut-off for growth deficiency, I chose to use a 9th percentile cut-off to be consistent with paediatric practice in the UK.[106]

## 4.2 Algorithm specifications

As I was developing the case ascertainment algorithms for FASD, it became apparent that there was an element of ambiguity in some of the criteria. For example, the FASD Canadian guidelines (2005) state that the pattern of PAE should be documented but do not specify a threshold, noting that "the evaluation of 'significant alcohol exposure' is often confusing." [72(p.S11)] Other guidelines suggest thresholds for PAE[73,74,212,227] (described in Appendix 3), but note that there is insufficient evidence to determine whether any amount of PAE can be considered safe.[227]

The Canadian guidelines also recommend that a diagnosis should only be made when "there is evidence of pervasive brain dysfunction…defined by severe impairment in three or more…neurodevelopmental domains."[227(p.193)] However, they do not offer clear direction about what to do when there are inconsistencies between measures that assess the same domain of functioning over time, or when using different tests. For example, should the adaptive functioning domain be classed as impaired only when there is consistent evidence of impairment across all

measures and/or informants, or is evidence of impairment on one, or some of these measures sufficient to indicate impairment?

To address these uncertainties, I created a series of algorithms that systematically explored a range of CNS and PAE criteria combinations. These algorithms corresponded to different levels of convergent evidence and symptom or exposure severity. Following the case conference validation process (described in Section 5), I also added a *Revised* CNS category to reflect further recommendations made by the expert panel (summarised in Appendix 8). Figure 8 provides a schematic representation of the different combinations of the CNS and PAE criteria, and the resulting algorithm names. Algorithm specifications are provided in Table 10. Full details of the measures included in the FASD algorithms are provided in Appendix 4. Consistent with the FASD Canadian guidelines (2005), the algorithms defined FAS as the presence of the full facial phenotype, growth deficiency, and CNS impairment, with or without confirmed PAE (all domains in Table 10). pFAS was defined as the partial facial phenotype, CNS impairment and confirmed PAE (all domains in Table 10, except domain 2). ARND was defined as CNS impairment with confirmed PAE (domains 1 and 4 in Table 10).

*Figure 8: Schematic representation of the case ascertainment algorithms for FASD that were generated by varying combinations of the central nervous system and prenatal alcohol exposure (PAE) criteria. Full definitions for the Liberal, Mid, Strict and Revised CNS criteria, and the terminology for all PAE categories are provided in Table 10.*

| | | | | Central nervous system (CNS)<br>Symptom severity and convergent evidence | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Liberal CNS** | **Mid CNS** | **Revised CNS** | **Strict CNS** |
| **Prenatal alcohol exposure (PAE)** | | **Duration (trimesters of exposure)** | **Any PAE** | Liberal CNS/Any PAE | Mid CNS/Any PAE | Revised CNS/Any PAE | Strict CNS/Any PAE |
| | | | **Mid PAE** | Liberal CNS/Mid PAE | Mid CNS/Mid PAE | Revised CNS/Mid PAE | Strict CNS/Mid PAE |
| | | | **Strict PAE** | Liberal CNS/Strict PAE | Mid CNS/Strict PAE | Revised CNS/Strict PAE | Strict CNS/Strict PAE |
| | | **Level of exposure** | **DSM-5 ND-PAE** | Liberal CNS/ND-PAE | Mid CNS/ND-PAE | Revised CNS/ND-PAE | Strict CNS/ND-PAE |
| | | | **NICE PAE** | Liberal CNS/NICE PAE | Mid CNS/NICE PAE | Revised CNS/NICE PAE | Strict CNS/NICE PAE |
| | | | **Canadian 2015 PAE** | Liberal CNS/Canadian PAE | Mid CNS/Canadian PAE | Revised CNS/Canadian PAE | Strict CNS/Canadian PAE |

*Table 10 (continued overleaf): ALSPAC FASD case ascertainment algorithm specifications*

| Domain | Subdomain | Case-definition specification | | | |
|---|---|---|---|---|---|
| | | **Liberal CNS** | **Mid CNS** | **Strict CNS** | **Revised CNS** |
| **1. Central Nervous System (CNS)[a]**<br><br>**Impairment in ≥ 3 subdomains** | ***a) Hard and soft neurologic signs*** | ≤5th percentile in ALSPAC coordination test **OR** ≥ 2 seizures not due to postnatal insult **OR** cerebral palsy | ≤5th percentile in ALSPAC coordination test **OR** cerebral palsy | As for Mid | ≤5th percentile in ALSPAC coordination test **OR** ≥ 2 seizures not due to postnatal insult) **OR** cerebral palsy |
| | ***b) Brain structure*** | Head circumference ≤ 2nd percentile at birth | Head circumference ≤ 2nd percentile at birth **AND** age 7 | As for Mid | Head circumference ≤ 2nd percentile at birth **OR** age 7 |
| | ***c) Cognition*** | Score ≤ 70 on total, verbal or performance IQ **OR** discrepancy of ≥ 1 SD between subdomains | As for Liberal | As for Liberal | Score ≤ 70 on total, verbal or performance IQ **OR** discrepancy of ≥ 1 SD between subdomains. Not impaired if discrepancy between subdomains but IQ ≥ 120 |
| | ***d) Communication: receptive and expressive*** | Score ≤ 2 SD from the mean for WOLD listening comprehension **OR** WOLD expressive language **OR** teacher-reported communication impairment at any time point | Impaired on any two of the measures in this domain | Score ≤ 2 SD from the mean for WOLD listening comprehension **AND** WOLD expressive language **AND** teacher-reported impairment at any time point | Impaired on any two of the measures in this domain (including consideration of SEN communication needs) |
| | ***e) Academic achievement*** | Failing to meet the expected level at school at any time point (Key Stage 1 Level 1 or W; Key Stage 2 < Level 4) **OR** SEN at any time point | Failing to meet the expected level at school at all time points (Key Stage 1 Level 1 or W; Key Stage 2 < Level 4) **OR** SEN at any time point | SEN at any time point | Failing to meet the expected level at school at all time points **OR** SEN at any time point. Do not mark as impaired if IQ ≤ 79. Do not mark as impaired if normal educational attainment and a reason other than |

| Domain | Subdomain | Case-definition specification | | | |
|---|---|---|---|---|---|
| | | **Liberal CNS** | **Mid CNS** | **Strict CNS** | **Revised CNS** |
| | | | | | cognitive and learning needs for SEN. |
| | ***f) Memory*** | Score ≤3rd percentile on Forward Digit Span **OR** Non-Word Repetition Task | As for Liberal | ≤3rd percentile on Forward Digit Span | Score ≤3rd percentile on Forward Digit Span **OR** Non-Word Repetition Task |
| | ***g) Executive functioning and abstract reasoning*** | Score ≤3rd percentile for any of the available measures: Opposite Worlds task, Counting Span task, Stop Signal task, Backwards Digit Span task | Score ≤3rd percentile for two of the available measures | Counting Span **AND** Backwards Digit Span score ≤3rd percentile **OR** Stop-Signal **AND** Opposite Worlds score ≤3rd percentile | Score ≤3rd percentile for two of the available measures |
| | ***h) Attention deficit/hyperactivity*** | Score ≤3rd percentile for Sky Search **OR** ADHD **OR** high SDQ hyperactivity | ADHD **OR** high SDQ hyperactivity | ADHD | Convergent evidence across 2 measures or impairment reported by both informants for SDQ (teacher and parent) **OR** ADHD |
| | ***i) Adaptive behaviour, social skills, social communication*** | High SDQ peer **OR** conduct problems **OR** ODD/CD **OR** ≥ 7 DANVA errors **OR** score ≥ 9 on SCDC **OR** autism **OR** teacher-reported emotional or behavioural difficulties | High SDQ peer **OR** conduct problems **OR** ODD/CD **OR** autism **OR** two of the following: ≥ 7 DANVA errors **OR** score ≥ 9 on SCDC **OR** teacher-reported emotional or behavioural difficulties | ODD/CD **OR** autism | Impaired on any two of the measures in this domain or impairment reported by both informants for SDQ (teacher and parent) including consideration of SEN behavioural, emotional and social development needs **OR** autism |
| **2. Growth** | | ≤ 9th percentile for: birth weight **AND/OR** birth length and postnatal height **AND/OR** birth and postnatal BMI | | | |
| **3. Face** | | Full facial phenotype (for FAS): short palpebral fissure length (≤ 2.5th percentile) **AND** smooth philtrum **AND** thin upper lip (equivalent to ranks 4-5 on the lip-philtrum guide)<br>Partial facial phenotype (for pFAS): any two features from the full facial phenotype | | | |

| Domain | Subdomain | Case-definition specification | | | |
|---|---|---|---|---|---|
| | | **Liberal CNS** | **Mid CNS** | **Strict CNS** | **Revised CNS** |
| **4. Prenatal alcohol exposure (PAE)** | | **Any PAE:** Any level of prenatal alcohol exposure at any time in pregnancy; **Mid PAE:** Two trimesters of prenatal alcohol exposure and/or binge drinking; **Strict PAE:** Three trimesters of prenatal alcohol exposure and/or binge drinking; **Canadian PAE**: Seven or more standard Canadian drinks per week (11.9 UK units) or any binge drinking[b]; **ND-PAE**: > 13 drinks per month, with > 2 drinks per occasion; **NICE PAE**: ≥ 1-2 drinks once or twice per week or ≥ 4 units of alcohol[d] | | | |

Abbreviations: CNS, central nervous system; DANVA, Diagnostic Analysis of Non-Verbal Accuracy; IQ, intelligence quotient; ND-PAE, Neurodevelopmental Disorder-Prenatal Alcohol Exposure; NICE, National Institute for Health and Care Excellence; ODD/CD, oppositional/conduct disorder; PAE, prenatal alcohol exposure; SD, standard deviation; SCDC, Social Communication Disorders Checklist; SDQ, Strengths and Difficulties Questionnaire; SEN, special educational needs; WOLD, Weschler Objective Language Dimensions.

[a] CNS criterion met if there is evidence of impairment in ≥ 3 subdomains (a - i). The Revised CNS case-definition requires that this includes impairment in the subdomains that measure adaptive functioning (e and i).

[b] The FASD Canadian 2016 guidelines suggest that more than one binge drinking episode is required to meet the PAE criteria; however, the ALSPAC data categorise binge drinking as: none, 1-2 days, 3-4 days, 5-10 days, > 10 days, or every day per month. Therefore, it was not possible to separate participants with one binge drinking episode from those with two or more.

[c] DSM-5 ND-PAE criterion of 'more than minimal exposure'. Exposed to alcohol at any time during gestation, including prior to pregnancy recognition, and the exposure level was more than minimal (i.e. more than 13 drinks in any one month, with more than two drinks on any drinking occasion.

[d] Drinking in excess of NICE 2008 antenatal guideline limits ≥1-2 drinks once or twice per week; equivalent to ~ 32g / 4 units of alcohol.

# 5 Validation of the FASD case ascertainment algorithms

## 5.1 Method

The data source and study approval process were described in Section 4.1.

### 5.1.1 Participants and sampling strategy

In the validation stage of this study, I selected a stratified random sample of 31 participant profiles from the eligible ALSPAC sample to be considered by an expert case conference panel. The sample was stratified to ensure that at least two participant profiles were considered for each of the 24 algorithm specifications and that at least three participants did not meet criteria for FASD under any of the case ascertainment algorithms. By definition, participants who met criteria for the more stringent FASD classifications (i.e. higher levels/duration of PAE or more convergent evidence or severe symptoms for the CNS criteria) also met criteria for the less stringent classifications. Figure 9 presents the number of participants in the validation sample who met criteria for each of the case ascertainment algorithms.

### 5.1.2 Case conference procedure

The case conference panel was hosted at Cardiff University on 17th January 2017 and consisted of a one-day meeting involving a consultant psychiatrist and national FASD specialist (RM), community paediatrician (AK) and educational psychologist (AH). The meeting was audio recorded and administrative support was provided by a member of staff from the professional and support services team at Cardiff University (JH). Appendices 4, 5 and 6, show the documents that were given to the panel members for the case conference. During the case conference, the expert

panel were given 31 participant profiles and asked to decide for each profile whether, on the balance of probability, a diagnosis of FASD would be made in clinic, based on the information provided. Panel members were given a score sheet, based on the FASD Canadian guidelines (2005), and a description of the relevant ALSPAC measures. Panel members were blind to the FASD classification status that was assigned to each participant based on the application of the case ascertainment algorithms. Panel decisions on FASD status were reached by consensus during the meeting.

*Figure 9: Sample for the FASD case ascertainment algorithm validation process. The number in each cell corresponds to the number of participants who met criteria for FASD under the corresponding algorithm specification. Total N = 31.*

| | | | | Central nervous system (CNS)<br>Symptom severity and convergent evidence | | | |
|---|---|---|---|---|---|---|---|
| | | | | Liberal | Mid | Revised | Strict |
| Prenatal alcohol exposure (PAE) | | Duration (trimesters of exposure) | Any | 28 | 19 | 13 | 9 |
| | | | Mid | 19 | 13 | 9 | 6 |
| | | | Strict | 10 | 7 | 5 | 3 |
| | | Level of exposure | ND-PAE | 8 | 5 | 2 | 3 |
| | | | NICE PAE | 10 | 7 | 4 | 3 |
| | | | Canadian 2015 PAE | 6 | 4 | 2 | 2 |
| | **No FASD** under all definitions | | | 3 | | | |

### 5.1.3 Analysis

I calculated diagnostic accuracy statistics to compare the performance of each of the FASD case ascertainment algorithms (index tests), with the FASD classifications assigned by the expert case conference panel (reference standard). Algorithm performance was quantified using sensitivity and specificity statistics and the 0,1 method, which identifies the shortest distance to the top left hand corner of a Receiver Operating Characteristic (ROC) plot, defined as $d = \sqrt{([1\text{-}Sensitivity]^2 + [1\text{-}Specificity]^2)}$.[243] The rationale behind this method is that an algorithm that had perfect agreement with the case conference panel would pass through the top left hand corner of the ROC plot. Therefore, shorter distances indicate greater agreement between the case ascertainment algorithm and the expert consensus panel. This method places equal importance on sensitivity and specificity. Further considerations about the relative importance of sensitivity and specificity are described later in this chapter, with reference to the qualitative results from the case conference panel. Confidence intervals for sensitivity and specificity estimates were generated using the Wilson method, which is recommended for sample sizes ≤ 40.[244] In addition to the quantitative analyses, I produced a qualitative summary of the panel discussion that took place for each participant profile and the corresponding FASD outcome decision. I also recorded the recommendations that members of the case conference panel made for refinement of the FASD case ascertainment algorithms.

## 5.2 Quantitative results

Diagnostic accuracy statistics for each of the FASD case ascertainment algorithms are presented in Table 11 and a ROC plot is provided in Figure 10. The Mid CNS/Any PAE case ascertainment algorithm had the highest level of agreement with the FASD classifications that were assigned by the expert consensus panel. Ninety-one percent of individuals who were classified as having FASD by the case conference panel were assigned a FASD classification by this algorithm (95% CI 62% - 98%). Specificity for the Mid CNS/Any PAE case ascertainment algorithm was 55% (95% CI 34% - 74%), indicating that nine out of 20 individuals who met criteria for FASD under this definition were not classified as having FASD by the panel. Overall, this algorithm had the shortest distance to the top left hand corner of the ROC plot (0,1 value = 0.46). The Mid CNS/Mid PAE and Revised CNS/Any PAE case ascertainment algorithms had the next highest level of diagnostic accuracy, relative to the case conference panel decision, with both algorithms having the same values for sensitivity, specificity and the 0,1 statistic (64% [95% CI, 35% - 85%]; 70% [95% CI, 48% - 85%]; and 0.47, respectively). However, confidence intervals were wide for all estimates, due to the small sample size. In summary, the three top-performing ALSPAC FASD case-definitions, based on the diagnostic accuracy statistics, were the Mid CNS/Any PAE, Mid CNS/Mid PAE and Revised CNS/Any PAE algorithms.

## 5.3 Qualitative results

A full qualitative summary of discussions from the case conference panel about FASD classification decisions is presented in Appendix 7. The case conference panel reached consensus for all participant FASD classifications. However, in many cases, they reported a lack of confidence in their classifications due to missing

information or discrepancies between test results in key domains. The panel noted that in real-life clinical settings they would have sought further testing and information, including an in-depth discussion with the child's caregivers, before reaching a FASD outcome decision for many of the cases.

During the case conference, panel members discussed the factors that most influenced their decisions about participants' FASD classification. The panel made several recommendations for refinements to the FASD case ascertainment algorithm, which are summarised in Appendix 8. These were incorporated into the *Revised* CNS case ascertainment algorithms.

*Table 11: Test accuracy statistics for ALSPAC FASD case ascertainment algorithms (index test), compared to case conference panel FASD classification (reference standard). Measures with shortest 0,1 distance highlighted in bold.*

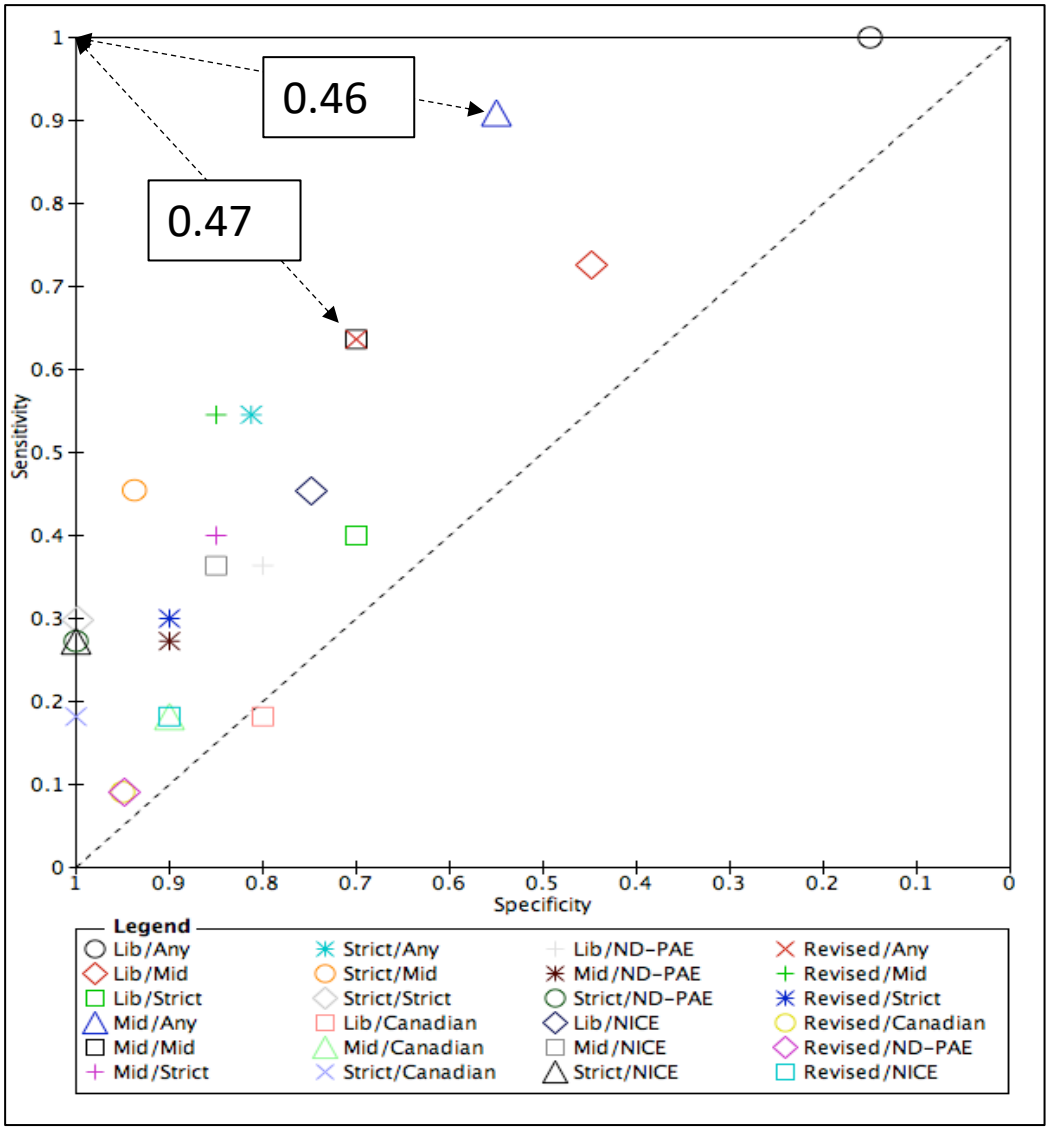
| FASD case-definition | TP | FP | FN | TN | N[a] | Sens % (95% CI) | Spec % (95% CI) | 0,1[b] |
|---|---|---|---|---|---|---|---|---|
| Lib CNS; Any PAE | 11 | 17 | 0 | 3 | 31 | 100 (74 - 100) | 15 (5 - 36) | 0.85 |
| Lib CNS; Mid PAE | 8 | 11 | 3 | 9 | 31 | 73 (43 - 90) | 45 (26 - 66) | 0.61 |
| Lib CNS; Strict PAE | 4 | 6 | 6 | 14 | 30 | 40 (17 - 69) | 70 (48 - 85) | 0.67 |
| Lib CNS; Canadian PAE | 2 | 4 | 9 | 16 | 31 | 18 (5 - 48) | 80 (56 - 94) | 0.84 |
| Lib CNS; ND-PAE | 4 | 4 | 7 | 16 | 31 | 36 (15 - 65) | 80 (56 - 94) | 0.67 |
| Lib CNS; NICE PAE | 5 | 5 | 6 | 15 | 31 | 45 (21 - 72) | 75 (51 - 91) | 0.60 |
| **Mid CNS; Any PAE** | **10** | **9** | **1** | **11** | **31** | **91 (62 - 98)** | **55 (34 - 74)** | **0.46** |
| **Mid CNS; Mid PAE** | **7** | **6** | **4** | **14** | **31** | **64 (35 - 85)** | **70 (48 - 85)** | **0.47** |
| Mid CNS; Strict PAE | 4 | 3 | 6 | 17 | 30 | 40 (17 - 69) | 85 (64 - 95) | 0.62 |
| Mid CNS; Canadian PAE | 2 | 2 | 9 | 18 | 31 | 18 (5 - 48) | 90 (68 - 99) | 0.82 |
| Mid CNS; ND-PAE | 3 | 2 | 8 | 18 | 31 | 27 (9 - 57) | 90 (68 - 99) | 0.73 |
| Mid CNS; NICE PAE | 4 | 3 | 7 | 17 | 31 | 36 (15 - 65) | 85 (64 - 95) | 0.65 |
| Strict CNS; Any PAE | 6 | 3 | 5 | 13 | 27 | 55 (28 - 79) | 81 (57 - 93) | 0.49 |
| Strict CNS; Mid PAE | 5 | 1 | 6 | 15 | 27 | 45 (21 - 72) | 94 (72 - 99) | 0.55 |
| Strict CNS; Strict PAE | 3 | 0 | 7 | 16 | 26 | 30 (11 - 60) | 100 (81 - 100) | 0.70 |
| Strict CNS; Canadian PAE | 2 | 0 | 9 | 16 | 27 | 18 (5 - 48) | 100 (81 - 100) | 0.82 |
| Strict CNS; ND-PAE | 3 | 0 | 8 | 16 | 27 | 27 (10 - 57) | 100 (81 -100) | 0.73 |
| Strict CNS; NICE PAE | 3 | 0 | 8 | 16 | 27 | 27 (10 - 57) | 100 (81 - 100) | 0.73 |
| **Rev CNS; Any PAE** | **7** | **6** | **4** | **14** | **31** | **64 (35 - 85)** | **70 (48 - 85)** | **0.47** |
| Rev CNS; Mid PAE | 6 | 3 | 5 | 17 | 31 | 55 (28 - 79) | 85 (64 - 95) | 0.48 |
| Rev CNS; Strict PAE | 3 | 2 | 7 | 18 | 30 | 30 (11 - 60) | 90 (70 - 97) | 0.71 |
| Rev CNS; Canadian PAE | 1 | 1 | 10 | 19 | 31 | 9 (2 - 38) | 95 (76 - 99) | 0.91 |
| Rev CNS; ND-PAE | 1 | 1 | 10 | 19 | 31 | 9 (2 - 38) | 95 (76 - 99) | 0.91 |
| Rev CNS; NICE PAE | 2 | 2 | 9 | 18 | 31 | 18 (5 - 48) | 90 (70 - 97) | 0.82 |

Abbreviations: CI, confidence interval; CNS, central nervous system; FN, false negative; FP, false positive; Lib; liberal; PAE, prenatal alcohol exposure; Rev, revised; Sens, sensitivity; Spec, specificity; TN, true negative; TP, true positive.

[a] N refers to number of participants out of the total case conference sample (N = 31) with sufficient information available to determine FASD classification for each case-ascertainment algorithm. For example, some participants had missing data on PAE for one or more trimester and, therefore, had insufficient data available to meet Strict PAE case ascertainment algorithms.

[b] 0,1 statistic indicates distance from the top left hand corner of a Receiver Operating Characteristic (ROC) plot with lower values indicating better test performance.

*Figure 10: Receiver operating characteristic (ROC) plot depicting diagnostic accuracy of the FASD case ascertainment algorithms, relative to the FASD classifications assigned by the expert case conference panel. Dashed arrows represent distance from the top left hand corner of the ROC plot (which would indicate perfect agreement with the case conference panel) and text boxes indicate the 0,1 statistic for the three FASD case ascertainment algorithms with the highest level of agreement with the case conference panel. Note: the Mid CNS/Mid PAE and Revised CNS/Any PAE data points overlap, as they appear in the same position in the plot.*



# 6 Discussion

## 6.1 Main results

In this chapter, I demonstrated that it was possible to develop FASD case ascertainment algorithms based on the FASD Canadian guidelines (2005)[72] and consultation with clinical experts, for application to a population-based birth cohort (ALSPAC). The Mid CNS/Any PAE algorithm[g] had the highest levels of agreement with the expert case conference panel (sensitivity 91%; specificity 55%; 0,1 value 0.46), offering support for its validity.

The algorithms with the next highest levels of agreement were the Mid CNS/Mid PAE and Revised CNS/Any PAE algorithms (for both: sensitivity 64%, specificity 70%; 0,1 value 0.46). To my knowledge, this is the first study to develop and validate FASD case ascertainment algorithms for use in an epidemiological study. The FASD classification algorithms are transparent and can potentially be applied to other international cohorts to enable valid comparison of prevalence across settings, subject to equivalence of measures and data availability.

## 6.2 Strengths and limitations

### 6.2.1 Data considerations

A key strength of the ALSPAC dataset was that it included extensive information that enabled each of the domains within the FASD Canadian guidelines for diagnosis (2005) to be assessed. Measures included an array of multidisciplinary, multi-rater assessments, which were conducted with a large population-based

[g] The Mid CNS/Any PAE classification corresponds to an intermediate level of FASD symptom severity, convergent evidence across a range of central nervous system measures, and evidence of any level of confirmed prenatal alcohol exposure.

sample. Tests were carried out by qualified personnel, including psychologists and speech and language therapists, and a range of informants, including parents and teachers. In addition, the involvement of a FASD specialist, paediatrician and educational psychologist in the algorithm development and validation steps, enabled a multidisciplinary approach to FASD case identification and validation of the algorithms, in a manner that approximated recommended FASD case conference procedures.[72]

However, it is important to note that the tests that were used to derive FASD classifications were not administered with this specific purpose in mind and, therefore, may not be optimal for FASD identification. The FASD Canadian guidelines for diagnosis include an appendix that lists the tests that are most commonly used by FASD assessment teams.[72] The ALSPAC dataset and resulting algorithms did include some of these measures, such as the WISC-III and the Movement Assessment Battery for Children, although these were administered in an abbreviated form.[235,245,246] Other tests that were listed in the Canadian appendix were not available in the ALSPAC dataset. For example, the Wilson-Nagrani et al. scale for lips[236] was used to assess the FAS facial phenotype, rather than the recommended University of Washington lip-philtrum guide.[247] The choice of tests used to ascertain FASD status will inevitably influence classification outcomes and, therefore, prevalence estimates. Nevertheless, it is reasonable to assume that measures that purport to measure the same domain of functioning should have convergent validity. Furthermore, I identified alternatives to the measures listed in the Canadian appendix in consultation with the expert group to ensure that the

measures selected from those available in ALSPAC were the most suitable for FASD assessment.

Measures of prenatal alcohol consumption were based on maternal self-report and are likely to be subject to measurement error. Although self-report measures of PAE have their limitations, the fact that most of the data were collected prospectively and anonymously should reduce the risk of recall bias and mitigate some of the issues that contribute to inaccuracies in self-reported PAE, such as fear of persecution and social desirability bias. Furthermore, the PAE questionnaires benefitted from the use of standard indicators for drink sizes and dose/frequency/type response formats for specific time points in pregnancy. These aspects have been shown to improve the validity of self-report measures of alcohol use.[62]

### 6.2.2 Algorithm validation

Qualitative data suggested that the expert panel lacked confidence in some of their classification decisions, often due to missing data in participant profiles and the lack of face-to-face contact with children and their caregivers to support clinical decision-making. Therefore, while the case conference panel provides some indication of the performance of the FASD algorithms, relative to expert consensus, the panel cannot be considered a gold standard reference test, as it did not replicate a thorough in-clinic assessment process.

It is also important to note that the expert panel were involved in both the algorithm development and validation stages. This may have increased the diagnostic accuracy performance of the algorithms. Finally, confidence intervals

were wide for diagnostic accuracy statistics due to the small sample size. Whilst a larger sample may have been preferable to increase precision in diagnostic accuracy estimates, panel members had a limited amount of time available to contribute to this process and, therefore, the sample size was restricted to ensure that it was feasible to conduct the validation step in a one-day face-to-face meeting format.

Follow-up studies that evaluate the performance of the FASD algorithms with alternative reference standards are warranted, but were beyond the scope of this thesis due to time and resource constraints. For example, it would be useful to evaluate algorithm performance relative to FASD classifications made by an independent expert panel, general population active case ascertainment screening methods (such as in-school screening),[95] FASD clinic-based assessments, and GP-linked datasets.[248]

### 6.2.3 Sensitivity and specificity of the algorithms

Specificity estimates indicated that the Mid CNS/Any PAE case ascertainment algorithm may have produced a high proportion of false positive results (i.e. the algorithm classified some participants as FASD when the case conference panel did not). This suggests that the algorithm may overestimate FASD. It is possible that the alcohol exposure criterion, which allowed for any PAE to be sufficient for consideration of a FASD classification, may have been too liberal. As described previously, evidence of the risk of harm at low levels of PAE is inconclusive.[24] However, Astley and Grant point out that one in seven of the children diagnosed with FAS in their Washington clinic have exposure in the low to moderate range (1 -

8 drinks per week),[43] and the absence of a threshold for exposure is the most consistent interpretation of the Canadian 2005 guidelines for diagnosis. Alternatively, it may be that the apparently low specificity values could be due to an imperfect reference standard. The qualitative results showed that many of the participant profiles that were classified as 'not FASD' by the panel were considered possible cases, subject to further investigation. The panel appeared to only classify participants as having FASD when they were fairly certain that they met criteria, and tended to classify participants as not having FASD when they were uncertain. Therefore, it seemed reasonable to favour high sensitivity, rather than high specificity, when choosing which of the case ascertainment algorithms with the highest overall accuracy (0,1 value) to select for the analyses that follow in this thesis.

### 6.2.4 Differential diagnosis

Many of the features of FASD are not specific to PAE and, therefore, guidelines characterise FASD as a 'diagnosis of exclusion.' To address this issue, I excluded participants who were known to have a genetic condition from the sample. However, it was not possible to rule out other exposures that may have contributed to developmental outcomes on a case-by-case basis (the impact of differential diagnosis on FASD prevalence estimates is discussed further in Chapter 4). Nevertheless, the implications of co-occurring exposures for FASD classifications were discussed with the expert group during the validation step. The group acknowledged that while prenatal exposure to other substances (such as cigarette smoking and illicit drug use) and perinatal insults (such as hypoxia) complicate

efforts to determine whether alcohol was the key contributor to a child's developmental profile, exposure to multiple adverse factors is the norm among children with FASD and does not rule out the potential contribution of alcohol as an important causal factor.

# 7 Conclusion and implications for this thesis

It was feasible to develop FASD case ascertainment algorithms to enable investigation of the epidemiology of FASD in the ALSPAC cohort. Since the Mid CNS/Any PAE FASD case ascertainment algorithm had the highest levels of agreement with the expert case conference panel, this algorithm will be used to identify individuals who meet criteria for FASD in the prevalence and risk factor analyses that follow in Chapters 4 and 6. The Mid CNS/Mid PAE and Revised CNS/Any PAE case-definition algorithms had the next highest levels of agreement with the validation panel and will be used in the sensitivity analyses for prevalence estimates in Chapter 4.

It is important to note that the FASD classifications that are derived from application of the algorithms in this thesis are not intended to provide formal 'diagnoses' of FASD. As described above, FASD diagnosis requires input from a multidisciplinary team in a clinic setting, with an opportunity to interact with caregivers, to allow a thorough analysis of a child's developmental profile and to support differential diagnoses. For the purposes of this research, 'cases' and reference to 'participants with FASD' refers to individuals who meet the case ascertainment algorithm criteria for FASD, rather than individuals who have received a formal FASD diagnosis.

# Chapter 4. Prevalence and characteristics of fetal alcohol spectrum disorder in England: a population-based birth cohort study

## 1 Overview

In this chapter, I present prevalence estimates for fetal alcohol spectrum disorder (FASD), using data from a population-based birth cohort in England (ALSPAC). First, I describe why there is a need for a study of FASD prevalence in the UK and present the chapter aims and objectives. As with many epidemiological studies, missing data are an issue in the ALSPAC cohort. Therefore, next, I describe the strategies that I used to address missing data and discuss their relative validity. I then present FASD prevalence estimates and describe the sociodemographic and clinical characteristics associated with FASD. In the final section, I present a discussion of the strengths and limitations of my approach, compare these results with the existing literature, and discuss the implications of this work.

## 2 Background

FASD is thought to be a major cause of developmental disability, affecting up to 10% of children in the general population in Europe and North America.[79-87] Prenatal alcohol use is common in the UK,[11-13] but the prevalence of FASD is unknown.[94] The International Charter on Prevention of FASD, published in 2014, describes the lack of public and professional awareness of FASD and calls for prevalence research as an important step in informing prevention efforts.[249] The All Party Parliamentary Group (APPG) on FASD noted that a lack of UK-based research on the epidemiology of FASD has led to inadequate service provision.[108] Gregory

and colleagues echoed the sentiments of the APPG, stating that "The most obvious difficulty in trying to persuade health commissioners of the need to fund services for assessments and management of cases of FAS/FASD is the lack of clarity regarding the number of children affected. It has wrongly been assumed that this is a rare disorder and that no additional support is needed to manage them."[104(p.233)]

Active case ascertainment methods, such as in-school screening methods, are the preferred approach for FASD prevalence studies; however, they are costly and resource intensive.[95] To date, active case ascertainment studies of FASD have not been possible in the UK due to a lack of funding and ethical issues.[108] Given these challenges, I applied the FASD case ascertainment algorithms that I developed in Chapter 3 to existing data from a population-based birth cohort in England to estimate the prevalence of FASD.

# 3 Aims and objectives

## 3.1 Aims

i. To estimate the prevalence of FASD in the ALSPAC cohort.
ii. To describe the sociodemographic and clinical characteristics associated with FASD.

## 3.2 Objectives

i. To consider the validity of using complete case, single imputation and multiple imputation methods to address missing data in the ALSPAC cohort.
ii. To estimate the prevalence of FASD within the ALSPAC cohort, using complete case, single imputation and multiple imputation methods.
iii. To use descriptive statistics to summarise the sociodemographic and clinical characteristics associated with FASD.

# 4 Method

## 4.1 Data source

The Avon Longitudinal Study of Parents and Children (ALSPAC; described in Chapter 3 section 4.1.1).

## 4.2 Study approval

Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee (IRB00003312) and the Local Research Ethics Committees.[232] Project approval was granted by the ALSPAC Executive Committee on the 2nd March 2016 (Project B2620).

## 4.3 Participants

This study included singleton pregnancies within the core ALSPAC sample. Participants with genetic conditions, participants who were not alive at one year of age, and participants who did not speak English as a primary language were excluded. Participants who were in the armed forces social class category were excluded due to sparse data, which caused computational problems in imputation and risk factor models (N = 28).

## 4.4 Outcome

The primary outcome was total FASD prevalence, defined as the proportion of participants who met criteria for any condition within the FASD continuum, based on the Mid CNS/Any PAE case ascertainment algorithm (described in Chapter 3; Table 10). Secondary outcomes were the prevalence of FASD subtypes, including

fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS) and alcohol-related neurodevelopmental disorder (ARND).

## 4.5 Statistical analysis: the analytical challenge posed by missing data

Missing data are common in longitudinal datasets, including ALSPAC. In this section, I describe missing data types and methods, in the context of FASD prevalence research.

### 4.5.1 Types of missing data

Little and Rubin provide a terminology to describe the different relationships between missing and observed data.[250] Data are missing completely at random (MCAR) if missingness is not related to the data values; for example, if data are missing due to a power cut during a computer task. However, unless built into the study design[h] MCAR is rarely assumed to hold.[251]

Data are missing at random (MAR) if any systematic differences between the missing and observed values can be fully explained by differences in the observed data. For example, if missing blood pressure measurements are lower than the observed measures but only because younger people are less likely to have their blood pressure monitored then data are said to be MAR given age.[252]

Data are missing not at random (MNAR) if there are still systematic differences between the missing and observed data, even after the observed data are taken into account.[252] It is not possible to determine whether data are MAR or MNAR based on the observed data, since knowledge of this relationship relies on knowing

[h] For example, in 'planned missing' data designs, participants may be randomly allocated to respond to a subset of measures.

the values of the unobserved data. As missing data are often beyond the control of the researcher, and the researcher is unlikely to be aware of, or have access to, all of the data that predict missingness, most data are expected to be at least partly MNAR.[253] I will describe the implications of the MAR and MNAR assumptions for the analyses in this chapter in the discussion section.

Data that are MCAR will not produce biased prevalence estimates but will lead to decreased precision due to reduced sample size. When outcome data are more likely to be missing given certain values of the exposure, or depend on the unobserved values of the outcome, then prevalence estimates will be biased.[252,254] For example, FASD prevalence will be underestimated if children with incomplete data have higher levels of a given causal risk factor (such as binge PAE) than those with complete data. Prevalence will also be underestimated if children with incomplete data have poorer performance than those with complete data on measures relevant to FASD, such as lower IQ.

### 4.5.2 Analysis methods for missing data

Missing data complicate attempts to determine FASD prevalence. In this section, I describe the different missing data strategies that have been used in existing FASD research. I also describe the missing data strategies that I used to estimate the prevalence of FASD in the ALSPAC sample, and how I evaluated the relative validity of each of these strategies.

#### *4.5.2.1 Complete case methods*

Complete case methods are ubiquitous in epidemiology. Under a complete case approach, analyses are restricted to include only participants with observed data

for all relevant variables. This approach is justified on the grounds of simplicity when the loss of information is minimal and the missing data represent a random sample of all participants (MCAR).[250] Complete case analysis is not usually appropriate as data are rarely MCAR.[252] Therefore, using this method can lead to biased results and a loss of precision. Nevertheless, since complete case methods have been used in FASD prevalence studies,[84,85] I decided to investigate the validity of applying this missing data strategy and its impact on prevalence estimates. For the complete case prevalence analyses, I excluded all children who had missing data on any of the measures that were included in the Mid CNS/Any PAE FASD case ascertainment algorithm. To determine whether the MCAR assumption was plausible, I investigated missing data patterns using the *misstable* command in Stata 14.2[155] and compared the distribution of key sociodemographic factors, risk factors and clinical characteristics among participants with complete versus incomplete data on the measures required to ascertain FASD status.

#### *4.5.2.2 Single imputation methods*

Single imputation is another approach for handling missing data and involves replacing missing data with one fixed value.[250] Examples include mean substitution (missing data are replaced with the average of the observed values) and last observation carried forward methods (missing data are replaced with the last observed value in longitudinal studies).[251] Single imputation methods benefit from being straightforward to implement. However, they do not account for uncertainty in the imputed values and lead to unrealistically small estimates of variability.[252]

In FASD prevalence studies, single imputation strategies have also been used and often involve replacing missing exposure and symptom data with zero (i.e. they interpret missing data as indicating no PAE and/or no impairment). For example, in their study of FASD prevalence, Okulicz-Kozaryn and colleagues used the following single imputation method: "All uncertainties and missing test results (unless it was clearly reported that a child was not able to do the test) were interpreted in the child's favour…It was also assumed that all missing data on prenatal alcohol exposure would be interpreted as no exposure." [81(p.64)] Accordingly, for the single imputation analyses in this chapter, I assumed that missing PAE data indicated no exposure and that missing CNS, growth and facial data indicated no impairment. This is a strong assumption and I explore the validity of this single imputation strategy later in this chapter, when I describe the missing data patterns within this sample.

#### *4.5.2.3 Multiple imputation methods*

Multiple imputation can be used to address missing data problems in epidemiological analyses, under the assumption that data are MCAR or MAR.[255] Multiple imputation is a statistical technique that generates multiple sets of possible values for missing data, based on the distribution of the observed data.

Multiple imputation comprises three stages. In the first stage, the imputation procedure creates multiple versions of the dataset, in which missing values are imputed by sampling from the distribution of possible unobserved values conditional on the observed values. This step incorporates uncertainty in the imputed values by adding variability into the values across the imputation sets. The

second stage of multiple imputation uses conventional statistical procedures to fit the models that are of interest in the substantive analysis to each of the imputation sets. The effect estimates from each of the imputation sets will differ due to the inherent variability in the imputation process. In the final stage, estimates from across the imputation sets are combined using Rubin's combination rules.[252,256] Rubin's rules attribute the appropriate amount of variability into pooled estimates from imputed data by accounting for both within- and between-imputation variability and the number of imputation sets, therefore taking into account the uncertainty of the imputed values. Using Rubin's rules, pooled prevalence estimates are calculated as the average proportion across the number of imputation sets, denoted as m, as follows: ($proportion_1$ + $proportion_2$ ... + $proportion_{20}$)/m. Estimated total variance is calculated as: ([estimated average within-imputation variance] + [1 + $m^{-1}$] x [estimated between-imputation variance]).

I used multiple imputation methods to address missing data in the ALSPAC cohort under the assumption that data were MAR. It is important to note that MAR is an assumption, rather than a property of the data. To increase the plausibility of the MAR assumption, I used an inclusive strategy for the imputation model, which included hypothesised risk factors for FASD, sociodemographic variables, clinical characteristics and auxiliary variables.[252,253] Auxiliary variables are not included in the main analyses of interest (i.e. are not key exposures, confounders or outcomes), but are included in the imputation model "solely to improve the performance of the missing data procedure." [253(p.331)] Types of auxiliary variables include variables that predict whether a value is missing and variables that predict

the values of missing data (e.g. the inclusion of binge drinking at 8 weeks postpartum to predict PAE).[253] I selected auxiliary variables based on whether they had been identified as potentially relevant to the causal context of FASD (described in Chapter 5).

To generate the imputation sets, I used multiple imputation by chained equations (MICE) in Stata 14.2.[155,257] MICE is appropriate for use with the mixed pattern of missing data in this sample.[258] The MICE procedure is as follows: given a dataset in which variables $x_1$, ….., $x_k$ have missing values, all missing values are initially imputed at random. Then, the first variable to have one or more missing values ($x_1$) is regressed on all of the other variables in the imputation model ($x_2$, ….., $x_k$). This estimation step is limited to participants with observed $x_1$. Missing values in $x_1$ are then imputed by random draws from the posterior predictive distribution of $x_1$. This process is repeated for all other variables with missing values $x_{2, ….,}$ $x_{k,}$ which are regressed on all of the other variables, including the imputed values of the preceding variables in this sequence. One iteration of this sequence is called a cycle. I used 10 cycles to produce each imputed data set and generated 20 imputation sets, following guidance from Sterne and colleagues.[252] Imputation models were specified as binary, ordered categorical, non-ordered categorical and continuous for each variable as appropriate.[255,257]

FASD status is a complex variable. It is a composite binary outcome (FASD/not FASD) that is derived from multiple subcomponent variables including PAE data, psychological test scores, clinical assessments and questionnaire data that are measured at multiple time points, using a range of informants. Models that impute

subcomponents of composite outcomes have been shown to lead to increased precision and reduced bias, relative to methods that impute the composite outcomes directly.[259,260] Therefore, rather than imputing FASD status as a composite outcome, I imputed the missing values of FASD subcomponents. Following imputation, I combined these subcomponent values using the *mi passive* command in Stata[258] and applied the FASD case ascertainment algorithm to construct the final FASD composite outcome. Appendix 9 provides the full specification for the multiple imputation model.

To assess the performance of the multiple imputation model, I used the *midiagplots*[509] command in Stata to compare observed, imputed and completed data values.

### 4.5.3 Prevalence estimation

I generated prevalence estimates for total FASD and FASD subtypes (FAS, pFAS and ARND) for each of the missing data strategies (complete case, single imputation, multiple imputation), by applying the Mid CNS/Any PAE FASD case ascertainment algorithm to the relevant sample. For analyses based on the complete case and single imputation strategies, prevalence was defined as the number of participants in the sample who met criteria for FASD, divided by the total relevant sample. For estimates based on multiply imputed data, I applied the FASD case ascertainment algorithm to each imputation set and generated pooled prevalence estimates, following Rubin's rules.[256]

I calculated 95% confidence intervals using the Wilson method for the complete case and singly imputed data.[261] The Wilson method is preferable to the standard

normal approximation method when sample sizes are small or proportions are close to 0 (for example, when exploring outcomes that are relatively rare, such as FAS).[244] For estimates based on multiply imputed data, I generated 95% confidence intervals using Rubin's rules.[256]

### 4.5.4 Sample characteristics by FASD status

As well as estimating the potential burden of FASD (prevalence), it is useful to know who is most likely to be affected by FASD and to describe their clinical presentation to inform efforts for targeted prevention and assessment. Therefore, I calculated descriptive statistics to summarise participant sociodemographic and clinical characteristics, according to FASD status, using the multiply imputed data. All statistics were calculated using Rubin's combination rules.[256]

I note that there will be an element of circularity in the description of some of the clinical characteristics that I present. For example, by definition, all children with FASD will have at least three subdomains of CNS impairment and confirmed PAE.[i] However, other characteristics such as patterns of PAE, the relative profile of specific subdomains of CNS impairment, growth and facial features are free to vary and may give some indication of the most common profiles of impairment among children with FASD.

[i] Except for FAS, which can be diagnosed without confirmation of PAE (expected to be a minority of cases).

# 5 Results

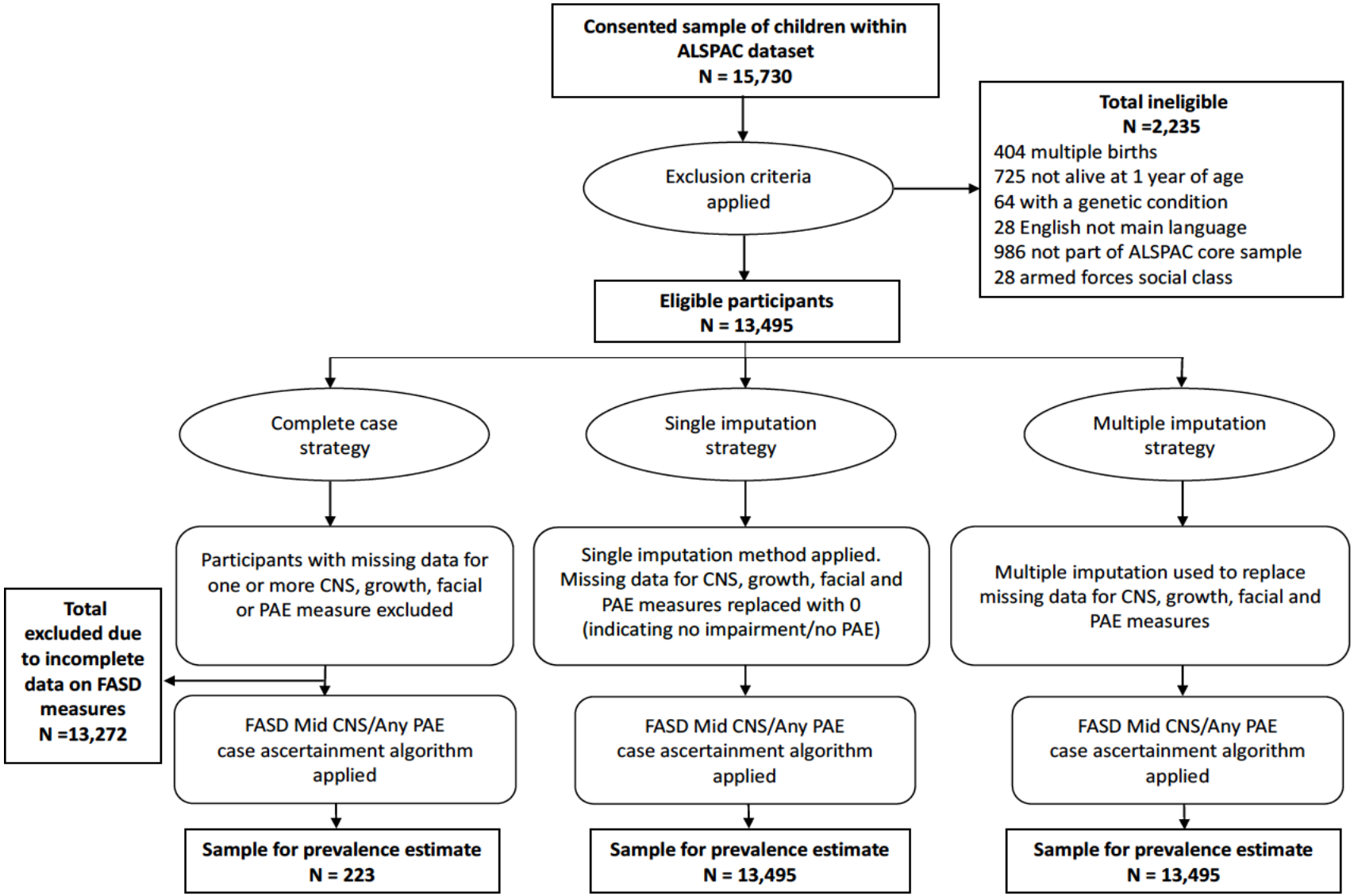
## 5.1 Participants

Figure 11 provides a summary of the number of participants included in each of the prevalence analyses, by the type of imputation strategy. There were 15,730 consented children in the ALSPAC dataset. After applying the eligibility criteria, 13,495 children remained in the sample. This sample size was preserved using the single and multiple imputation strategies to replace missing data. However, missing data led to substantial attrition in the complete case sample. 13,272 children had missing data for one or more of the measures in the FASD case ascertainment algorithm, producing a final complete case sample of 223.

### 5.1.1 Missing data patterns

Appendix 9 describes the proportion of missing data in the eligible sample for each of the variables included the imputation model. The proportion of missing data ranged from 0% for maternal age, gestational age at delivery, and child sex variables to 70% for teacher-reported communication problems at school. Forty-nine percent of participants had incomplete PAE data. Missing data patterns included a combination of monotone missing (where participants dropped out at one time point and did not complete any further measures in ALSPAC), unit missing (where participants had missing information for an entire questionnaire or clinic session) and item missing (where participants had missing data for some items of a measure, but not others).[250] Appendix 10 presents the characteristics of participants with complete data, compared to those who had missing data for one or more of the measures required to ascertain FASD status.

*Figure 11: Flow diagram depicting eligible and final sample for the FASD prevalence estimates, by missing data strategy*



Participants with complete data differed from those with incomplete data on a range of demographic characteristics, prenatal exposures and clinical characteristics, indicating that data were not MCAR. For example, compared to those with complete data, mothers of children with incomplete data were younger at delivery (aged 30 years or over: incomplete 37%; complete 53%), were more likely to report that pregnancy was unplanned (incomplete 31%; complete 16%), and were of lower socioeconomic status according to a range of indicators (e.g. home owner: incomplete 73%; complete 92%). During pregnancy, mothers of children with incomplete data were less likely to report drinking alcohol overall (incomplete 69%; complete 73%), but more likely to report binge drinking (incomplete 22%; complete 19%). They were also more likely to have smoked (incomplete 28%; complete 14%), less likely to have taken vitamin supplements (incomplete 54%; complete 57%), and were more likely to have significant depression (incomplete 21%; complete 13%) and anxiety (incomplete 24%; complete 18%) symptoms. Children with incomplete data had poorer outcomes on a range of measures including lower IQ, lower academic attainment, special educational needs, conduct problems, emotional and behavioural problems at school, and growth deficiency. However, outcomes were in the opposite direction for some measures; for example, a higher proportion of children with complete data had a smooth philtrum and thin upper lip (full details in Appendix 10).

### 5.1.2 Validity of missing data strategies for estimating FASD prevalence

The preceding analyses indicated that the missing data in this sample imposed several limitations that compromised the validity of the complete case and single

imputation strategies for estimating FASD prevalence. First, missing data led to significant attrition in the complete case sample. Only 2% of eligible participants had sufficient data to remain in the complete case sample. Second, comparison of the characteristics of participants with complete versus incomplete data revealed that missingness was associated with risk factors for FASD,[j] including lower socioeconomic status, adverse prenatal exposures and poorer outcomes on the clinical characteristics that contribute to FASD status. This suggests that both the complete case strategy and the single imputation strategy (which assumed that missing data were indicative of no PAE/no impairment), were likely to be invalid and lead to underestimates of FASD prevalence. Since these strategies have been widely used in existing FASD studies, in the next section, I present prevalence estimates based on the complete case, single imputation and multiple imputation strategies to demonstrate their impact on FASD prevalence estimates. However, given the limitations described above, I consider the prevalence estimate based on multiply imputed data to be the most valid.

## 5.2 FASD prevalence

### 5.2.1 Complete case prevalence estimates

Based on the complete case sample, 16 children (7.2%; 95% CI 4.5% - 11.3%) met criteria for any FASD. Fifteen children (6.7%; 95% CI 4.1% - 10.8%) met criteria for ARND and one child (0.4%; 95% CI 0.08% - 2.5%) met criteria for pFAS. None of the children met criteria for FAS.

[j] FASD risk factors are discussed further in Chapters 5 and 6.

### 5.2.2 Single imputation prevalence estimates

In the analyses that used the single imputation method to address missing data, 6.0% (95% CI 5.7% - 6.5%) of children met criteria for FASD. ARND was the most common subtype of FASD, accounting for 5.8% (95% CI 5.5% - 6.2%) of FASD cases. None of the children met criteria for FAS, and 0.2% (95% CI 0.1% - 0.3%) met criteria for pFAS.

### 5.2.3 Multiple imputation prevalence estimates

In analyses with multiply imputed data, 17.0% (95% CI 16.1% - 17.8%) of children met criteria for any FASD. ARND was the most common subtype of FASD, affecting 15.4% (95% 14.4% - 16.4%) of children. For the remaining FASD subtypes, 1.6% (95% CI 1.1% - 2.1%) of children met criteria for pFAS, 0.02% (95% CI 0.00% - 0.04%) met criteria for FAS with confirmed PAE, and 0.01% (95% CI 0.00% - 0.02%) met criteria for FAS without confirmed PAE.

#### *5.2.3.1 Sensitivity analyses with multiply imputed data*

I conducted sensitivity analyses to explore the impact of using different case ascertainment algorithms to estimate total FASD prevalence. The primary algorithm was the Mid CNS/Any PAE case ascertainment algorithm (results described above). I also applied the Mid CNS/Mid PAE and the Revised CNS/Any PAE algorithms. These algorithms have been described in full in Chapter 3 (see Table 10 for algorithm criteria and Table 11 for test accuracy statistics). Briefly, compared to the Mid CNS/Any PAE algorithm, the Mid CNS/Mid PAE algorithm had a more stringent PAE criterion (which required evidence of two trimesters of PAE and/or binge drinking). The Revised CNS/Any PAE algorithm had the same PAE,

facial and growth criteria as the Mid CNS/Any PAE algorithm but incorporated the changes to the CNS criteria that were recommended by the expert consensus panel (panel recommendations described in Appendix 8). The Mid CNS/Mid PAE and Revised CNS/Any PAE algorithms had lower sensitivity and higher specificity values than the Mid CNS/Any PAE case ascertainment algorithm (sensitivity 64% and specificity 70% for both; compared to 91% sensitivity and 55% specificity for the Mid CNS/Any PAE algorithm), but similar performance on the overall accuracy metric (0,1 statistic 0.47 for both; compared to 0.46 for the Mid CNS/Any PAE algorithm).

Based on the Mid CNS/Mid PAE case ascertainment algorithm, 12.7% (95% CI 11.9% - 13.4%) of children met criteria for any FASD. Using the Revised CNS/Any PAE case ascertainment algorithm, 12.8% (95% CI 12.0% - 13.5%) of children met criteria for any FASD. This indicates that the FASD prevalence estimates remained relatively high, even when algorithms with lower sensitivity, higher specificity, and more stringent PAE and CNS criteria were used.

## 5.3 Participant characteristics by FASD status

In this section, I present participant sociodemographic and clinical characteristics, according to FASD status, using the multiply imputed data.

### 5.3.1 Sociodemographic and pregnancy characteristics

Appendix 11 presents full details of the sociodemographic and pregnancy characteristics of participants, according to FASD status. Overall, FASD was more common among children whose mothers were of lower socioeconomic status. For example, 22% of mothers of children who met criteria for FASD had a partly skilled

or unskilled occupation compared to 14% of mothers whose children did not have FASD, and mothers of children with FASD were less likely to be homeowners (57%) than mothers of children who did not have FASD (76%). Patterns were similar for all other indicators of socioeconomic status, including maternal and paternal education and paternal social class. Mothers of children with FASD were younger than those without FASD. Sixty-nine percent of mothers of children with FASD were under 30 years old at the time of delivery, compared to 62% of mothers of children without FASD. Children with FASD were more likely to be male (66%). Thirty-nine percent of mothers of children with FASD reported that their pregnancy was unplanned, compared to 30% of mothers of children without FASD.

### 5.3.2 Prenatal alcohol exposure and clinical characteristics

Appendix 12 provides a full overview of the PAE patterns and clinical characteristics of participants, by FASD status. These results are summarised below.

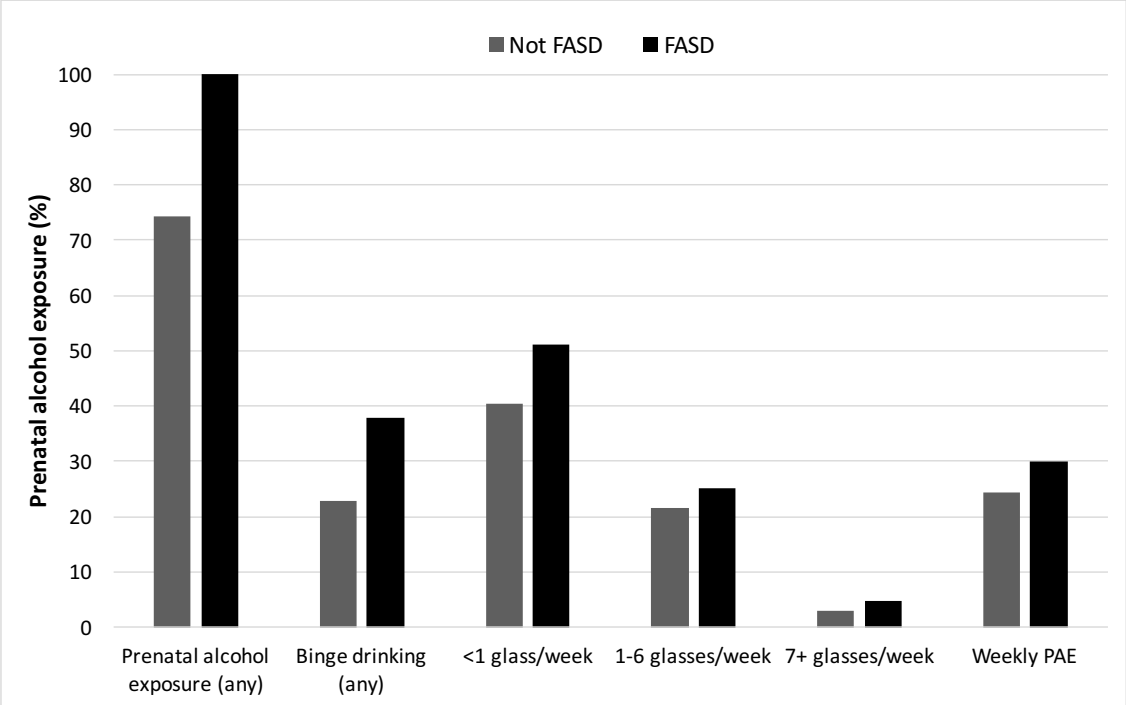
#### *5.3.2.1 Prenatal alcohol exposure (PAE)*

PAE was common in this sample. Overall, 79% of mothers drank some amount of alcohol while pregnant and 25% reported binge drinking during pregnancy. According to weekly dose/frequency measures, levels of PAE were generally low. Most women reported that they drank less than one glass of alcohol per week during pregnancy (42%). Twenty-two percent of participants reported drinking up to six glasses of alcohol per week and 3% reported drinking seven or more glasses of alcohol per week.

Figure 12 presents a summary of reported maternal alcohol use by FASD outcome. Across all measures, children who met criteria for FASD had higher levels of PAE

than children without FASD, although differences were relatively modest. Children with FASD were more likely to have been exposed to binge-level PAE at any point in pregnancy and to have been exposed to alcohol on a weekly basis.

*Figure 12: Patterns of prenatal alcohol exposure by FASD status based on multiply imputed data*




### *5.3.2.2 Clinical characteristics*

Figure 13 presents the proportion of participants who met criteria for each of the core FASD criteria (growth, facial phenotype, CNS and PAE), by FASD status. Consistent with the low prevalence of the dysmorphic subtypes of FASD (FAS and pFAS), there was a low prevalence of growth deficiency (FASD 12%; not FASD 8%) and the FAS facial phenotype (FASD 0.7%; not FASD 0.5%). By definition, all individuals with FASD had a CNS impairment in three or more subdomains, compared to 6% of individuals without FASD.

*Figure 13: Proportion of participants who met criteria for each of the primary FASD domains by FASD status based on multiply imputed data*

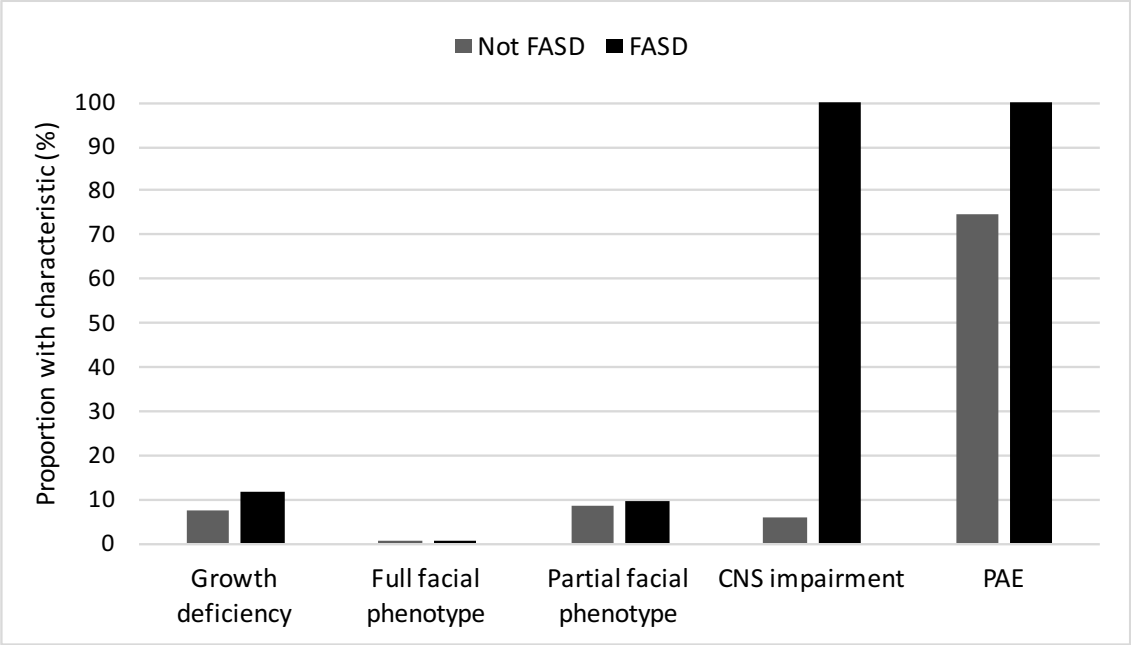



Figure 14 describes the proportion of individuals with impairment in each of the CNS subdomains by FASD status. The most commonly impaired subdomains were cognition (FASD 77% impaired; not FASD 52% impaired), attention deficit/hyperactivity (FASD 60% impaired; not FASD 10% impaired) and adaptive behaviour (FASD 85% impaired; not FASD 29% impaired). In the sample as a whole, 56% of children met criteria for cognitive impairment, predominantly due to having a difference of at least one standard deviation between IQ subscale scores (55%).

### 5.3.3 Comparison of participant characteristics by missing data strategy

To investigate which factors were most influential in accounting for the higher prevalence of FASD in the analysis with multiple imputed data, I compared the patterns of PAE and clinical characteristics across each of the missing data strategies. The relative pattern of sociodemographic, PAE and clinical characteristics of individuals with and without FASD was broadly consistent across

analyses. Compared to the complete case and single imputation methods, the multiply imputed data contained a higher proportion of individuals with PAE (up to 10% increase), growth deficiency (up to 4% increase) and CNS impairment in at least three subdomains (up to 13% increase) (see Figure 15).

*Figure 14: Proportion of participants who met criteria for impairment in each CNS subdomain by FASD status based on multiply imputed data*

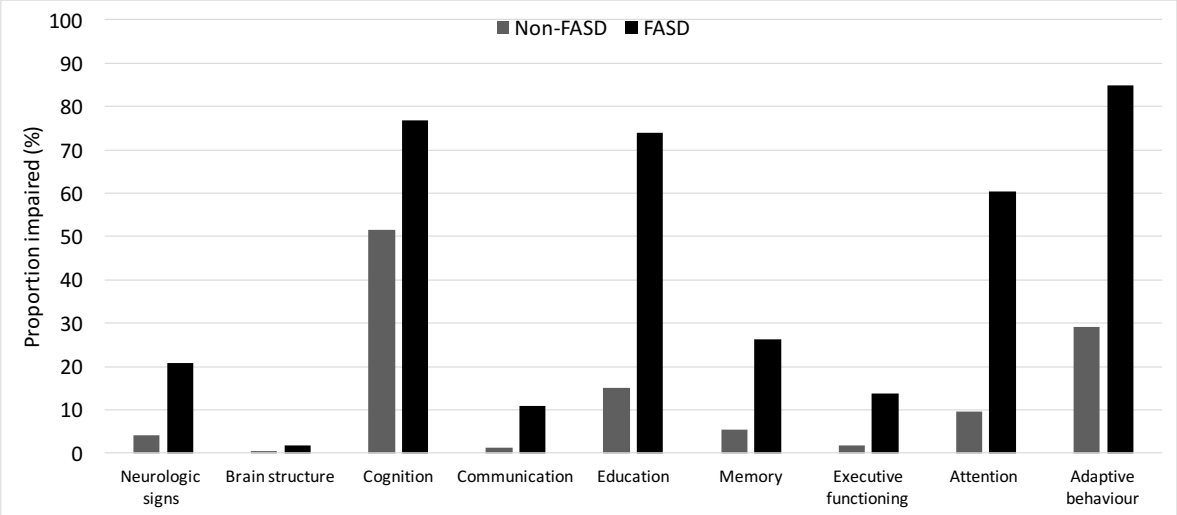



*Figure 15: Proportion of participants who met criteria for each of the main FASD domains in analyses with complete case, single imputation, and multiple imputation methods*

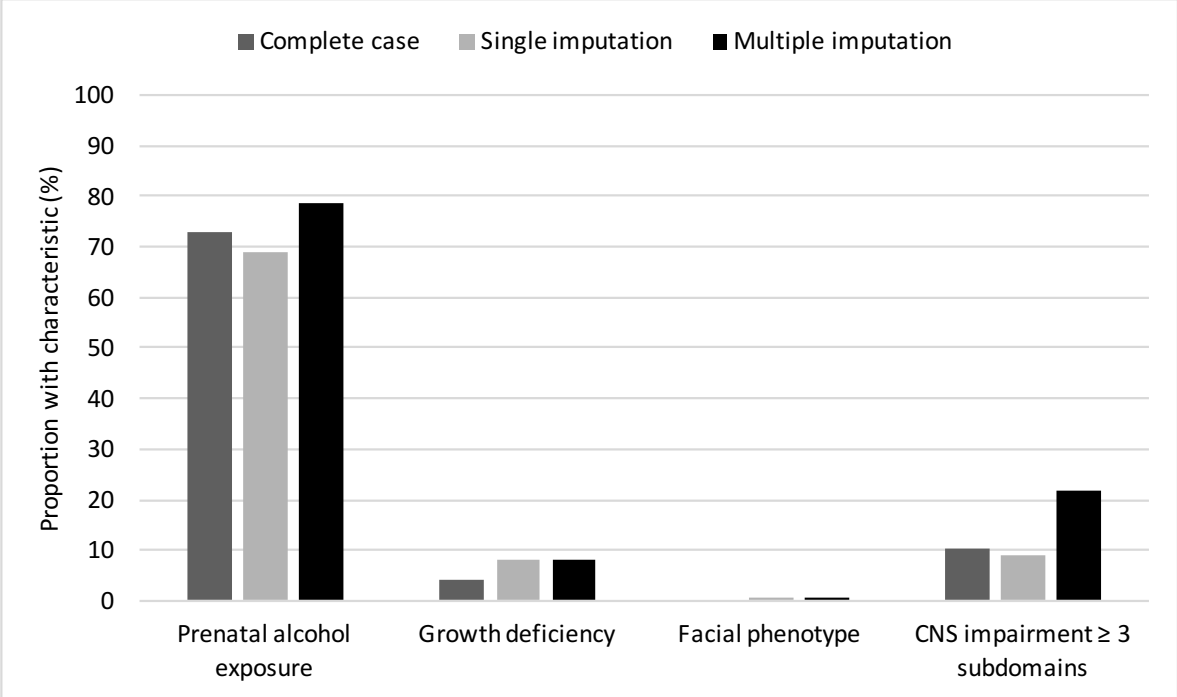
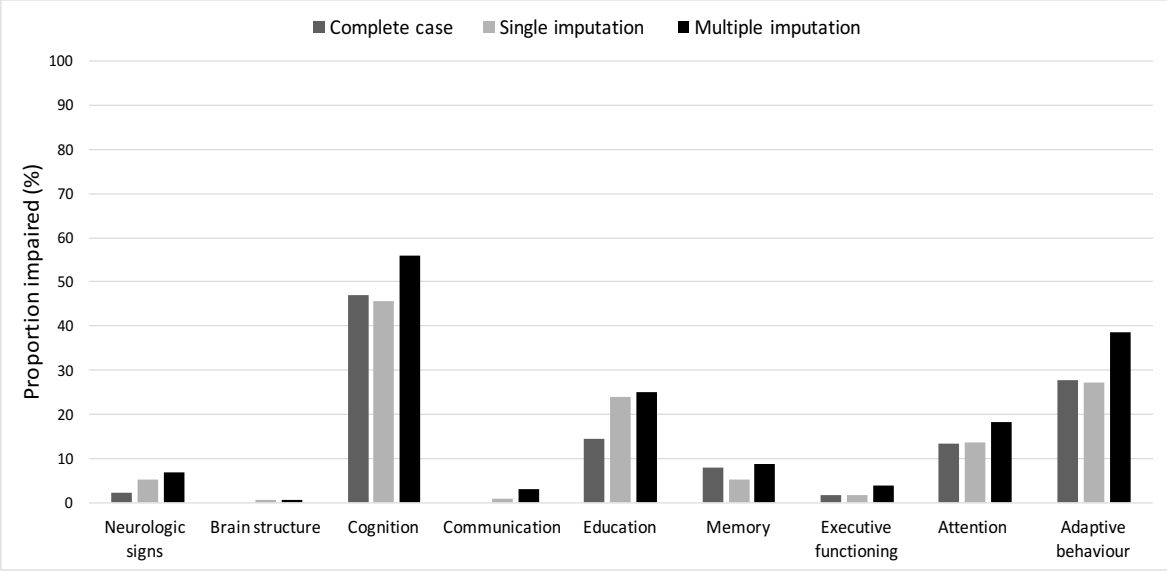


Figure 16 presents a comparison of the proportion of participants with impairment in each CNS subdomain, by missing data strategy. For all CNS subdomains, the multiply imputed data had the highest proportion of participants with impairment. The largest increases, relative to the complete case and singly imputed data, were in the cognitive (up to 10% increase) and adaptive functioning (up to 12% increase) subdomains. Overall, the increased prevalence of impairment across CNS subdomains, combined with the increased prevalence of PAE accounted for the higher prevalence of FASD in the analyses with multiply imputed data, compared to the complete case and single imputation methods.

*Figure 16: Proportion of participants who met criteria for impairment in each CNS subdomain in analyses with complete case, single imputation and multiple imputation methods*




# 6 Discussion

## 6.1 Main results

### 6.1.1 Total FASD prevalence

The estimated prevalence of FASD in the ALSPAC cohort was 6.0% (95% CI 5.7% - 6.5%) in the analyses that used a single imputation method, 7.2% (95% CI 4.5% -

11.3%) in complete case analyses and 17.0% (95% CI 16.1% - 17.8%) in analyses with multiply imputed data. The estimates for total FASD that I have presented, based on the complete case and single imputation strategies, are broadly consistent with the upper limit of studies in other European countries. To date, the only countries in Europe with estimates of FASD prevalence based on active-case ascertainment methods in the general population are Poland,[81] France (Reunion Island)[262] and Italy.[82,83] These studies, and recent meta-analyses, published in 2016 and 2017, have produced prevalence estimates of 1% to 6% for FASD in Europe.[80,86] Although the prevalence estimates that I have presented based on the complete case and single imputation methods are similar to other European studies and, therefore, have some face validity, exploration of missing data patterns indicated that these estimates were likely to be biased. Participants with incomplete data experienced more adverse prenatal exposures, including prenatal binge drinking, and had poorer performance on the cognitive and behavioural measures that were relevant to FASD. Therefore, analyses based on the complete case data and the single imputation strategy (which assumed that missing PAE and phenotype data indicated no exposure and no impairment) were likely to underestimate FASD prevalence.

The FASD prevalence estimate of 17%, based on multiply imputed data, is the most plausible prevalence estimate in this study, due to the ability of this method to reduce the bias introduced by missing data.[252,255] This prevalence estimate is significantly higher than existing estimates from other countries in Europe and the USA, which report a maximum prevalence of 10%,[79-87] but lower than estimates from South Africa, where prenatal binge drinking is more common and FASD

prevalence is up to 28%.[89] The FASD prevalence estimates based on multiply imputed data remained relatively high (13%) in sensitivity analyses that applied two FASD case ascertainment algorithms with lower sensitivity and higher specificity to the multiply imputed data, demonstrating that the high prevalence estimate in the primary analysis was not fully explained by the higher sensitivity and lower specificity of the Mid CNS/Any PAE case ascertainment algorithm.

### 6.1.2 PAE prevalence

Given that the UK has one of the highest rates of PAE in the world, FASD prevalence would also be expected to be relatively high. A recent meta-analysis produced a pooled prevalence estimate for PAE of 41% in the UK, representing the fourth highest prevalence of PAE in the world (behind Denmark [45.8%]; Belarus [46.6%] and Ireland [60.4%]).[11] Recently published studies that have prospectively assessed PAE, suggest that 75% to 79% of women in the UK drink some amount of alcohol while pregnant, and that up to 33% binge drink.[2,13] Consistent with these estimates, the data from the ALSPAC cohort indicate that up to 79% of women drank some amount of alcohol during pregnancy and 25% drank at binge levels. Compared to the levels of PAE reported in this UK sample, European studies of FASD in the general population suggest lower levels of alcohol consumption. Thirty-six percent of women in Poland, and up to 69% of women in Italy reportedly consume alcohol while pregnant.[81-83] According to the World Health Organisation, the prevalence of heavy episodic drinking among women aged 15 years or more in Italy is 0.7%, compared to 20.9% in the UK.[263,264] Binge pattern alcohol

consumption is particularly harmful to fetal development and therefore would be expected to increase FASD prevalence, as described further in Chapter 5.

The reported level of PAE based on dose/frequency measures, however, was generally low. Fifty-one percent of participants who met criteria for FASD had mothers who reported a maximum intake of less than one glass of alcohol per week during pregnancy. Interpretation of the effect of the reported quantity of PAE on FASD risk is complicated, as maternal self-report is likely to underestimate true levels of exposure.[124] The fact that a quarter of women reported binge drinking points to underreporting on dose/frequency measures. Furthermore, multiple co-occurring risk factors and maternal characteristics influence blood alcohol concentrations, the duration of fetal alcohol exposure and, therefore, alcohol teratogenicity.[8] This has led some to question whether it will ever be possible to determine a 'safe' threshold for PAE.[265] In summary, evidence of FASD among individuals with low level PAE could represent a true harmful effect of low levels of exposure or underreporting of higher levels of exposure, and of course will have been influenced by the chosen FASD case ascertainment algorithm, which considered evidence of any PAE as sufficient to meet the alcohol criterion for FASD.

### 6.1.3 FASD symptomology

Results indicated that the most common symptoms among individuals who met criteria for FASD were not specific to PAE. Symptoms were similar to those shown by children with a range of emotional, behavioural and developmental disorders. For example, the most common areas of impairment among individuals with FASD were cognition (77% impaired), adaptive functioning (85% impaired), education

(74% impaired) and attention deficit/hyperactivity (60% impaired). Such results are consistent with a recent meta-analysis that indicated that 51% of individuals with FASD had attention deficit/hyperactivity disorder, 69% had a cognitive disorder and 91% had conduct and/or behavioural problems.[90] Cognitive impairment, particularly a discrepancy in performance and verbal IQ, was common in the ALSPAC sample (56% had impaired cognition, mainly due to a significant discrepancy between verbal and performance IQ subdomains). This, coupled with a high prevalence of educational problems (25% of whole sample) and adaptive behaviour problems (39% of whole sample), in conjunction with high levels of PAE (79% of whole sample), contribute to the relatively high prevalence of FASD that I reported in this study.

FASD was more common among children of lower socioeconomic status and among children whose mother reported having an unplanned pregnancy. These results are consistent with previous studies[111] and the proposed causal basis for these associations are investigated further in Chapter 5. The finding that FASD occurred in twice as many boys as girls in this sample was unexpected, as most general population studies of FASD have found no clear sex difference.[79,81,83,266,267] A study of referrals for FASD assessment in Washington State found that boys were more likely to be diagnosed with FASD than girls.[268] Conversely, studies in ALSPAC and the UK Millennium Cohort Study have found that boys are less likely than girls to have cognitive, mental health and/or behavioural problems following PAE.[5,14,35,269,270]

### 6.1.4 FASD subtypes

ARND was the most common subtype of FASD, accounting for 5.8% of FASD cases in the analyses that used single imputation and 15.4% of FASD cases in analyses with multiply imputed data. The dysmorphic subtypes of FASD (FAS and pFAS) were less common. Across the analyses, a maximum of 0.03% of participants were classified as FAS and 1.6% as pFAS.

The prevalence of ARND in this sample is higher than estimates from existing European studies, while the prevalence of FAS is lower. Active case ascertainment studies from Poland,[81] Croatia,[84,85] and Italy[82,83] have produced estimates of 0.05% to 0.8% for ARND, 0.4% to 1.7% for FAS,[81-85] and 0.8% to 5.0% for pFAS.[81-85] As described in Chapter 1, existing UK studies of FAS have relied on surveillance methods, which are thought to significantly underestimate prevalence. Previously, the highest reported prevalence of FAS in the UK was 0.19 per 1,000 (~0.02%) based on data from a FAS surveillance study in Scotland between 2010 and 2015.[96] Using a simulation method that incorporated PAE data to estimate likely FAS prevalence, a recent study estimated that 0.6% of children in the UK may have FAS.[11] Therefore, the FAS prevalence estimate of 0.03% in the ALSPAC sample may represent an underestimate. It is important to note that the facial scan data were collected at age 15 and there is evidence to suggest that the facial features associated with PAE may become less prominent over time.[271,272] This may have led to reduced detection of children with FAS in this sample, but will not have influenced prevalence estimates for total FASD.

The higher prevalence of ARND that I report in this chapter, relative to the existing literature, may be explained in part by key differences in study design. Existing active case ascertainment studies of FASD in Europe[81-83] and many others within the USA[79,273] and South Africa,[89,274-278] follow a tiered screening protocol based primarily on child dysmorphology (growth, facial features and head circumference), with some brief neurobehavioural measures. Since studies that follow this design prioritise FASD assessment based on the visible features of FASD, ARND is likely to be "severely undercounted." [82(p.2346)] Furthermore, most of these studies have used the IOM-revised guidelines for FASD.[73] These guidelines provide more liberal facial phenotype criteria than the Canadian guidelines,[72] which may also contribute to higher FAS prevalence estimates than those reported in this chapter. Finally, the evaluation of child physical and neurobehavioural outcomes in the ALSPAC dataset are much more extensive than the assessments that have been possible in the active case ascertainment studies, which are subject to more resource and time restrictions. This too is likely to have contributed to higher prevalence estimates for FASD, relative to existing studies.

## 6.2 Strengths and limitations

To the best of my knowledge, this study is the first to produce an estimate of FASD prevalence and associated characteristics in a general population sample from England. It provides a novel approach to FASD case ascertainment, including the use of multiple imputation to reduce bias due to missing data. To date, FASD prevalence studies have included three main approaches: i. Surveillance and record review methods; ii. Clinic-based studies; iii. Active case ascertainment (e.g. in-school screening).[95] The study presented in this chapter introduces a fourth

potential approach, based on a form of retrospective active case ascertainment. The design of this study has the following advantages. It is likely to increase capture of the full spectrum of FASD, since it does not rely on dysmorphology screening as a gateway to assessment; it facilitates a large population-based investigation of FASD using a comprehensive range of measures to assess child phenotype in a manner that is significantly less costly, resource intensive and time consuming than traditional active case ascertainment methods; and, as it uses existing data, it can be conducted without additional requirements for consent from participants, which maximises participation rates. Low participation rates are an issue in FASD prevalence studies. For example, 49% to 83% of eligible children did not participate in recent FAS and FASD active case ascertainment studies in Italy, Croatia and Poland, leading to imprecise prevalence estimates, small sample sizes, and raising questions about the generalisability of the sample to the general population.[81,83,84] Therefore, given that it has not yet been possible to conduct an active case ascertainment study of FASD in the UK, the method described in this chapter arguably provides the best currently available means of exploring the epidemiology of FASD at a population level.

However, there are several important limitations of this study. First, the validity of the prevalence estimates necessarily depend on the validity of the case ascertainment algorithm. Sensitivity analyses showed that the prevalence of total FASD varied between 13% to 17% based on application of the three best-performing case ascertainment algorithms to the multiply imputed data. The validation process, and strengths and limitations of the FASD case ascertainment algorithms have been described in Chapter 3. Furthermore, an ideal clinical

assessment for FASD would include an in-person evaluation, including genetic microarray testing to support differential diagnosis.[279] This was not possible in the present study due to resource constraints (only a limited number of participant profiles could be reviewed due to time limitations in the expert case conference panel), and the use of secondary data that did not include microarray testing. In a study of 80 children with suspected FASD who were referred for genetic testing in the UK, Douzgou and colleagues found that 9% received an alternative diagnosis, due to the presence of a chromosome disorder.[280] Therefore, it must be emphasised that the prevalence estimates provided in this chapter represent the number of children who met the criteria for FASD based on the Mid CNS/Any PAE case ascertainment algorithm. This is not equivalent to a formal diagnosis. It may be that given the opportunity for a gold standard clinical and genetic assessment, some of the children would not be considered to have FASD based on differential diagnosis (i.e. that other pre- or postnatal factors might be considered to offer a more plausible explanation for the aetiology behind their clinical profile). Nevertheless, results still indicated that there were a significant proportion of children who had symptoms that suggest compromised development and who could potentially benefit from early intervention and additional support.

Other limitations stem from the concept of FASD as a whole. As described previously, many of the diagnostic criteria are non-specific to FASD. Therefore, as emphasised by the authors of the 4-Digit Diagnostic Code, in a child with prenatal alcohol (and other) exposure(s), and with the exception of those with the FAS facial phenotype, alcohol exposure may be fully, partially or not accountable for an observed pattern of impairment. Therefore, in the simplest terms, the prevalence

estimates reported in this chapter indicate that up to 17% of children in the ALSPAC sample were exposed to alcohol prenatally and have evidence of impairment in three or more CNS domains, based on the pre-specified criteria. It is not possible to prove conclusively that PAE was the key causal factor in determining the outcomes of these children. Equally, it is not possible to rule out alcohol as an important causal factor.

As described in Chapter 3, some of the tests in ALSPAC, such as the Weschler Intelligence Scale for Children (WISC-III), were administered in an adapted form. This may have influenced participant FASD status classifications. Most notably, 55% of participants in this study had a discrepancy of 15 points or more in verbal IQ versus performance IQ scores. While the authors of the WISC note that such discrepancies are not infrequent, their norms, based on a standardisation sample from the USA, indicate that such discrepancies tend to occur in a smaller proportion (24%) of the general population.[281] Furthermore, much of the data on prenatal exposures and child behaviour were measured using self-report. These data are likely to be subject to measurement error.[61,62]

Data on prenatal alcohol use in the ALSPAC cohort were collected between 1991 and 1992, at which time there were no formal guidelines for drinking in pregnancy.[282] Therefore, there is a question around the generalisability of these results to the present day. Despite the changes in guidance, the prevalence of PAE in the ALSPAC sample was similar to recently published estimates of PAE in terms of both the prevalence of any alcohol consumption and binge level alcohol use.[12,13] This suggests that results from this chapter may reflect present day patterns of PAE

and, therefore, FASD. However, it is important to note that because FASD is determined by a complex interplay of multiple risk factors that co-occur with maternal alcohol use, FASD prevalence could be subject to change based on the relative prevalence of co-occurring risk and protective factors (see Chapters 5 and 6). Mothers in the ALSPAC sample were slightly more affluent and children had higher levels of educational achievement than the general population, which poses further limitations on the ability to generalise findings from this sample to the general population of the UK.[228,229] Specifically, the estimates of FASD prevalence in this sample may be lower than estimates derived from samples with lower socioeconomic status and those that include children with poorer educational outcomes on average.

It is important to note that the effective use of multiple imputation methods to reduce bias due to missing data rests on the assumption that data are missing at random (MAR); in other words, that any systematic differences between the observed and missing values can be fully explained by the observed data. If data are missing not at random (MNAR) then analyses based on multiple imputation may not be valid. Sterne and colleagues[252] and Graham[283] note that some data will inevitably be MNAR. For example, missing data on prenatal alcohol use are likely to be determined by the true unobserved data for prenatal alcohol use, since women with high levels of consumption may be less likely to respond. Nevertheless, the inclusion of multiple measures of maternal alcohol use in the imputation model and correlates of PAE, such as socioeconomic status and prenatal smoking, increases the likelihood that data are at least partly MAR. Overall, the important point to consider is not whether data are 'purely' MAR or MNAR, but rather what

impact the MNAR data are likely to have on the results.[252,283] Of key importance to the prevalence analyses in this chapter is the extent to which the imputed prevalence estimate, based on the final composite FASD status outcome (FASD/not FASD), is likely to reflect the true prevalence of FASD in this sample. The inclusive component-based multiple imputation strategy that I used is likely to increase the plausibility of the MAR assumption, and therefore increase confidence in analyses with multiply imputed data for the following reasons. First, the imputation model included a comprehensive range of hypothesised risk factors for FASD that would be expected to convey information about likely FASD outcome. Second, the availability of the FASD status outcome is more likely to be determined by specific aspects of the child's phenotype (MAR), rather than their (unobserved) FASD status classification (MNAR). For example, a child's level of hyperactivity, conduct problems and cognitive ability are likely to influence whether they attend the in-clinic assessments, and/or whether their caregiver has completed the study questionnaires, and consequently whether a child has an observed FASD status outcome. Therefore, inclusion of the specific phenotype variables in the imputation model increases confidence in the MAR assumption. Furthermore, data in the ALSPAC cohort were collected without a specific research question in mind. Since participants were blind to the purpose of this study, missingness is less likely to depend on FASD status.

Finally, the imputation model included a range of auxiliary variables, which have been shown to reduce the bias introduced by MNAR missingness.[253] In summary, even with the expectation that some data were not MNAR, the analyses with multiply imputed data benefit from a substantially increased sample size and are

likely to be less biased than the estimates that used complete case and single imputation methods to address missing data.

## 6.3 Implications for research and practice

The results from this chapter indicate that FASD may be a significant public health concern in England. FASD is more common among children born to parents of lower socioeconomic status and children from unplanned pregnancies, suggesting that these subgroups may be particularly useful to target for prevention, assessment and intervention strategies.

Further studies are required to corroborate results. In particular, active case ascertainment studies of FASD in the UK may be particularly informative in terms of shedding further light on the epidemiology of FASD in the general population. As the British Medical Association note, UK-based epidemiological data on the prevalence of FASD are essential for supporting the case for improved diagnostic and management services in order to improve outcomes among individuals with FASD and their families.[10] Prevalence data are also important for informing policy on PAE and supporting the development of health economics models for FASD in the UK.[284]

# 7 Conclusion

FASD may be a common cause of developmental disability in England, affecting up to 17% of children in the general population. Results require corroboration and should be considered a starting point for further investigation of the potential burden of FASD in the UK.

# 8 Implications for this thesis

Results from this chapter indicate that a significant proportion of children in the ALSPAC sample met criteria for FASD. Therefore, it is possible and necessary to investigate risk factors for FASD among this group. In Chapter 5, I will present a narrative synthesis of the literature on FASD risk factors, using causal diagram methodology. This will be followed by a multivariable analysis of causal risk factors for FASD in Chapter 6.

# Chapter 5. The causal web of FASD: a causal diagram approach

## 1 Overview

In this chapter, I present a causal diagram (directed acyclic graph; DAG) that I constructed to describe the aetiological context of FASD. First, I describe causal inference theory and introduce causal diagrams. Next, I present the aims and objectives of the chapter and describe the literature review methodology. I then present the results of the literature synthesis and the corresponding DAG. I conclude the chapter with a discussion of the strengths and limitations of my approach and the implications of this work for the remaining research in this thesis.

## 2 Background

Prenatal alcohol exposure (PAE) is the sole necessary cause of FASD, but it is not always sufficient.[285] Among women who drink any amount of alcohol in pregnancy, an estimated one in 13 will have a child with FASD and one in 67 will have a child with FAS.[11,86] Fetal alcohol exposure interacts with multiple factors in a complex process to determine offspring outcome. Accordingly, rather than a simple causal chain, the image of a spider's web has been considered most appropriate for describing the causal context of FASD.[286,287]

Much of the existing FASD literature simply lists risk factors and reports associations with little consideration of the underlying causal structure.[109,111] The term 'risk factor' obscures the distinction between a predictor variable and a cause.[288] It is important to try to distinguish between causal and non-causal

associations and, specifically, to consider the combinations of causal structures, available data, and analysis strategies that may give rise to non-causal associations. While knowledge of predictor variables is important for identifying who is most at risk of FASD and for targeting interventions, causal knowledge is important for identifying effective mechanisms for prevention and intervention programmes.[289]

In this chapter, I present a causal diagram (DAG) to describe my assumptions about the causal structure of the variables that are involved in the pathways to FASD. DAGs are emerging as a gold standard method for supporting causal inference and reducing bias in epidemiological studies.[290-294] They offer a novel approach to the synthesis of the risk factor literature for FASD. Results from this chapter will guide the analyses and interpretation of results in my study of FASD risk factors, which I present in Chapter 6.

## 2.1 Causal inference

Causal inference, defined as the science of inferring the presence and magnitude of cause-effect relationships from data, is a central aim of epidemiology.[290,295] The counterfactual theory of causation has been used as a framework to support causal inference within epidemiology.[296] Under a counterfactual definition, causality refers to the notion "had the exposure differed, the outcome would have differed."[297(p. 1)] Measures of association such as risk differences, risk ratios and odds ratios can be given a causal interpretation, subject only to strong assumptions, the central of which is exchangeability. Exchangeability refers to the idea that the risk of the outcome in Group 1 would have been the same as the risk in Group 2, had the participants in Group 2 received the exposure given to Group 1,

and vice-versa. Given exchangeability, the observed risk is equal to the counterfactual risk. In randomised controlled trials, the participants in different exposure groups are exchangeable, since treatment allocation is independent of participants' characteristics. Therefore, the measure of association equals the causal effect, provided there is no additional bias for reasons such as differential loss to follow up or failure of the randomisation process.[298] However, for many public health issues, including PAE, randomisation of exposure is unethical and/or unfeasible and, therefore, it is necessary to rely on observational data. Measures of (conditional)[k] association from observational designs can be used to estimate causal effects if conditional exchangeability is created by appropriate control of bias, such as adjustment for confounders.[288]

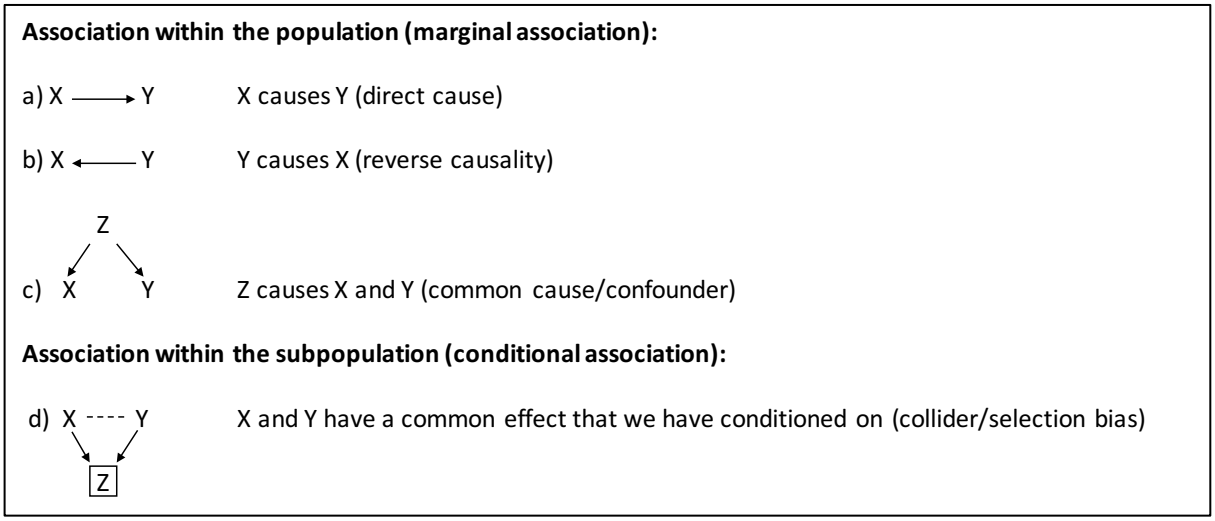
The presence of a valid statistical association is the starting point for causal inference. However, associations do not provide information about underlying causal structure. A statistical measure that conveys the strength of the relationship between variables X and Y will be identical regardless of whether X causes Y or Y causes X. In fact, four possible causal structures may account for an association between variables X and Y. Exposure X may lead to outcome Y, and thus X has a causal effect on Y. Alternatively, Y may cause X. This is an example of reverse causation. Another possibility is that there is a common cause (Z) of both X and Y. In this case, Z is a confounding variable. Finally, conditioning on[l] a common effect

[k] Conditional associations refer to adjusted effect estimates. Marginal associations are unadjusted (crude) effect estimates. This language is used to aid comparison with the wider causal diagram literature.

[l] Or equivalently: stratifying on, adjusting for, selecting on the basis of.

can create an association between X and Y. Figure 17 presents a schematic representation of possible causal structures.

*Figure 17: Four causal structures that can produce statistical association. Adapted from Daniel, R. (2015). An Introduction to Causal Inference. Advanced Course in Epidemiological Analysis. London School of Hygeine and Tropical Medicine. X, exposure; Y, outcome; Z, covariate.*




## 2.2 Approaches for strengthening causal inference in epidemiology

Several methods have been proposed to support causal inferences in observational epidemiology, including criterion-based methods such as the Bradford-Hill viewpoints.[299] However, there are no steadfast rules that can prove that an observed association is due to a causal effect and each rule is subject to limitations and/or exceptions.[300] Triangulation offers an approach for supporting causal inferences, using evidence synthesis. During triangulation, results are collated from studies that used different methodologies, each of which is assumed to have different sources of bias. The rationale is that if the results across a range of approaches indicate the same conclusion, then this increases confidence in aetiological inferences.[301,302]

Contemporary study designs, which include negative control studies and Mendelian randomisation studies, have also been used to support causal inferences. For

example, in studies of prenatal exposures, negative control studies have compared the strength of maternal versus paternal associations to distinguish between effects that are due to in-utero exposure and those that are due to confounding. The rationale is that if maternal and paternal associations are similar, effects may be due to confounding (e.g. due to shared environmental factors). Conversely, if maternal exposures are more strongly associated with child outcomes than paternal exposures, there is support for intrauterine causal processes.[303] For example, one study found that children born to mothers who smoked while pregnant had lower birthweight, while no association was observed for paternal smoking during that period,[289] thus lending support to an intrauterine causal mechanism.

Mendelian randomisation studies are based on the fact that genes are randomly assigned at conception and are, therefore, free from confounding. Some studies have used variations in alcohol-metabolising genes as instrumental variables for PAE to demonstrate that alcohol exposure has a causal influence on developmental outcomes (described later in this chapter).[303]

Causal diagrams, known as DAGs, have also been used to strengthen causal inferences in epidemiology. Judea Pearl devised the unifying framework that provided this graphical method and formal language for causal inference. Pearl reasoned that the combination of observed data plus causal knowledge allows individuals to move beyond the realm of statistical association to that of causality.[304] DAGs provide a tool for explicitly characterising assumptions about the causal relationships between exposures, covariates, and outcomes (these variables

are known as nodes in DAG terminology).[305] The graphical rules that underlie DAGs are supported by mathematical theory.[304] They provide a mechanism for identifying which variables should be controlled for, and which should not, to minimise bias in effect estimates on the basis of the assumptions encoded in the DAG.[304] In scenarios where DAGs contain a large number of variables they become less clear as visual overviews of the causal context, but maintain their utility as a theory driven approach to statistical modelling strategies. In contrast to data-driven approaches, it is the hypothesised causal relationships depicted by the DAG, rather than measures of statistical significance that guide variable selection.[294] A detailed description of DAG language and theory is provided in Appendix 13.

Since DAGs have utility both as visual representations of hypothesised causal processes and as tools to support statistical modelling strategies, I chose to develop a DAG to describe the causal context of FASD (this Chapter), and to use this DAG to inform the statistical modelling strategy for the FASD risk factor analyses (Chapter 6).

# 3 Aim and objectives

## 3.1 Aim

i. To describe what is known about the aetiology of FASD.

## 3.2 Objectives

i. To conduct a systematic literature search to identify FASD risk factors and their potential mechanism(s) of action.

ii. To consider each risk factor identified in the literature search and its role within the DAG, using a combination of narrative synthesis plus a priori knowledge and hypotheses.

iii. To consider other factors which may not be risk factors but may play an important role in the DAG, using a combination of narrative synthesis plus a priori knowledge and hypotheses.

iv. To use causal diagram theory and the results from objectives i - iii to create and interpret a DAG of the causal context of FASD.

# 4 Method

## 4.1 Literature search

I searched Medline from inception to 2nd March 2016 for existing systematic reviews of FASD risk factors using the search terms "(fasd or fetal) adj1 alcohol" AND "Risk Factors/" AND "systematic review.mp." This search produced two references, one of which, by Esper and colleagues (2014),[111] was relevant to the aims of this chapter.

Esper et al.'s review provided an overview of the demographic, psychological and social factors that are associated with FASD. However, it had little discussion of causality. To obtain more detailed information about possible causal structures, I searched for evidence in the full text articles of the included studies from this review. I also conducted separate searches for each risk factor in Medline to identify further information and to find studies that were published after Esper et al.'s review. In each search, I combined search terms for FASD and the relevant risk factor. For example, the search for prenatal stress was: (fasd or fetal) adj1 alcohol

AND stress* AND pregnan*. When available, review articles of specific risk factors were used as an efficient way to gain a summary of evidence and to identify key references.

I carried out supplementary searches of the electronic table of contents (eTOC) pages for relevant journals including: *Pediatrics; Alcoholism: Clinical and Experimental Research; the Canadian Medical Association Journal; and the Journal of Developmental & Behavioral Pediatrics*. I also searched for relevant UK-based evidence from the Infant Feeding Survey;[17,52,306] the Millennium Cohort Study;[307] and ALSPAC.[229] Other supplementary sources included the National Organization on Fetal Alcohol Syndrome (NOFAS) and EUFASD electronic newsletters, a manual search of the reference lists of relevant studies, ResearchGate and FASD conference abstracts.[308-310] Supplementary searches were concluded on the 22nd December 2017.

## 4.2 Evidence synthesis

DAGs convey assumptions about how the world works for a particular causal question. They are constructed based on subject-matter knowledge and hypotheses. To determine the most plausible structure for the aetiology of FASD, I used informal triangulation methods to evaluate the evidence from the literature search. Unlike systematic reviews, which seek to compare results from studies that are relatively homogenous, triangulation seeks to compare evidence from studies from a diverse range of approaches. The advantage of triangulation is that any biases are assumed to differ across the diverse study types. This increases confidence in causal inferences if similar effect estimates are obtained.[311] In this

narrative synthesis, I sought to compare results from studies that used at least two different approaches to address the same causal question. For example, I considered evidence from studies that used Mendelian randomisation, negative controls, multivariable regression with observational data, qualitative data, twin studies, cross-setting comparisons, randomised controlled trials, and animal studies.

To provide a framework for categorising the FASD risk factors, I used Abel and colleagues' distinction between provocative and permissive factors.[285] The risk factors described in the DAG fall into the category of permissive factors. Permissive factors are "predisposing behavioural, social, or environmental characteristics (e.g. alcohol consumption patterns, socioeconomic status, culture) that can produce certain biological conditions. These conditions…increase fetal vulnerability to alcohol's teratogenic effects."[285(p. 446)] Provocative causes, by contrast, are "the biological conditions (e.g. high blood alcohol levels, decreased antioxidant status) resulting from these permissive factors, which create the internal milieu responsible for the increased fetal vulnerability to alcohol at the cellular level."[285(p. 446)] Provocative factors have typically been explored in animal studies. In humans, the precise biological mechanisms that account for alcohol teratogenicity and result in FASD remain poorly understood.[10] Where available, I will discuss evidence about provocative factors with the aim of supporting inferences about the causal nature of permissive factors.

## 4.3 Consideration of factors other than risk factors that are relevant to the causal structures in the DAG

Based on the principles of DAG theory, and my subject matter knowledge of the challenges associated with FASD identification and the measurement of prenatal exposures, I specified a series of nodes to represent my assumptions about the most relevant sources of measurement error and residual bias. These are described in more detail in Section 5.1.3.

## 4.4 DAG construction

I used DAGitty (www.dagitty.net/)[312] to draw a DAG, based on the information that I collated in objectives i - iii. DAGitty is a platform for creating and analysing causal diagrams. It operationalises the graphical rules (described in Appendix 13) to identify which set of covariates should be adjusted for, and which should not, to minimise bias in multivariable causal effect estimates.[312] DAGitty code is provided in Appendix 14.

Although DAGitty is a useful tool for analysing complex DAGs, the space in the browser window is finite and the DAG becomes of limited use as a visual representation when the causal structure is particularly complex. Therefore, I used DAGitty as an analytical tool to identify biasing pathways, but created a Figure of the DAG using Microsoft PowerPoint[313] to provide a clearer visual representation of the hypothesised causal pathways to FASD.

# 5 Results

In this section, I present the literature review and corresponding DAG that I created to describe the causal context of FASD. In Chapter 6, I describe how I used the DAG to inform the statistical modelling strategy for FASD risk factors.

The DAG (Figure 18) provides a visual description of the hypothesised aetiological context of FASD, based on a synthesis of current evidence. To enable comparison of the literature review with the causal relationships depicted in the DAG, I first describe the groupings of variables that were implied following the evidence synthesis. I then present the supporting evidence from the literature synthesis for each variable (node).

## 5.1 Categories of relationships implied by the literature review and DAG

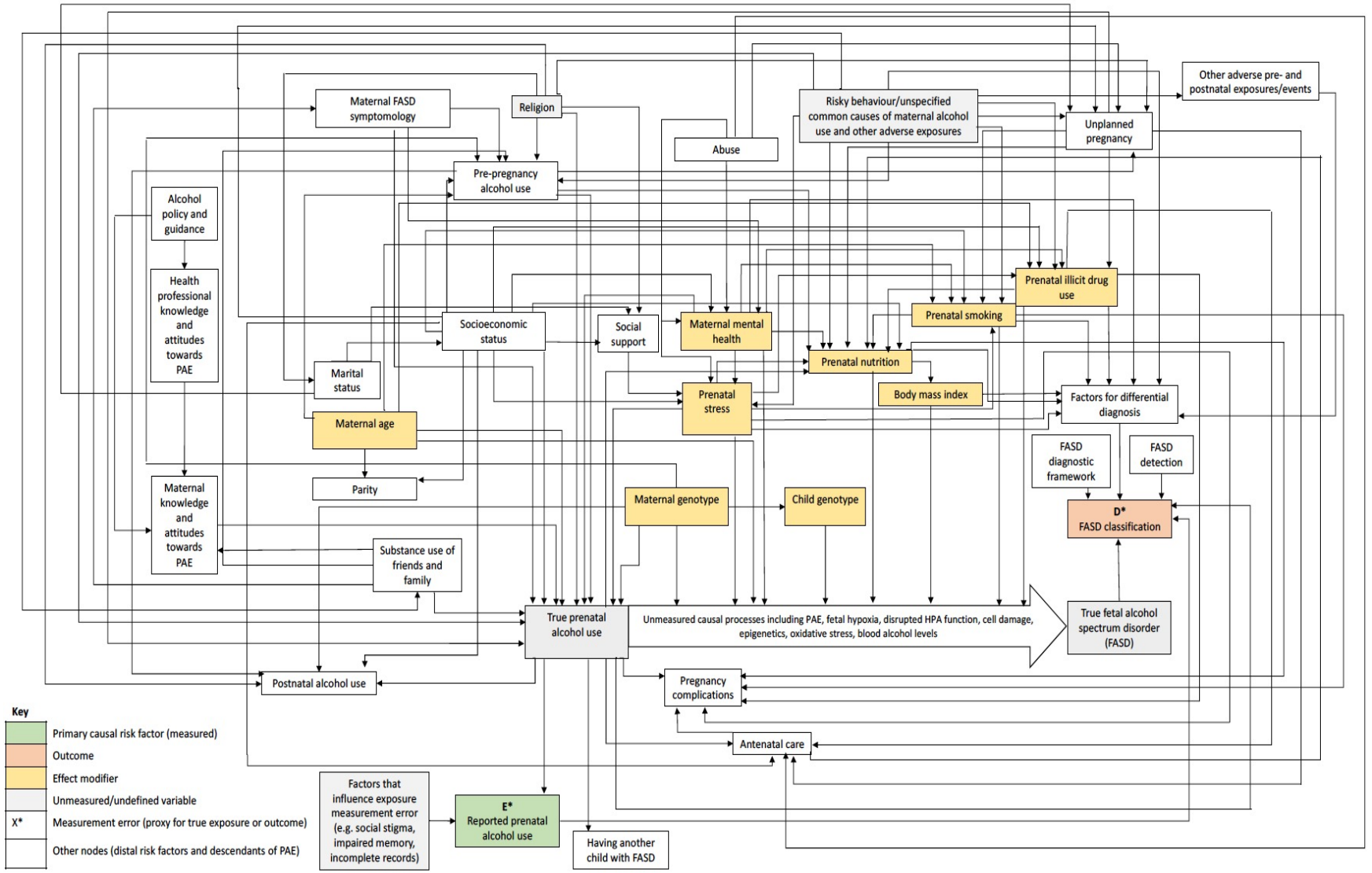
### 5.1.1 Causal risk factors

Given that alcohol is the single necessary cause for FASD, other causal risk factors were hypothesised to influence the risk of FASD in two ways: by changing the risk of alcohol exposure during pregnancy and/or by changing the effect of alcohol on the fetus. These causal risk factors are described below. The supporting evidence follows in Section 5.2.

#### *5.1.1.1 Primary causal risk factor*

Prenatal alcohol use is the sole necessary cause of FASD. The effect of maternal alcohol use is mediated by proximal unmeasured causal processes, or provocative factors, that include fetal alcohol exposure, hypoxia, free radical damage, epigenetic changes and disrupted neurotransmitter functioning.[10]

*Figure 18: Directed acyclic graph (DAG) depicting the hypothesised causal pathways to FASD*



#### *5.1.1.2 Effect modifiers*

Although PAE is the sole necessary cause of FASD, it is also useful to study exposures that modify the effect of PAE on FASD to identify opportunities for harm reduction. Identified effect modifiers include maternal age, genotype, prenatal stress, prenatal mental health, prenatal nutrition, BMI, prenatal smoking, and prenatal illicit drug use.[110,111,314] Within this group of effect modifiers, some are potentially amenable to intervention (prenatal mental health, prenatal stress, prenatal nutrition, BMI, prenatal smoking, and prenatal illicit drug use) and some are not (maternal age, genotype). Modelling strategies that estimate the effect of proposed causal effect modifiers on FASD can suggest useful potential targets for intervention. Therefore, I will focus on a subset of these potentially modifiable risk factors in the analyses that I present in Chapter 6.

#### *5.1.1.3 Distal causal risk factors FASD*

Most of the risk factors that have been identified for FASD feature at a more distal stage in the causal pathway. These factors become independent from FASD once the proximal causal factors (patterns of PAE) and effect modifiers have been accounted for. Distal factors include: substance use of friends and family, social support, socioeconomic status, marital status, maternal FASD symptomology, alcohol use before pregnancy, religion, abuse and unplanned pregnancy.

Although these factors feature at a more distal stage of the causal pathway to FASD, some may be useful targets for intervention. For example, if low social support increases the risk of FASD by increasing prenatal stress, which in turn

increases the risk of PAE, then social support initiatives may be a useful focus for intervention. I will investigate the effect of social support on FASD in Chapter 6.

### 5.1.2 Non-causal factors (risk markers)

Having another child with FASD and postnatal alcohol use are depicted as risk markers for FASD. These factors do not lie on the causal pathway between substance use in the current pregnancy and FASD, since they occur outside of the index pregnancy. Nevertheless, they may be useful indicators of adverse prenatal exposures and, therefore, may be informative for identifying children who require follow-up and assessment.

### 5.1.3 Other categories

In the preceding sections, I described categories of FASD risk factors and risk markers. Here, I specify other nodes that were included in the DAG to represent potential sources of bias and measurement error.

#### *5.1.3.1 Confounding: clustering of adverse exposures/events*

Adverse exposures and events such as substance use, unhealthy diet, life stressors and adverse pre- and postnatal environments, tend to co-occur.[315-319] Rather than one of these factors simply causing the others, it is likely that their co-occurrence reflects some latent common cause. This common cause has been labelled 'risky behaviour' in some DAGs that link alcohol use and smoking, and in studies that explore binge drinking and unplanned pregnancy.[320,321] Therefore, for the DAG that I present in this chapter, I have included an overarching node labelled "*risky behaviour/unspecified common causes of maternal alcohol use and other adverse*

*exposures"* to represent the latent factors that cause adverse exposures to co-occur. The clustering of adverse exposures may be due to a variety of unknown and unmeasured factors. Therefore, this common cause node was added to the DAG to represent this potential source of residual confounding. In the analysis of FASD risk factors that follows in Chapter 6, I describe a factor in the ALSPAC dataset that I used to partially control for this source of confounding.

#### *5.1.3.2 Measurement error*

In many epidemiological studies, it is not possible to directly measure the true exposure and true outcome. Therefore, it is necessary to rely on surrogate measures that approximate but do not perfectly match the values of the true exposure and outcome.[322] The degree of bias depends on the extent to which the measured values deviate from the true values.[323] Measurement error of PAE and of FASD is a particular concern. Therefore, for reasons of transparency, I represented these sources of error as extra nodes in the DAG. It is important to note that measurement error is implicitly acknowledged for all reported exposures in the DAG (e.g. prenatal smoking, prenatal stressful life events), however I chose not to depict these factors as extra nodes for reasons of visual clarity.

##### *5.1.3.2.1 Measurement error of the exposure: PAE*

Ascertainment of PAE often relies on maternal self-report or incomplete medical records, which are thought to underestimate true maternal alcohol use.[61,62] As described in Chapter 2, established biomarkers for PAE are not currently available.

True PAE is influenced not only by patterns of maternal alcohol use, but also by maternal and fetal metabolism, and a range of other factors that modify the

teratogenic effects of alcohol.[26] In the absence of measures of fetal blood alcohol concentration, there will be measurement error of true PAE. Therefore, in the DAG, I depicted self-reported maternal alcohol use as a source of measurement error. It is a factor that is associated with, but distinct from, true maternal alcohol use. Measurement error of the primary exposure (true maternal alcohol use) will also contribute to measurement error of the outcome (FASD diagnosis), since alcohol use is a diagnostic criterion for FASD.

#### *5.1.3.2.2 Measurement error of the outcome: FASD diagnosis*

FASD diagnosis is a surrogate for true FASD status. FASD diagnosis is influenced by a range of factors. These factors are represented as separate nodes within the DAG and include: the choice of diagnostic framework (described further in Appendix 3), adequacy of FASD detection (e.g. the availability of diagnostic services for FASD), and consideration of factors for differential diagnosis (described further in Appendix 5).[76] The FASD Canadian guidelines for diagnosis (2005), describe the issue of differential diagnosis as follows: "The face of FAS is the result of a specific effect of ethanol teratogenesis altering growth of the midface and brain. Those exposed to other embryotoxic agents may display a similar, but not identical, phenotypic facial development, impaired growth, a higher frequency of anomalies and developmental and behavioural abnormalities... Knowledge of exposure history will decrease the possibility of misdiagnosing FASD."[72(p S7)] As FASD shares many overlapping features with other disorders and exposures, it has been described as a diagnosis of exclusion.[324]

#### *5.1.3.3 Biological mechanisms (provocative factors)*

The specific biological mechanisms that cause FASD are still under investigation.[10] Therefore, within the DAG, I depicted these provocative factors as a broad category of unmeasured factors that mediate the relationship between PAE and FASD (denoted as a large connecting arrow between true prenatal alcohol use and true FASD).

## 5.2 Supporting evidence by variable type

In this section, I present the narrative synthesis of evidence that supports the inclusion of factors in the DAG, and their position, under the subheadings: lifestyle factors, sociodemographic factors, pregnancy factors, stress and social support, maternal characteristics and family factors.

### 5.2.1 Lifestyle factors

#### *5.2.1.1 Patterns of prenatal alcohol exposure (PAE)*

The impact of PAE is thought to vary according to the dose, frequency and timing of exposure. However, residual confounding, measurement error and individual variability across a vast range of covariates complicate efforts to determine what pattern of maternal alcohol use will lead to FASD in an individual case. Nevertheless, on average, binge and heavy chronic patterns of maternal alcohol use are most likely to result in FASD.[19,79,276,325-327] While these results provide insight into the effects of heavy episodic drinking, evidence about the impact of lower-level PAE on FASD is less clear. As described in Chapter 1, evidence on the effects of low to moderate PAE on developmental outcomes is limited and inconsistent, ranging from evidence of harm to evidence of slight

benefit.[20,21,26,27,328] For example, in an observational study that used data from the Millennium Cohort Study, Kelly and colleagues found that 3-year old boys who were born to mothers who reported drinking no more than one to two units of alcohol per week or per occasion during pregnancy had a lower risk of hyperactivity and conduct problems than those born to abstainers.[14] Negative control studies that compare the strength of association for maternal and paternal exposures have also been used to investigate the causal effects of low to moderate PAE, but did not find evidence to support intrauterine effects on child IQ or head circumference.[329,330] In contrast, Mendelian randomisation studies have offered evidence that low to moderate PAE can cause persistent conduct problems and adversely affect cognitive and academic outcomes.[26,27,331] Despite inconsistencies in the evidence, Mendelian randomisation studies provide stronger causal evidence than observational studies that low to moderate alcohol use can cause adverse developmental outcomes. They suggest that the apparent null or protective effects of low to moderate PAE, are likely to be due to residual confounding, owing to the socioeconomic patterning of prenatal alcohol use.[21,24,26]Overall, Clarren and colleagues argue that "based on the variabilities between mothers and fetuses, metabolism, genetics, interactions of environmental factors, it is very unlikely that 'absolute risk' for the harmful effects of alcohol consumption during pregnancy will be established and the question of 'how much is too much' will remain unanswered. Thus, the best advice is no exposure equals no risk."[265]

#### *5.2.1.2 Alcohol use before pregnancy*

Alcohol use before pregnancy predicts FASD.[111,266] Mothers of children with FASD are more likely to drink at high levels before pregnancy, to have had longer 'drinking careers' (i.e. more years of drinking alcohol), to have a history of alcohol abuse and to have been referred to alcohol treatment, relative to controls.[276,325,327,332] Chronic alcohol consumption impairs the functioning of alcohol metabolising enzymes and can lead to malnutrition due to reduced intake and absorption of key nutrients, thus increasing the risk of FASD.[285,333-336] Therefore, alcohol use prior to pregnancy can influence FASD risk by influencing alcohol metabolism and creating a nutritional state that can compromise fetal development.

In the UK, up to 91% of women aged 16 to 45 report drinking some level of alcohol and excessive alcohol consumption is common.[337,338] Up to 47% of women aged 16 to 44 drink above national guideline recommendations (> 3 units per day) and approximately 30% binge drink (> 6 units per day).[337] Few women follow guidance for prenatal alcohol use when planning a pregnancy[58] and alcohol use increases the risk of unplanned pregnancy.[321,339-341] If pregnancy is unplanned, women are less likely to modify their alcohol intake, due to delayed pregnancy recognition.[321,339-341] Therefore, alcohol use prior to pregnancy is a significant risk factor for PAE and FASD.

#### *5.2.1.3 Postnatal alcohol use*

Postnatal levels of alcohol consumption are higher among mothers of children with FASD.[82,83,266,274,276,278,342-345] Of course, heavy postnatal alcohol use cannot cause

FASD among children who have already been born. It can, however, indicate a continuing pattern of heavy alcohol use and serve as a risk marker for previous and future alcohol-exposed pregnancies. Heavy postnatal alcohol consumption may be a useful proxy for PAE, as women may be more likely to disclose heavy current drinking than prenatal drinking. Studies from the USA and Italy found that self-reported prenatal alcohol use was not consistently different between mothers of children with FASD and controls, but current consumption was significantly higher.[79,82,345] In contrast, studies in the Western Cape of South Africa (where maternal self-report is believed to be more reliable, due to normative binge drinking patterns and higher levels of PAE) show consistently higher levels of self-reported PAE and current consumption among mothers of children with FASD.[266,274,276,278,342,344] In summary, postnatal drinking patterns may be particularly useful as a risk marker for PAE and for future FASD risk in populations that are particularly susceptible to underreporting of PAE.

#### *5.2.1.4 Smoking during pregnancy*

Prenatal cigarette smoking is more common among mothers of children with FASD than those without.[111,266] Smoking and PAE may interact synergistically to increase the risk of FASD-related outcomes including low birth weight.[346] Proposed biologic mechanisms for the interaction between tobacco and alcohol include common effects on nutrient availability, oxidative stress and vasoconstriction of the placenta and umbilical cord, which can lead to hypoxia and prolonged uterine exposure to ethanol.[8,285,346]

In addition to its interactive effect with PAE, prenatal smoking is independently associated with reduced fetal growth, low birthweight and cognitive and behavioural impairment.[315,347-353] Evidence suggests that the impact of smoking on decreased birth weight is 1.5 to 3 times greater than that of PAE.[315,354] Therefore, prenatal tobacco exposure is also an important factor for differential diagnosis in FASD.

Prenatal smoking is associated with several other factors in the DAG. It is more common among mothers with an unplanned pregnancy, mothers with other forms of substance use, mothers who report prenatal stress, younger mothers, and mothers of lower socioeconomic status.[52,316,347,351]

#### *5.2.1.5 Illicit drug use during pregnancy*

Prenatal illicit drug use is more prevalent among mothers of children with FASD[273,325] and it can lead to physical, cognitive and behavioural impairments that are relevant to FASD diagnosis.[317,355] Since prenatal drug exposure can lead to FASD-relevant symptomology, it is an important consideration for differential diagnosis. Cocaine, opiates and amphetamines have been found to increase the risk of intrauterine growth restriction, low birth weight, small head circumference and congenital anomalies.[317,350,355,356] However, the effect of illicit substance exposure on birthweight and fetal growth is thought to be less severe and persistent than that of PAE and prenatal smoking.[315,349] Evidence suggests that children with prenatal illicit substance exposure may show catch-up growth after infancy[357] and that effects are significantly attenuated following adjustment for co-occurring adverse social factors.[318] Evidence on the effect of marijuana exposure on

growth outcomes has been inconsistent.[315,349,358] However, there is some evidence that prenatal marijuana use may lead to subtle impairments in executive functioning, motor skills, attention and memory in childhood and adolescence.[359-361] In a mouse model study, synthetic cannabinoids were found to interact synergistically with ethanol to increase the prevalence of ocular defects. Investigations indicated that the defects may have been caused by suppression of the Shh signalling pathway, which is involved in brain and facial development.[362]

Evidence about the long-term effects of cocaine, opiates and amphetamines on neurodevelopmental outcomes in humans is inconclusive. Prenatal cocaine exposure may impair attention, speech and language development.[363-365] Opiate exposure can lead to neonatal abstinence syndrome, which is characterised by abnormal arousal and irritability.[355,356] Prenatal amphetamine exposure has been linked to smaller subcortical volume, externalising behaviour, and deficits in attention and memory.[366-368]

It is unclear whether illicit drugs modify the effects of PAE. Schempf and colleagues failed to find an interaction between PAE and maternal illicit drug use on birth outcomes.[318] However, evidence suggests that prenatal illicit drug exposure and PAE share common biological mechanisms of harm including restricted blood flow to the fetus and altered neuroendocrine regulation.[317,369]

In relation to other variables in the DAG, prenatal illicit drug use is associated with a range of social and psychological factors including low socioeconomic status, stress, mental health problems, abuse and low social support.[317,318,370] Although maternal drug use may contribute to subsequent adverse social and psychological

outcomes, such factors are primarily perceived as preceding factors that increase the likelihood of drug use as a strategy for self-medication.[318] Illicit drug use increases the risk of inadequate prenatal care, pregnancy complications, poor nutrition and is more common among older women and those with an unplanned pregnancy.[64,317,318,369]

#### *5.2.1.6 Prenatal nutrition*

Optimal nutrition is important for fetal development.[314] NICE guidelines recommend that pregnant women should eat a varied and healthy diet, take folic acid supplements before pregnancy and throughout the first trimester, and take vitamin D supplements during pregnancy and while breastfeeding.[371] Folic acid and vitamins C and D are available to eligible low-income women in the UK via the Healthy Start Programme.[372] The Royal College of Obstetricians and Gynaecologists advises pregnant women against taking some vitamin supplements, particularly vitamin A, which may be teratogenic in high doses.[373] Nutrient deficiency during pregnancy increases the risk of a range of adverse outcomes including neural tube defects, restricted fetal growth, low birth weight and skeletal deformity.[374-376] Therefore, prenatal nutrition is an important consideration for differential diagnosis when assessing FASD.

Prenatal nutrition also features at several other points in the causal context of FASD. Nutrition modifies the impact of PAE on FASD, influences maternal BMI, and is influenced by maternal substance use, maternal mental health, stress, antenatal care, whether pregnancy was planned or not, and socioeconomic status.[109,285,314,377,378] Recent evidence from the ALSPAC cohort found that greater

consumption of processed foods in pregnancy was associated with heavier alcohol intake and that a healthier diet was associated with light-to-moderate alcohol intake.[379] These associations may be due to latent factors such as tendencies towards healthy or unhealthy lifestyle behaviours (depicted as the "risky behaviour" node in the DAG).

Among children with PAE, lower calorific intake has been found to increase the risk of FASD, while evidence from animal studies suggest that specific nutrients (including: vitamin A, docosahexaenoic acid, folate, zinc, choline, vitamin E, selenium, riboflavin, calcium, docosapentaenoic acid, zinc, b-vitamins, iron and protein) may reduce the risk of FASD-relevant outcomes including physical malformations, growth deficiency, behavioural regulation and memory.[109,314,380-383]

During pregnancy, mothers of children with FASD report a lower intake of key nutrients and report being hungry more often than controls.[274,384] Deficient nutrient intake does not, however, fully explain increased risk for FASD. Even when dietary intake is equivalent, alcohol-exposed rats weigh less and produce offspring with poorer outcomes than unexposed rats.[381,385] Alcohol competes with nutrients that are essential for fetal development due to shared metabolic pathways.[386,387] Alcohol consumption can also lead to impaired placental blood flow and nutrient transportation.[314,336,388,389] Nutritional supplementation has been found to attenuate FASD symptomology in some animal models.[314] Optimal nutrition may protect offspring from alcohol-related harm by reducing the oxidative stress caused by alcohol metabolism.[390,391]

Several biological mechanisms have been proposed to account for ethanol-nutrition interactions. Nutrition influences the rate of ethanol metabolism and blood alcohol concentrations.[392] For example, alcohol-exposed rats given a low-calorie diet have slower rates of ethanol metabolism and greater levels of fetal toxicity (reduced litter size and more fetal deaths) than adequately nourished rats.[381] Low-calorie and low-protein diets have been found to reduce the activity of alcohol-metabolising enzymes, leading to sustained blood alcohol concentrations.[336,393] Finally, inadequate nutrition may interact with PAE to influence gene expression. Shankar and colleagues demonstrated that alcohol-exposed, undernourished rats showed altered expression of several genes, including those associated with growth, stress regulation, cell proliferation and apoptosis.[393]

Trials of pre- and postnatal nutritional interventions with alcohol-exposed children have found complex and inconsistent results.[394-396] The Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) is carrying out a prospective cohort study in Ukraine that explores the effect of prenatal multivitamin and choline supplementation on children with and without PAE. Follow-up at six to twelve months failed to find an interaction between gestational alcohol use and nutritional supplementation on developmental outcomes (Bayley Scales of Infant Development)[394] or information processing (habituation paradigm).[395] However, there was a significant interaction between periconceptual alcohol use, nutritional supplementation and child sex. Bayley Mental Development Index scores were lowest in males who had been exposed to alcohol in the periconceptual period and who did not receive multivitamin supplementation.[394] A small (N = 55) randomised

controlled trial of postnatal choline supplementation among school-aged children with FASD found no improvement in neurobehavioural symptoms.[397] However, the consequences of PAE may be subtle and are often difficult to detect until later in childhood.[43]. Therefore, the effects of pre- and postnatal nutritional supplementation on FASD symptoms in children are not yet established.

### 5.2.2 Sociodemographic factors

#### *5.2.2.1 Socioeconomic status*

Indicators of socioeconomic status (SES) including low maternal education, low income and unemployment have been identified as risk factors for FASD.[111,266] Perhaps counterintuitively in light of these results, the UK Infant Feeding Survey has consistently found that women within higher social classes are more likely to drink during pregnancy than those from lower social classes.[16,18,52,306] UK-based cohort studies have echoed these results, showing that women who drink in pregnancy are more highly educated, less likely to live in deprived areas, and more likely to be employed than abstainers.[13,26,398] Although high SES is associated with an increased risk of prenatal alcohol use, mothers of low SES who do drink during pregnancy are more likely to do so in a binge pattern.[26] As well as differences in the social patterning of alcohol use, children born to high SES mothers may be relatively protected against the harms of PAE due to factors associated with social advantage.[328] Based on the available evidence, it is hypothesised that SES is associated with FASD via the causal pathways proposed by Abel and colleagues,[285] in which low SES contributes to FASD through its influence on drinking patterns, stress, mental health, substance use and nutrition.

#### *5.2.2.2 Maternal age*

Several studies have found that older maternal age is associated with an increased risk of FASD.[111,266] Older mothers are more likely to drink during pregnancy than younger mothers.[13,16,18,52,306] Blood alcohol concentration (BAC) per unit dose increases with age, leading to higher levels of exposure per unit consumed among the offspring of older mothers.[399-402] Higher BACs may be partly explained by age-related changes in body composition including an increased body-fat-to-water ratio among older individuals.[399,401-403] Some studies have also found differences in rates of ethanol metabolism among older participants, although results are mixed.[400-402,404-409] Chronic alcohol exposure, which is associated with maternal age, can impair the function of enzymes responsible for transporting nutrients that are important for fetal development.[389,410] Thus, older maternal age is proposed to increase the risk of FASD due to increased PAE and reduced nutrient availability.

#### *5.2.2.3 Marital status*

Mothers of children with FASD are less likely to be married and more likely to be cohabiting with a partner than controls.[111,274,276,325,343,344,411,412] Existing studies do not offer an explanation as to the causal basis of this relationship, however it is possible that marriage may protect against FASD by offering a form of social support. It may also indicate fewer relationship problems, which have been cited as a source of prenatal stress and predictor of PAE among mothers of children with FASD (see Section 5.2.4.1). In addition, marriage is associated with higher socioeconomic status, which in turn predicts a lower risk of FASD. Unplanned

pregnancy, a further risk factor for PAE, is lower among married women and this too may reduce the risk of FASD (see Section 5.2.3.4).

#### *5.2.2.4 Religion*

In their review of FASD risk factors, Esper and colleagues concluded that 'less religious' women had an increased risk of having a child with FASD.[111] However, the evidence base is mixed. Three studies in South Africa found that mothers of children with FASD reported a lower frequency of church attendance and prayer than controls.[274,276,344] However, in Italy, women categorised as more religious were more likely have a child with FASD than those classed as less religious.[82,83] Other studies have found no significant differences in the religious practices of mothers of children with FASD and those without.[413] These studies do not provide any insight into possible causal relationships between religion and FASD. However, the extent to which religion may protect against, or be a risk factor for, FASD is likely to depend on its association with more proximal risk factors for FASD, such as alcohol use, unplanned pregnancy and social support. For example, religions differ in their stance towards alcohol consumption. The Islamic faith promotes abstinence from alcohol and abstinence is high among Muslims.[414] The prevalence of FASD is 50 times lower than the global average in the World Health Organisation (WHO) Eastern Mediterranean Region, where the population is predominantly Muslim.[11] In contrast, wine is part of Communion in the Catholic faith and abstinence is much less common among Catholics.[414] Astley and colleagues found that mothers of children with FAS were more likely to become abstinent in the future if they reported a religious affiliation and more satisfactory support

networks.[70] Finer and colleagues found that religion was associated with a reduced risk of unplanned pregnancy.[415] Therefore, it is possible that religion may protect against FASD if the faith promotes abstinence from alcohol, or drinking in moderation, reduces the risk of unplanned pregnancy, and offers a satisfactory form of social support for mothers.

### 5.2.3 Pregnancy factors

#### *5.2.3.1 Parity*

Parity is higher among mothers of children with FASD, relative to controls.[111,266] Some authors have suggested that higher parity could increase susceptibility to alcohol teratogenicity due to greater levels of uterine collagen and elastin, which reduce blood flow and contribute to fetal hypoxia.[285] However, experimental studies suggest that it is maternal age, rather than parity that modifies the effect of PAE on offspring outcomes.[403,416] When rats of the same age, but different parity, are exposed to equivalent levels of alcohol the number of birth defects are comparable across groups. In contrast, older rats are more likely to produce offspring with birth defects following PAE than younger rats, when parity is equivalent.[416,417] Based on this evidence, in the DAG, I depicted parity as an effect of maternal age in the DAG, rather than a direct causal factor for FASD.

#### *5.2.3.2 Pregnancy complications*

Mothers of children with FASD report more complications including preterm delivery, fetal distress, miscarriage, stillbirth, placental abruption and admission to special care baby units.[276,326,411,412] Pre- and perinatal complications are an effect of PAE and therefore may serve as a marker for FASD risk.

Complications during pregnancy and delivery have also been linked to other exposures including prenatal smoking, prenatal illicit drug use, poor nutrition and high levels of prenatal stress.[276,326,369,374,411,412,418-420] The risk of pregnancy complications may be mitigated by access and adherence to antenatal care recommendations and monitoring.[25,64]

#### *5.2.3.3 Antenatal care*

Compared to controls, mothers of children with FASD are less likely to have received antenatal care, more likely to have accessed care late in pregnancy and more likely to have attended fewer antenatal appointments.[273,325,326,411,412] Substance misuse may act as a barrier to antenatal care. The NICE *Pregnancy and Complex Social Factors* guideline suggests that women who misuse substances are less likely to access prenatal care due to negative staff attitudes, fear about the involvement of children's services, and feelings of guilt.[64]

As well as influencing uptake of antenatal care, prenatal substance use may be influenced by antenatal care. For example, mothers accessing antenatal care via the NHS are provided with information about the risks of prenatal substance use and are given details of organisations including Alcohol Concern, the Drug and Alcohol Helpline and smoking cessation services.[421] In the USA, pregnant women who have health insurance are approximately 50% less likely to have consumed alcohol in the last month, further suggesting that antenatal care can influence PAE (although this relationship may also be due to the association between PAE and socioeconomic status).[422] Therefore, there is a time-dependent causal relationship between prenatal substance use and antenatal care. As it is not possible to have

feedback loops within DAGs, the full sequence can be conceptualised as a series of time-dependent factors: *substance use before pregnancy → prenatal substance use at time 1 → attendance at antenatal care → prenatal substance use at time 2.* For clarity of presentation, in the DAG I depicted antenatal care as a consequence of prenatal substance use (time 1 relationship).

#### *5.2.3.4 Unplanned pregnancy*

Worldwide, 40% of pregnancies are unplanned,[423] ranging from 16% in the UK,[341] 35% in Africa, and 51% in North America.[423] Unplanned pregnancy is a risk factor for FASD. Among a sample of mothers of children with FAS in the USA, 73% of live births were unplanned.[70] The median time to pregnancy recognition among women with an unplanned pregnancy is five weeks.[321] Unplanned pregnancy is more common among women who drink regularly and/or binge drink,[321,339-341] and binge drinking is associated with inadequate methods of contraception.[424] Therefore, the risk of unintended PAE during the periconceptual period is high.

Studies from England, Ireland and the USA indicate that women who have an unplanned pregnancy are less likely than those with a planned pregnancy to follow advice for prenatal lifestyle behaviours including alcohol use, smoking and diet.[57,58,425] A qualitative study of pregnant women who attended alcohol establishments in South Africa suggested that some women with unplanned pregnancies continued to drink because they did not feel a connection or responsibility for their unborn baby, and some women reported drinking in an attempt to abort the fetus.[426]

Other factors associated with an increased risk of unplanned pregnancy include low socioeconomic status, single marital status, exposure to intimate partner violence, smoking, illicit drug use and younger maternal age; while religion is associated with a decreased risk.[70,321,339,341,415,427] Some of these factors may be causal and others may be risk markers for unplanned pregnancy due to their association with other causal factors. For example, low socioeconomic status may have a causal relationship with unplanned pregnancy, as in some countries, women may be less likely to commit limited financial resources to contraceptives. Studies conducted in the USA show that unplanned pregnancy is more common among poorer women,[415] and in one study 43% of women who gave birth to a child with FAS reported that they could not afford contraception.[70] Conversely, socioeconomic status was not found to be associated with unplanned pregnancy in a UK study, where contraception is available for free.[341] Intimate partner violence may be causally associated with unplanned pregnancy due to an increased risk of sexual coercion, and sabotage of contraception.[427,428] Substance use before pregnancy may be causally associated with unplanned pregnancy, as it increases the likelihood of not using contraception.[424,429] Further consequences of unplanned pregnancy include late prenatal care and complications during pregnancy.[321,339,340,415,430]

### 5.2.4 Stress and social support

#### *5.2.4.1 Prenatal stress*

Prenatal stress may increase vulnerability to FASD.[285] Mothers of children with FASD are more likely to report that pregnancy was a particularly stressful time and to have experienced stressful life events, including abuse and interpersonal

violence.[111,287] Qualitative evidence suggests that some mothers may use alcohol to alleviate stress during pregnancy.[70,344,426] Prenatal stress has been found to predict co-occurrence of PAE and prenatal smoking, illicit drug use, and poor prenatal nutrition.[316,318] It is important to acknowledge that substance use may also cause prenatal stress.[276] However, in the literature, stress is typically thought to precede substance use. This implies the following causal structure: *prenatal stress at time 1 → PAE → prenatal stress at time 2*. To aid clarity of presentation and prevent feedback loops, I presented the time 1 relationship from *stress → PAE* in the DAG.

As well as influencing drinking behaviour, prenatal stress may exacerbate the teratogenic effects of alcohol.[431] In a Ukrainian study, prenatal depression and PAE jointly influenced neurodevelopmental outcomes in infants. This effect may be partially mediated through prenatal stress.[294] Reviews of animal studies have concluded that PAE and stress operate synergistically to impair developmental outcomes.[286] For example, one primate study explored the impact of prenatal exposure to alcohol and a noise stressor on attention and neuromotor outcomes in offspring.[432] Concurrent exposure to PAE and stress produced deficits in coordination, response speed, and birthweight, while exposure to PAE alone did not significantly affect these outcomes. Another primate study found a significant interaction between prenatal stress and PAE on behavioural outcomes. Animals that had been exposed to both prenatal stress and PAE showed increased levels of hyperactivity and stereotypies postpartum compared to animals exposed only to one of these factors, and non-exposed controls.[433] However, evidence is inconsistent and not all studies have found an interaction between PAE and stress

in producing outcomes relevant to FASD symptomology, possibly as a result of small sample size.[434-436]

Several biological mechanisms have been proposed to account for observed interactions between PAE and stress in FASD. One hypothesis is that PAE and prenatal stress produce lasting changes in the functioning of the fetal hypothalamic-pituitary-adrenal (HPA) axis, which is involved in the stress response. This effect is heightened in the presence of both PAE and stress, relative to their individual contribution.[437,438] Adrenocorticotrophic hormone responses to a postnatal stressor have been found to be higher in primates exposed to both prenatal stress and PAE than those exposed to PAE alone.[301,437] Endocrine dysregulation may contribute to the risk of FASD by influencing behavioural, cognitive and emotional functioning.[432,438] Another suggestion is that dual exposure contributes to fetal hypoxia by restricting uterine blood flow and suppressing fetal breathing. Hypoxia promotes cell damage, which may lead to midfacial abnormalities[431] and can be particularly damaging in areas such as the hippocampus and cerebellum which are involved in attention, motor function and learning.[285,432] Prenatal stress has been independently linked to adverse child outcomes and congenital anomalies and is also a consideration for differential diagnosis.[431,439-441]

#### *5.2.4.2 Maternal mental health*

Mental health disorders are common among mothers of children with FASD.[70,111,327] Among a clinic-based sample of 80 mothers of children with FAS in the USA, 96% had one or more mental health disorders.[70] The mechanisms that link

prenatal mental health to child outcomes are poorly understood; however, effects are thought to be facilitated primarily via increased stress hormone circulation and altered HPA functioning, as described in the previous section.[442-444]

Bandoli and colleagues found that unmedicated maternal depression and PAE interacted to predict poorer outcomes on the Bayley Scales of Infant Development (BSID) at ages six and twelve months; while maternal depression, alone, was not independently associated with neurodevelopmental outcomes.[294] Santucci and colleagues also failed to find an association between prenatal depression and BSID scores.[445] Other studies have found an independent association between prenatal depression symptoms and poorer emotional-behavioural and cognitive outcomes in children up to age eight.[377,378] These effects were thought to be partially mediated through an unhealthy prenatal diet. Overall, results are inconsistent with regards to the independent influence of prenatal mental health on FASD-related symptomology. Following a review of the evidence, Waters and colleagues concluded that, with the exception of conduct problems, prenatal depression does not appear to independently influence child neurodevelopmental outcomes.[443] Therefore, associations between prenatal mental health and FASD are thought to be largely mediated by the prenatal stress response, rather than mental health itself.

In addition to its interaction with PAE, maternal mental health may influence drinking behaviour due to the use of alcohol for self-medication.[426,446] A prospective study that investigated the comorbidity of alcohol use disorder and major depression found that depression often preceded alcohol use disorder.[447]

Furthermore, mothers who have a child with FAS and subsequently receive mental health treatment are more likely to report abstinence, suggesting a promising target for FASD prevention.[70] Maternal mental health issues may also affect fetal development by increasing the risk of other adverse exposures including illicit substance use, smoking and poor prenatal nutrition.[318,377,378,448]

#### *5.2.4.3 Social support in pregnancy*

Social support has been defined as "the degree to which a person's basic needs are gratified through interaction with others."[449(p. 54)] Social support is typically provided by family and friends and can include general support (when the recipient feels liked, loved or respected) and instrumental support (when the recipient has opportunities for practical assistance including financial support and child care).[449] Social support during pregnancy is associated with reduced alcohol intake in European and American samples.[449,450] Mothers who have given birth to a child with FASD are more likely to achieve abstinence in the future if they report having a large, satisfactory support network.[70] However, the beneficial effects of social support on alcohol use may be less apparent among women in poverty, and within subcultures that normalise drinking in pregnancy.[426,451] In summary, social support may protect against PAE by attenuating the impact of stressors and reducing the likelihood that alcohol will be used as a coping strategy.

#### *5.2.4.4 Abuse*

FASD is more common among children born to women who have experienced childhood physical or sexual abuse and/or intimate partner violence.[70,111,276,327] Substances including alcohol may be used as a self-medication strategy to alleviate

stress and mental health symptoms among those who have experienced abuse.[452] Abuse influences whether mothers reduce their alcohol intake following pregnancy recognition. Seventy-two percent of mothers of children with FAS reported that they did not want to reduce their alcohol use because they were in an abusive relationship.[70] Abuse may also influence risk of FASD by acting as a barrier to adequate antenatal care. Women experiencing domestic abuse may be prevented from accessing antenatal care by the perpetrator or may be reluctant to access antenatal care due to concerns that disclosure of abuse would make the situation worse, or would lead to involvement of child protection services.[64]

### 5.2.5 Other maternal psychological and physical characteristics

#### *5.2.5.1 Maternal physical characteristics*

In a review of the evidence, May and colleagues concluded that maternal "body mass significantly and obviously moderates risk for FASD." [109(p. 21)] Lower maternal height, weight and BMI have been found to consistently predict FASD in studies in South Africa.[266,274,276,342,343,413] Studies conducted in Italy and the USA have been less consistent with regards to BMI, but found that mothers of children with FASD tended to be shorter or weighed less than those without FASD.[79,109,273,345]

Several mechanisms have been proposed to account for the effect of maternal BMI on FASD risk. Maternal physical characteristics influence the distribution of alcohol after it is consumed. As alcohol is distributed in body water, blood alcohol concentrations (BACs), and therefore PAE, tends to be higher in smaller women.[110,453] As well as influencing BACs, higher maternal weight and BMI may indicate adequate nutrition. Although higher BMI may protect against FASD, it is

important to note that maternal obesity is associated with a range of adverse outcomes including gestational diabetes mellitus, induction of labour and preterm delivery.[454]

#### *5.2.5.2 Maternal FASD symptomology*

Mothers of children with FASD are more likely to have symptoms of FASD themselves, including cognitive impairment and small head circumference.[266,274,327] Maternal FASD symptomology leads to an increased risk of mental disorders. Up to 94% of individuals with FASD have comorbid mental health disorders,[93] including conduct disorder (91%), ADHD (51%), and depression (35%).[90] In turn, impaired mental health increases the risk of PAE (as described in Section 5.2.4.2). Fifty-five percent of individuals with FASD experience drug or alcohol dependence later in life,[90] thus increasing the risk of FASD in subsequent generations.

#### *5.2.5.3 Knowledge and attitudes towards PAE and FASD*

Public awareness of the risks of PAE is low. Inconsistencies in UK guidance and mixed advice from health professionals are likely to contribute this lack of awareness.[94] A UK study, published in 2015, sought to determine what the general public knew about PAE and FASD.[56] This study found that 40% of participants did not know the current government guidance on PAE. Most participants (71%) also said that government guidance on PAE was unclear, and some said that this lack of clarity was likely to lead to messages being disregarded.[56] Health professionals in the UK have been found to give mixed advice to women about PAE. The Infant Feeding Survey 2010 reported that 30% of expectant mothers were not given any information about alcohol use in pregnancy. Among women who received advice,

36% were given information on reducing their intake and 29% on stopping drinking.[52]

In the absence of clear evidence about a 'safe' threshold for PAE, opinions can be polarised. Some groups endorse a 'no alcohol no risk' message, while others warn against the 'policing of pregnancy' and suggest that women should be free to make an educated choice about whether they drink when trying to conceive and during pregnancy.[53,54]

FASD is not well understood by the public and health professionals in the UK and internationally.[10] A UK study of 674 individuals found that while 87% had heard of FASD, knowledge of the condition was limited.[56] A 2011 survey of over 600 midwives in East Anglia found that only 10% were able to identify the core features of FASD, and less than 2% reported that they were 'very prepared' to deal with the condition.[116] Among professionals who are aware of FASD, some are reluctant to consider a diagnosis over concerns that it is stigmatising and because of a lack of FASD services.[106]

For the purposes of DAG construction, public health guidance on alcohol use was hypothesised to influence public and professional knowledge on the risks of PAE, which in turn was proposed to influence PAE.

### 5.2.6 Family factors

#### *5.2.6.1 Alcohol use of friends and family*

Mothers of children with FASD report higher levels of alcohol consumption by their partner, family and friends.[79,111] Ceccanti and colleagues found that alcohol

problems in the family increased the likelihood of having a child with FASD by nine times.[345] Alcohol use by friends and family may impact on PAE in various ways. First, the drinking behaviour of those in close social networks may represent the norms within that context. For example, a binge pattern of drinking may not be viewed as problematic in some communities in South Africa where heavy episodic alcohol use is commonplace.[413] Second, some women may feel coerced into drinking before and during pregnancy as a result of the behaviour of friends and family. In a study of mothers of children with FAS, Astley and colleagues reported that 36% of women said that they did not want to reduce their alcohol intake because their partner did not want them to, and 20% because their family and friends did not want them to.[455]

Strong correlations between maternal substance use and the substance use of friends and family could also be due to processes of self-selection. Self-selection theories posit that individuals pick companions who are similar to themselves and who are supportive of their behaviour. Evidence supports self-selection as a mechanism that accounts for similarities in substance misuse behaviour within social networks.[456] In the DAG, I represented this mechanism via the unspecified "risky behaviour" node that influences both maternal substance use and also the drinking behaviour of those who have been selected as part of the mother's social network.

Finally, as described above, mothers of children with FASD are more likely to have symptoms of FASD themselves, and to report that the maternal grandmother has a history of alcohol problems.[274,327] This raises the possibility of intergenerational

transmission of FASD – a phenomenon that has received support via controlled animal studies.[457] Heavy alcohol use by the maternal grandmother could indicate the presence of a risk genotype for heavy alcohol use,[458] could result in epigenetic changes that increase the risk of heavy alcohol use in later generations[457] and could provide a model of problematic alcohol use that is adopted by offspring via social learning.[459]

Emerging evidence from animal studies suggests that paternal alcohol consumption could influence the epigenetics of sperm DNA by influencing methylation patterns in sites that are important to developmental outcomes.[460] Paternal alcohol use has been associated with FASD symptomology including low birth weight, reduced brain size, microcephaly, impaired learning and hyperresponsiveness,[330,461] although results have been inconsistent.[462] In a 2016 review, Day and colleagues stated that "the alcohol consumption of the father during his lifetime can lead to FASD in his offspring."[461(p.16)] However, the authors note that the interaction between maternal and paternal alcohol use requires further investigation, and that studies have not been able to demonstrate that epigenetic inheritance, solely due to paternal drinking, can cause a specific phenotype.[461] Furthermore, with the exception of FAS, all diagnoses within the FASD continuum require evidence of maternal alcohol consumption. By definition, therefore, paternal alcohol use cannot currently be considered a sole cause of FASD. Further research is required to elucidate the precise mechanisms through which familial alcohol consumption influences the risk of FASD in future generations.[462]

#### *5.2.6.2 Having another child with FASD*

Population-based data from the USA indicate that between nine and 29% of mothers identified within the FAS Surveillance Network had another child with FASD.[325] An early study, published in 1979, found that 70% of children with FAS had a sibling with confirmed or suspected FAS.[463] Therefore, having a sibling with FASD is a strong risk marker for FASD.

Having another child with FASD is also a strong predictor of subsequent alcohol-exposed pregnancies.[69,325,326,463] In a cross-sectional study of mothers of children with FAS, 80% subsequently had an unplanned pregnancy, of which 75% were alcohol-exposed.[70] In the absence of intervention, women who are at risk of having another child with FASD exist within the same causal context as that which surrounded their first affected pregnancy. Thus, identifying mothers who have had a child with FASD is an important focus for targeted prevention.[69]

#### *5.2.6.3 Genetics*

Genetic factors influence vulnerability to FASD. Twin studies show that dizygotic twins have differential susceptibility to FASD, while monozygotic twins have high levels of concordance.[326,464-467] Animal models of FASD demonstrate that different strains of mice have diverse outcomes according to their genotype.[468,469] Finally, observational studies have found that particular genetic variants are more common among alcohol-exposed children who develop FASD, compared to those who do not.[470,471]

Existing reviews have demonstrated that genetic factors modify susceptibility to alcohol-related harm.[458,472-474] A range of genes have been investigated in animal

models of FASD, including aldehyde dehydrogenase (ALDH), Fancd2, Cdon, Gli2, Shh, Nos1, PDGFRA, hinfp, foxi1, mars, plk1 and vangl2; however, these genes are yet to be explored in human studies.[474] In humans, polymorphisms of alcohol dehydrogenase (ADH) genes, have been the primary focus of investigation.[472-474] ADH enzymes oxidise ethanol to acetaldehyde and account for up to 95% of alcohol metabolism. ALDH and CYPE2E1 also influence ethanol metabolism in humans, however their role in FASD susceptibility is yet to be established.[470,473]

Polymorphisms at the ADH1B locus produce enzymes that affect the rate of ethanol clearance. In populations of European ancestry, the slow-metabolising allele, ADH1B*1, is the typical variant and occurs in approximately 95% of individuals. The rare ADH1B*2 and ADH1B*3 alleles convert ethanol to acetaldehyde 75-88 times faster than ADH1B*1.[475] Maternal and infant ADH1B genotype has been found to influence risk of FASD as, with the exception of one study,[476] fast-metabolising alleles have been associated with a reduced risk of FASD symptomology following PAE.[470,477-480]

Using data from the ALSPAC cohort, Lewis and colleagues[27] explored the impact of ADH polymorphisms on children's IQ following PAE. Rare variants of four child single nucleotide polymorphisms (SNPs; ADH7 rs284779, ADH1B rs4147536, ADH1A rs975833 and ADH1A rs2866151; herein referred to as the SNP set) were negatively associated with IQ at age eight for children with low to moderate alcohol exposure in-utero (1 to 6 units per week). These rare variants were hypothesised to be associated with slower metabolism, leading to relatively increased alcohol exposure and teratogenicity.[27,481] However, a study of in-vivo alcohol metabolism

failed to show that these SNPs influenced blood or breath alcohol concentrations.[482] Within Lewis and colleagues' study another SNP, ADH4 rs4148884, had divergent effects. The rare allele was associated with decreased offspring IQ when present in the maternal genotype, but increased IQ when present in the child's genotype. These SNPs were not associated with IQ among children of non-drinking mothers, suggesting that genotype acted as an effect modifier following maternal alcohol intake. In a separate study, the SNP set identified by Lewis et al. was found to modify the relationship between alcohol use and cognitive decline in older age, providing further evidence that these factors are meaningful indicators of genetic vulnerability to alcohol-related harm.[481]

As well as influencing alcohol metabolism, genotype may influence drinking behaviour, hence the interest in ADH variants as instrumental variables in Mendelian randomisation studies.[289] Patterns of alcohol consumption tend to run in families and several genes have been found to contribute to risk of alcoholism.[458,462] ADH1B has been widely studied and research shows that individuals with the rare allele consume less alcohol than those with the typical allele, and have a reduced risk of alcoholism.[483-486] In pregnancy, mothers with the rare ADH1B allele have been found to consume significantly less alcohol before pregnancy and to be 50% less likely to binge drink during pregnancy.[487] In summary, genotype may influence the risk of FASD through its influence on both alcohol metabolism and drinking behaviour.[26]

# 6 Discussion

The aetiology of FASD is multifaceted and complex. Following prenatal exposure to alcohol, FASD risk is determined by a range of lifestyle, sociodemographic, maternal, social, gestational, and genetic factors that mutually influence children's outcomes. The DAG that I have presented provides a summary of the distal and proximal factors that causally influence FASD risk, as well as the factors that are proxies for causal factors or (non-causal) risk markers for FASD. The principles of causal diagram theory can be applied to this DAG to inform statistical modelling strategies for epidemiological studies of FASD.

## 6.1 Strengths and limitations

### 6.1.1 Literature review and DAG methodology

The literature review and DAG build upon evidence from existing reviews of risk factors for FASD[109,111,488] to provide an updated synthesis using the latest evidence from observational human studies and animal experiments. The use of DAG methodology represents a novel approach to the study of FASD and adds clarity to causal inferences in an area that has mostly been confined to discussions of association. As a visual representation, the DAG presents a unified summary of the current evidence on the aetiology of FASD.

Since the DAG has been constructed based on current subject-matter knowledge and my interpretation of plausible causal structures, it may be subject to change as new evidence emerges. This is not a limitation of the DAG approach, but rather reflects the realities of making causal inferences within a developing evidence base. In its current form, the DAG that I have presented may be a useful working

template for researchers of FASD, who can use this tool to guide their analyses and update the causal structure of this diagram, as necessary.

It is important to note that the causal inferences derived from the DAG rest on the assumption that the diagram is valid. The DAG is based on my interpretation of the evidence and therefore, it is possible that given the same evidence another researcher may depict the relationships differently. Furthermore, the limitations inherent in the evidence base will necessarily apply to the results from this review and the DAG. Many proposed risk factors for FASD, such as prenatal substance exposure and maternal stress, cannot be manipulated for ethical and practical reasons.[489] The evidence that I have used to construct the DAG was, therefore, based on experimental studies with animals and observational studies with humans. Animal studies have allowed the causal status of ethanol as a teratogen to be established[490] and have enabled causal conclusions about the influence of covariates such as stress and genetics on outcomes relevant to FASD.[434,469,491] Furthermore, in contrast to human studies, in which adverse exposures and health behaviours tend to co-occur, animal studies can isolate and evaluate the effects of specific exposures. However, animal models are imperfect substitutes for human studies for reasons including differences in alcohol metabolism, gestational processes, and their inability to replicate higher-level behavioural outcomes.[286] No single animal model has replicated all of the attributes of FASD.[490] Observational studies of humans pose fewer concerns than animal studies in terms of exploring associations between a variety of co-occurring social and lifestyle factors and the full spectrum of FASD. However, this advantage is countered by the potential for bias due to lack of experimental control. For example, Esper and colleagues[111] used

the Newcastle-Ottawa Scale (NOS)[492] to assess quality evidence for the studies of maternal risk factors for FASD that were included in their review. They noted that quality was generally high for the selection criteria (maximum quality score for eight out of 13 eligible studies; indicating appropriate FASD case definition, selection and definition of controls, and representativeness) but low for the comparability criteria (10 out of 13 eligible studies had a score of one out of a maximum of two; indicating limitations in the case-control matching procedures and/or a lack of adjustment for key confounders), and low for the exposure criterion (one to two stars out of four for all studies; indicating that ascertainment of exposure was not based on appropriate records, that interviewers were not blind to case/control status, that there were different methods of ascertainment for cases and controls and/or that there was a differential response rate for cases and controls). In general, observational studies are at risk of bias due to residual confounding, misclassification of the exposure and/or outcome, and differential loss to follow-up. In the evidence synthesis for the DAG, as well as studies with traditional observational designs, I included negative control and Mendelian randomisation studies. Although these studies are also subject to potential limitations (the contribution of paternal epigenetic factors to prenatal development in studies with negative controls remains to be established; and effect estimates may be biased towards the null in Mendelian randomisation studies if genetic instruments are weakly correlated with the exposure of interest),[303,330,486,487] the sources of bias differ for each approach and therefore, under a triangulation method, the fact that the evidence from these different study designs points to a similar causal conclusion for many of the nodes of interest

increases confidence in the causal hypotheses presented in the DAG.[302,303] However, it may never be possible to determine whether the pathways suggested by the observational and animal studies represent the true causal pathways to FASD. Although this is a limitation of causal inference in general, compared to conventional modelling strategies, DAG methodology benefits from providing transparency about causal assumptions and likely remaining sources of bias.

### 6.1.2 The utility of DAGs in casual inference

Recently, there have been some criticisms about the gain in popularity of DAGs as tools to support causal inference. Kreiger and Davey Smith argue that DAGs have become synonymous with 'casual inference' and that this unnecessarily narrows the scope of causal inquiry in epidemiology.[302] They assert that "robust causal inference…comprises a complex narrative, created by scientists appraising, from diverse perspectives, different strands of evidence produced by myriad methods. DAGs can of course be useful, but should not alone wag the causal tale." [302(p.1789)] I agree, and this is why I have drawn upon a broad body of evidence to support the causal pathways depicted in the DAG. Daniel and colleagues attribute Kreiger and Davey Smith's criticism to a misinterpretation of the role of DAGs in causal inference. As they point out, DAGs do not purport to provide a substitute for careful consideration of the evidence base. Instead they are a result of this.[493] Under this view, I would contend that the narrative synthesis that I have conducted is the causal inference *approach,* while the DAG is the *tool* that summarises key causal assumptions and enables application of graphical rules to guide appropriate analyses and interpretation within a causal framework.

Finally, DAGs are nonparametric and debate is ongoing as to whether it is possible to represent effect modification and, if so, how it should be represented. This is particularly problematic when trying to describe the aetiological context of FASD. In this context, while there is a sole necessary cause (alcohol), several effect modifiers may be present that influence whether PAE results in FASD. In the DAG (Figure 18), I have followed Weinberg[494] and Thompson's[495] suggestion by representing effect modification with an arrow that intersects the intermediate causal pathway from PAE to FASD,[494] although other representations have been suggested.[496,497] VanderWeele and Robins indicate effect modification as separate arrows from exposures that each lead to the outcome variable.[496] However, when considering FASD, this conceptualisation does not acknowledge the fact that in the absence of PAE, another exposure cannot independently influence true FASD. Other conceptualisations of effect modification suggest that interacting variables can be represented as separate nodes that lead to a single composite node that contains both exposures. However, for the purposes of this chapter this representation would become too visually complex and would interfere with the ability the interpret the DAG.[497] It is important to reiterate that, since DAGs are non-parametric, they are entirely agnostic as to whether or not there is effect modification. My representation of effect modification as an intersecting arrow in the direct pathway between PAE and FASD is purely for visual illustration. To enable analysis of the DAG in the DAGitty platform (i.e. to identify covariate adjustment sets for the risk factor analysis in Chapter 6), I directly connected the arrows from the proposed effect modifiers to the FASD outcome to allow

application of the graphical rule algorithms (DAGitty code is available in Appendix 14).[498]

# 7 Implications for this thesis

The DAG presented in this chapter formalises and unifies current knowledge about the causal context of FASD. I believe that this DAG provides a useful synthesis of evidence for those interested in the epidemiology of FASD, and will strengthen my analysis plan and interpretation in subsequent chapters of my thesis.

In the next chapter (Chapter 6), I will describe how I used the information from the DAG to design and execute the statistical analysis strategy in my analysis of FASD risk factors using data from the ALSPAC cohort.

# Chapter 6. Risk factors for fetal alcohol spectrum disorder: a population-based birth cohort study

## 1 Overview

In this chapter, I investigate risk factors for FASD using data from a population-based birth cohort in England (ALSPAC). First, I present the aims and objectives of this chapter, followed by the methods, which include a description of how I used the causal diagram that I developed in Chapter 5 to inform the statistical modelling strategy. I then present the results from univariable and multivariable logistic regression analyses. I conclude the chapter with a summary of the main results and a discussion of the strengths and limitations of this work.

## 2 Background

There is significant variability in the outcomes of children who have been exposed to alcohol prenatally. Chapter 4 showed that although 79% of children in the ALSPAC cohort had PAE, only 17% met criteria for FASD. Furthermore, there were only modest differences in patterns of PAE between children with and without FASD. Therefore, it is important to identify which factors influence susceptibility to FASD to inform strategies for prevention and intervention.

In Chapter 5, I reviewed the published literature on FASD risk factors and produced a causal diagram (DAG) to synthesise this evidence. This review indicated that the aetiology of FASD is complex. Following PAE, a child's risk of developing FASD is influenced by lifestyle factors, sociodemographic characteristics, pregnancy factors,

stress, social structures and maternal psychological and physical characteristics. Based on the DAG, I identified a subset of potentially modifiable causal risk factors for FASD, which will be explored in the multivariable analyses that I present in this chapter. These factors are: patterns of prenatal alcohol use, smoking, illicit drug use, nutrition, mental health, stress and social support.

# 3 Aim and objectives

## 3.1 Aim

To quantify the effect of potentially modifiable risk factors for FASD among children who have been exposed to alcohol prenatally in the ALSPAC cohort.

## 3.2 Objectives

i. To use the causal diagram (DAG) that I developed in Chapter 5 to develop statistical models to evaluate the association between potentially modifiable risk factors and FASD.
ii. To quantify the association between different patterns of prenatal alcohol use, smoking, illicit drug use, nutrition, mental health, stress and social support and FASD among children who have been exposed to alcohol prenatally.

# 4 Method

## 4.1 Data source

The ALSPAC cohort (described in Chapter 3, Section 4.1.1).

## 4.2 Study approval

Ethical approval for this study was obtained from the ALSPAC Ethics and Law Committee (IRB00003312) and the Local Research Ethics Committees.[232] Project approval was granted by the ALSPAC Executive Committee on the 2nd March 2016 (Project B2620).

## 4.3 Participants

This study included participants from the core ALSPAC sample with confirmed PAE, based on maternal self-report. I excluded twins and triplets, participants who were known to have a genetic condition, participants who were not alive at one year of age and participants who did not speak English as a primary language. Participants who were in the armed forces social class category were excluded due sparse data, which caused problems with convergence in imputation and risk factor models (N = 28).

## 4.4 Variables

### 4.4.1 Outcome

The outcome was any FASD based on the Mid CNS/Any PAE case ascertainment algorithm (described in Chapter 3), which was coded as a binary (FASD/not FASD) variable.

### 4.4.2 Exposures

The exposures of interest were the potentially modifiable causal risk factors for FASD that I identified using a causal diagram (DAG; described in Chapter 5). These

variables are described further below. Full details of the original data collection methods and variables are available in the ALSPAC data dictionary.[233]

#### *4.4.2.1 Prenatal substance use*

##### 4.4.2.1.1 Patterns of prenatal alcohol exposure

*Dose/frequency (categorical):* Mothers completed self-report questionnaires that described the number of glasses[m] of alcohol that they consumed per week or per day in the first trimester (data collected at 18 weeks gestation), around the time they first felt the baby move (data collected at 32 weeks gestation) and in the third trimester (data collected at 8 weeks postpartum). To overcome issues with convergence in multiple imputation models and to reduce sparse data categories, I generated a summary variable that described the maximum amount of PAE consumed at any time during pregnancy as follows: less than 1 glass per week,[n] 1 to 6 glasses per week, 7 or more glasses per week.

*Weekly alcohol consumption (categorical):* Using the categorical dose/frequency measure described above, I generated a binary (yes/no) variable to indicate alcohol consumption on a weekly basis during pregnancy.

*Measures of alcohol (continuous):* Mothers reported the number of measures of alcohol that they consumed per weekday and weekend day for each of the

[m] The ALSPAC questionnaire defined a glass of alcohol as equivalent to a pub measure of spirits, ½ pint lager/beer, wine glass of wine etc.

[n] This category also included participants who reported 'never' drinking on this measure. These participants were identified as having drank some amount of alcohol while pregnant based on other drinking measures (including binge drinking, continuous measures of alcohol per week, and/or a lack of reduction in consumption compared to alcohol use before pregnancy). Therefore, these participants were considered to drink occasionally in pregnancy.

following types of alcohol: beer/lager (half-pints), wine (glasses), spirits (pub measures), and other (asked to describe), at 8 and 32 weeks gestation. Responses were summed to provide a continuous measure of weekly consumption.

*Binge drinking (categorical):* Information on binge drinking, defined as drinking the equivalent of two pints of beer, four glasses of wine or four pub measures of spirits on a single occasion, was collected using a self-report questionnaire at 18 and 32 weeks gestation. From this information, I created a binary (yes/no) variable to indicate binge drinking at any time during pregnancy.

#### 4.4.2.1.2 Prenatal smoking

Mothers completed questionnaires that asked them whether they smoked cigarettes during each trimester and to report the quantity that they smoked. Data were collected at 8, 18 and 32 weeks gestation and 8 weeks postpartum. I created a binary (yes/no) variable to indicate smoking at any time during pregnancy.

#### 4.4.2.1.3 Prenatal illicit drug use

Mothers reported whether they had used amphetamines, barbiturates, cannabis, crack cocaine, cocaine, heroin, methadone, ecstasy, 'hard drugs' and 'other drugs' at any point during pregnancy, and the frequency with which each drug had been taken. Information about first and second trimester use was collected at 18 weeks gestation and information about third trimester use was collected at 8 weeks postpartum. I created a binary (yes/no) variable to indicate any drug use at any time in pregnancy.

#### 4.4.2.1.4 Prenatal nutrition

*Vitamin supplements:* At 18 and 32 weeks gestation, mothers completed a self-report questionnaire that asked whether they had taken any of the following vitamin supplements during pregnancy: iron, zinc, calcium, folic acid and multivitamins. I generated binary (yes/no) variables to indicate whether the mother had taken each of the supplements at any time during pregnancy.

*Daily nutrient intake:* Mothers completed a food frequency questionnaire at 32 weeks gestation, which asked them about their consumption of 43 different foods. Information from the food frequency questionnaire was used to calculate approximate daily nutrient intakes, as reported previously.[499] I used dietary reference values from the British Nutrition Foundation,[500] recommendations from the European Food Safety Authority,[501] Weichelbaum and colleagues,[502] the Drug and Therapeutics Bulletin,[374] and the National Institutes of Health[503] to create binary variables that indicated whether participants had met the recommended daily intake for each nutrient (met/not met).[o]

### *4.4.2.2 Prenatal mental health*

Maternal prenatal mental health symptoms were assessed using self-report measures at 18 and 32 weeks gestation. Maternal anxiety symptoms were measured using the Crown-Crisp Experiential Index (CCEI)[504] and maternal depression symptoms were measured using the Edinburgh Postnatal Depression

[o] Within the ALSPAC study, scores have recently been derived from factor analyses to represent a variety of composite dietary patterns including: 'healthy', 'processed', 'confectionary', 'vegetarian' and 'traditional'. These were not available at the time that I conducted the analyses for this chapter, but may be of interest for future study.

Scale (EPDS), which has been found to be a valid indicator of prenatal depression symptoms.[505] I used a threshold of 9 or more to indicate prenatal anxiety and a threshold of 13 or more to indicate prenatal depression (the highest 15% of scores), following previous work by O'Connor and colleagues.[506] I created a binary variable to indicate whether mothers exceeded the threshold for anxiety or depression at any time during pregnancy (yes/no).

#### *4.4.2.3 Prenatal stress*

Mothers completed a 42-item life-events inventory at 18 weeks gestation and 8 weeks postpartum, which asked about stressful life events that occurred in early to mid pregnancy. I created binary variables to indicate whether mothers had experienced each of the following life events during pregnancy: bereavement, relationship difficulties, major financial problems, moving house, and accident or illness during pregnancy. I also generated variables to indicate whether mothers reported having been 'very affected' by each of these life events (yes/no).

In addition, I derived continuous variables that indicated the total number of life events that mothers experienced across pregnancy, and the total number of life events weighted by how affected the mother reported being by each event ('very affected' = 4; 'moderately affected' = 3; mildly affected = 2; 'not affected' = 1; 'did not happen' = 0), following the procedure reported in the ALSPAC documentation.[507]

#### *4.4.2.4 Social support during pregnancy*

At 12 weeks gestation, mothers completed a 10-item questionnaire that asked them about their perceived social, emotional and financial support during pregnancy. Items were scored from 0 to 3 with higher scores indicating more support, yielding a maximum total score of 30.

### 4.4.3 Confounding variables

I used the DAGitty platform[312] to analyse the DAG that I developed in Chapter 5 to identify which potential confounders to adjust for in each of the multivariable risk factor models. DAGitty is a tool for drawing and analysing causal diagrams. It applies the graphical rules (described in Appendix 13) to identify which covariates should be adjusted for, and which should not, in multivariable statistical models to minimise bias in causal effect estimates. Since DAGs are non-parametric, DAGitty was used to identify the appropriate covariate set and subsequent multivariable statistical analyses were carried out in Stata 14.2.[155]

Due to differences in the hypothesised causal and biasing pathways that connected each of the exposures of interest with the FASD outcome, adjustment sets differed across most of the multivariable models. Table 12 describes the variables that were identified as confounders, based on application of the graphical rules in DAGitty, for each of the multivariable risk factor models.

All causal diagrams are presented in Appendix 15. Figure 19 provides an example of one of the causal diagrams, using the smoking risk factor model. There were six

hypothesised causal pathways that accounted for the total causal effect of prenatal smoking on FASD classification among children with PAE. These were:

i. Prenatal smoking -> 'true' FASD (unobserved) -> FASD classification
ii. Prenatal smoking -> prenatal nutrition -> 'true' FASD (unobserved) -> FASD classification
iii. Prenatal smoking -> prenatal nutrition -> BMI -> 'true' FASD (unobserved) -> FASD classification
iv. Prenatal smoking -> differential diagnosis (unobserved) -> FASD classification
v. Prenatal smoking -> prenatal nutrition -> differential diagnosis (unobserved) -> FASD classification
vi. Prenatal smoking -> prenatal nutrition ->BMI -> differential diagnosis (unobserved) -> FASD classification

Pathways i. to iii. describe participants who have a pattern of symptoms that met the criteria for FASD and whose symptoms were caused by PAE. These are the participants with 'true FASD.' In contrast, pathways iv. to vi. describe participants who met the criteria for FASD but whose presentation may have been better explained by factors other than alcohol, if the opportunity was available for comprehensive differential diagnosis. All of these pathways contribute to the total prevalence of FASD that is observed in this research. Based on the graphical rules described in Chapter 5, the least biased estimate of the total causal effect of prenatal smoking on FASD could be obtained by including the following variables as covariates in the logistic regression model: maternal age at pregnancy, prenatal depression, prenatal anxiety, maternal 'risky behaviour' personality trait, indicators of socioeconomic status, prenatal stress and unplanned pregnancy.

*Table 12 (continued overleaf): Description of variables that were identified as potential confounders in one or more of the multivariable analyses of risk factors for FASD.*

| DAG node (confounder) | ALSPAC variable(s) | Categories | Time point of measurement | Risk factor model(s) that included this variable as a confounder |
|---|---|---|---|---|
| Abuse | Physical, sexual or domestic abuse towards the mother in childhood or during pregnancy | Yes, no | 18 & 32 weeks gestation, 2 years postpartum | Mental health<br>Nutrition<br>PAE<br>Stress |
| Age | Maternal age at pregnancy | <20, 20-29, 30+ years | Pregnancy baseline sample data | Smoking |
| Drug use | Prenatal illicit drug use[a] | Yes, no | 8 weeks gestation & 8 weeks postpartum | Nutrition |
| Genotype (maternal) | Maternal rs1229984 genotype (≥ 1 rare allele) | Yes, no | Not applicable | PAE |
| Marital status | Marital status | Not married, married | 8 weeks gestation | Social support |
| Maternal FASD | Maternal grandmother had alcoholism[b] | Yes, no | 12 weeks gestation | Mental health |
| Mental health | Prenatal anxiety[a] | Score ≥9 on CCEI, score <9 on CCEI | 18 & 32 weeks gestation | Nutrition<br>PAE<br>Smoking<br>Stress |
| | Prenatal depression[a] | Score ≥13 on EPDS, Score <13 on EPDS | 18 & 32 weeks gestation | |
| Patterns of maternal prenatal alcohol consumption | Alcohol dose/frequency during pregnancy[a]<br><br>Binge drinking during pregnancy[a] | <1 glass per week, 1-6 glasses per week, 7+ glasses per week<br><br>Yes/no | 18 & 32 weeks gestation, 8 weeks postpartum | Nutrition |
| Pre-pregnancy alcohol use | Pre-pregnancy alcohol use | ≤ 1 - 2 glasses per day, 3+ glasses per day | 18 weeks gestation | Nutrition<br>PAE |
| Religion | Maternal religion | None, Christian, other | 12 weeks gestation | Social support |

| DAG node (confounder) | ALSPAC variable(s) | Categories | Time point of measurement | Risk factor model(s) that included this variable as a confounder |
|---|---|---|---|---|
| Risky behaviour | Mother considers herself to be impulsive[c] | Doesn't apply, applies a bit, moderately/certainly applies. | 9 years postpartum | Nutrition<br>PAE<br>Smoking<br>Stress |
| Smoking | Prenatal smoking[a] | Yes, no | 32 weeks gestation & 8 weeks postpartum | Nutrition |
| Socioeconomic status (SES) | Maternal education<br><br>Paternal education | CSE, vocational, O Level, A Level, degree. | 32 weeks gestation | All |
| | Maternal social class<br><br>Paternal social class | Professional, managerial/technical, skilled non-manual, skilled manual, partly skilled, unskilled. | 32 weeks gestation | |
| | Home ownership status | Mortgaged/owned, council/housing association, rented [private], other. | 8 weeks gestation | |
| Stress | Prenatal stress[a] | Continuous (weighted life events score) | 18 weeks gestation & 8 weeks postpartum | Nutrition<br>PAE<br>Smoking |
| Support | Prenatal social support score[a] | Continuous | 12 weeks gestation | Mental health<br>Stress |
| Unplanned | Unplanned pregnancy | Yes, no | 18 weeks gestation | Nutrition<br>PAE<br>Smoking |

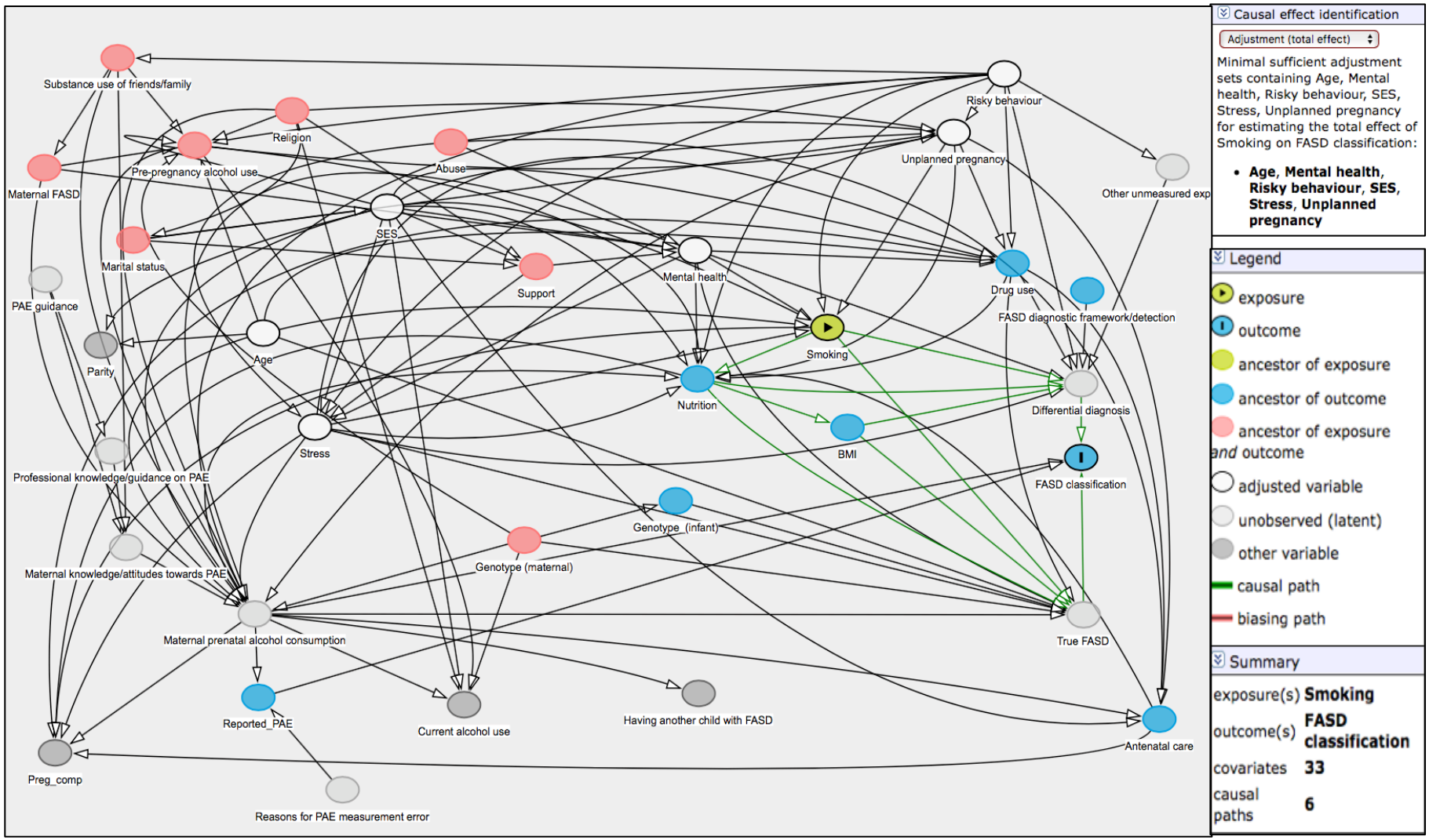
Abbreviations: CCEI, Crown-Crisp Experiential Index; CSE, Certificate of Secondary Education; EPDS, Edinburgh Postnatal Depression Scale.

[a] Variable is also an exposure of interest in other multivariable models.

[b] Proxy for maternal FASD.

[c] Proxy for 'risky behaviour'.

*Figure 19: DAGitty output depicting which confounding variables should be included in the multivariable statistical model to minimise bias in the estimate of the total casual effect of prenatal smoking on FASD classification (i.e. the minimal sufficient adjustment set). Hypothesised causal pathways are depicted in green.*



## 4.5 Statistical analyses

All statistical analyses were conducted in Stata 14.2.[155]

### 4.5.1 Missing data

As described in Chapter 4, missing data can lead to bias and imprecision in epidemiological analyses. To evaluate the impact of missing data for the risk factor analyses in this chapter, I used the *misstable* command in Stata 14.2[155] and created a flow chart to describe the frequency of missing data. To investigate whether data were missing completely at random (MCAR), I compared the distributions of sociodemographic, exposure and outcome data among participants who had complete versus incomplete data for multivariable risk factor analyses.

### 4.5.2 Participant characteristics

I calculated descriptive statistics to summarise participant characteristics, based on multiply imputed data. I described categorical variables using percentages with 95% confidence intervals and normally distributed continuous variables using means and standard deviations. Confidence intervals and pooled estimates were calculated using Rubin's combination rules.[256]

### 4.5.3 Regression analyses

I used logistic regression models to derive odds ratios to quantify the strength of the association between hypothesised causal risk factors and FASD. Westreich and colleagues coined the term the 'table 2 fallacy' to describe the issues that arise from interpreting multiple exposure effect estimates from the output of a single statistical model.[508] These include the inappropriate control of variables that lie on

mediating pathways, which can lead to biased estimates of the total causal effect for one or more of the variables of interest. To overcome these issues, I fitted separate regression models for each exposure.

### 4.5.4 Population attributable fractions

While odds ratios are useful for indicating the strength of the relationship between a candidate risk factor and FASD, they are less informative for predicting the impact of public health interventions.[510] To investigate the impact of potential interventions on the proportion of children affected by FASD, I calculated population attributable fractions (PAFs) using the *punaf* package for Stata,[511] adapted for use with multiply imputed data.[512]

PAFs are calculated as:

$$\frac{\text{Prevalence}(FASD) - \text{Prevalence}(FASD \mid no\ exposure)}{\text{Prevalence}(FASD)}$$

In this context, PAFs indicate the proportion of FASD that might be prevented in a counterfactual scenario where the exposure has been altered. They provide an estimate of the public health impact of intervening upon an exposure, based on the assumption that the association between this exposure and FASD is causal. For example, for a hypothesised binary risk factor, such as prenatal smoking, the PAF indicates the proportion of FASD that would be prevented if no mothers smoked during pregnancy and all other factors remained as they were. Since PAFs depend on the prevalence of the exposure, as well as the strength of the association between the exposure and the outcome, exposures that have the largest association with FASD (odds ratio) will not necessarily have the largest PAF.

# 5 Results

## 5.1 Missing data

Appendix 16 presents a flow diagram that shows the number of participants at each stage of this study. There were 15,730 consented children in the ALSPAC sample. After applying the pre-specified inclusion criteria, 9,135 children were eligible for the risk factor analyses. Missing data led to significant attrition in the complete case sample. 9,026 eligible participants were excluded from one or more of the risk factor analysis models due to missing data on exposure, confounding and/or outcome variables. Appendix 9 provides a full summary of the proportion of missing data for key variables.

Children with incomplete data differed from those with complete data on a range of characteristics, suggesting that data were not MCAR (full details provided in Appendix 17). Compared to children with complete data, children with incomplete data were more likely to have been exposed to prenatal binge drinking (47% versus 34%), smoking (28% versus 13%) and illicit substance use (4% versus 2%). Mothers of children with incomplete data were more likely to have prenatal depression (21% versus 14%), prenatal anxiety (24% versus 18%), and were less likely to have taken prenatal vitamin supplements (45% versus 49% for iron; 20% versus 22% for folic acid). Children with incomplete data were more likely to meet criteria for FASD than those with complete data (13% versus 9%). Compared to mothers of children with complete data, mothers of children with incomplete data were younger (41% versus 51% were aged 30 years or over at delivery), were more likely to live in council housing (14% versus 3%) and were less likely to have a professional

occupation (12% versus 21%). This pattern was consistent across all indicators of socioeconomic status.

To address the issues introduced by missing data (substantial reduction in sample size and potential bias due to differential loss to follow-up for exposures and outcome), I used multiple imputation to account for missing values for exposure, confounder and outcome variables, resulting in a restored sample size of 9,135 for all risk factor analyses. The multiple imputation method has been described previously in Chapter 4 and the imputation model specification is presented in Appendix 9.

## 5.2 Sample characteristics

Table 13 describes the sociodemographic characteristics of participants included in the main risk factor analyses, based on multiply imputed data. In this sample of children, who had all been exposed to alcohol prenatally, 20.3% met criteria for FASD. Compared to children without FASD, children with FASD were more likely to be male (FASD 66%; not FASD 48%), born to mothers of younger age (mothers aged 30 years or over at delivery: FASD 33%; not FASD 43%), and to be of lower socioeconomic status. For example, children with FASD were more likely than those without FASD to live in council housing (FASD 26%; not FASD 11%), and to have mothers with a less skilled occupation (partly skilled/unskilled occupation: FASD 21%; not FASD 11%).

*Table 13: Participant sociodemographic characteristics, based on multiply imputed data.*

| | Total sample N = 9,135 % (95% CI) | Not FASD[a] % (95% CI) | FASD[a] % (95% CI) |
|---|---|---|---|
| **FASD status** | - | 79.7 (78.7 - 80.8) | 20.3 (19.2 - 21.3) |
| **Child gender** | | | |
| Female | 48.5 (47.5 - 49.6) | 52.2 (51.0 - 53.4) | 34.0 (31.4 - 36.6) |
| Male | 51.5 (50.4 - 52.5) | 47.8 (46.6 - 49.0) | 66.0 (63.4 - 68.6) |
| **Maternal ethnicity** | | | |
| White | 98.0 (97.7 - 98.4) | 98.2 (97.9 - 98.5) | 97.4 (96.4 - 98.3) |
| Non-White | 2.0 (1.6 - 2.3) | 1.8 (1.5 - 2.1) | 2.6 (1.7 - 3.6) |
| **Maternal age at pregnancy (years)** | | | |
| <20 | 3.7 (3.3 - 4.1) | 3.1 (2.6 - 3.5) | 6.1 (4.7 - 7.5) |
| 20-29 | 55.3 (54.3 - 56.4) | 53.9 (52.8 - 55.1) | 60.9 (58.5 - 63.3) |
| 30+ | 41.0 (40.0 - 42.0) | 43.0 (41.8 - 44.1) | 33.0 (30.7 - 35.3) |
| **Home ownership** | | | |
| Mortgaged/owned | 75.2 (74.3 - 76.1) | 79.1 (78.0 - 80.1) | 59.8 (57.0 - 62.6) |
| Council/housing association | 14.0 (13.3 - 14.8) | 10.9 (10.1 - 11.7) | 26.4 (23.8 - 28.9) |
| Rented (private) | 7.3 (6.8 - 7.9) | 6.8 (6.1 - 7.4) | 9.7 (8.0 - 11.4) |
| Other | 3.5 (3.1 - 3.8) | 3.3 (2.8 - 3.7) | 4.1 (3.0 - 5.3) |
| **Maternal highest educational qualification** | | | |
| CSE | 18.5 (17.6 - 19.3) | 15.2 (14.3 - 16.1) | 31.2 (28.8 - 33.7) |
| Vocational | 9.5 (8.8 - 10.1) | 8.9 (8.2 - 9.6) | 11.7 (9.9 - 13.5) |
| O level | 34.2 (33.1 - 35.2) | 34.8 (33.7 - 36.0) | 31.5 (28.8 - 34.2) |
| A level | 23.8 (22.9 - 24.7) | 25.2 (24.2 - 26.3) | 18.1 (16.0 - 20.2) |
| Degree | 14.1 (13.4 - 14.8) | 15.8 (14.9 - 16.6) | 7.5 (6.0 - 9.0) |
| **Maternal social class** | | | |
| Professional | 5.7 (5.2 - 6.2) | 6.6 (6.0 - 7.2) | 2.3 (1.4 - 3.1) |
| Managerial/technical | 30.9 (29.8 - 31.9) | 32.8 (31.6 - 33.9) | 23.4 (21.1 - 25.7) |
| Skilled non-manual | 42.1 (40.9 - 43.2) | 42.0 (40.7 - 43.2) | 42.5 (39.5 - 45.4) |
| Skilled manual | 8.0 (7.3 - 8.7) | 7.4 (6.6 - 8.1) | 10.5 (8.7 - 12.3) |
| Partly skilled/unskilled | 13.4 (12.6 - 14.2) | 11.3 (10.5 - 12.2) | 21.3 (18.9 - 23.8) |
| **Marital status** | | | |
| Not married | 25.3 (24.4 - 26.2) | 22.6 (21.6 - 23.7) | 35.7 (33.2 - 38.2) |
| Married | 74.7 (73.8 - 75.6) | 77.4 (76.3 - 78.4) | 64.3 (61.8 - 66.8) |

[a] Total sample size for multiply imputed data = 9,135. The number of participants with and without FASD varies for each imputation set.
Abbreviations: CI, confidence interval; CSE, Certificate of Secondary Education; FASD, fetal alcohol spectrum disorder; N, sample size.

## 5.3 Regression results

Investigation of the output from multiple imputation diagnostics indicated that imputed values were plausible, based on the comparison of observed, imputed and completed data distributions. For reasons of increased precision and bias reduction, the following sections will present the results for analyses with multiply imputed data only.

### 5.3.1 Prenatal substance use

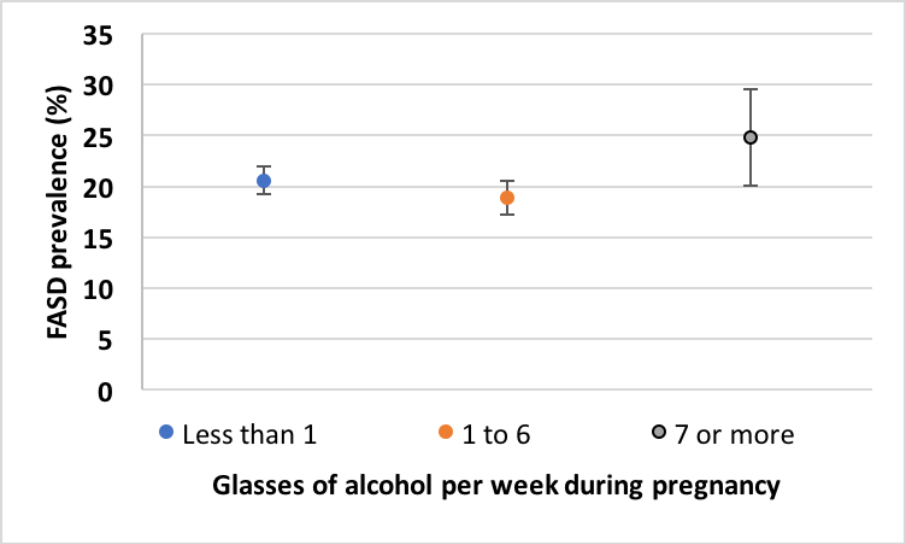
Table 14 presents unadjusted and adjusted odds ratios for FASD for the prenatal substance exposure variables.

#### *5.3.1.1 Patterns of prenatal alcohol exposure (PAE)*

There was no clear evidence of a dose-response relationship between the level of alcohol consumed among women who drank while pregnant and FASD. Point estimates, based on categorical data, provided weak evidence of a potential J-shaped relationship between the number of drinks per week and FASD (see Figure 20 and Table 14). Confidence intervals were wide and all odds ratios included the null value. Tests of the linear and non-linear (quadratic)[513] relationship between continuous measures of PAE and FASD showed no evidence of an association (adjusted odds ratio [aOR] for linear relationship per 1 unit increase in measures of alcohol at 8 weeks gestation 1.00 95% CI 1.00 - 1.02; and 32 weeks gestation aOR 1.01 95% CI 0.99 - 1.02; aOR for quadratic relationship at 8 and 32 weeks gestation both 1.00 95% CI 0.99 - 1.00). Graphs of the unadjusted association between continuous measures of PAE and FASD are presented in Appendix 18.

Among women who drank while pregnant, there was some evidence that binge drinking increased the odds of FASD. Unadjusted models showed an increase in the odds of FASD following binge drinking, compared to not binge drinking (OR 1.52 95% CI 1.33 - 1.74), however this effect was significantly attenuated in adjusted models (aOR 1.10 95% CI 0.95 - 1.28).

*Figure 20: FASD prevalence with 95% confidence intervals by glasses of alcohol per week during pregnancy. Alcohol use categories indicate maximum consumption at any point during pregnancy.*




#### *5.3.1.2 Prenatal smoking*

Mothers of children with FASD were more likely to have smoked while pregnant. Among children with PAE, prenatal smoking increased the odds of FASD by 19% (aOR 1.19 95% CI 1.03 - 1.36).

#### *5.3.1.3 Prenatal illicit drug use*

Mothers of children with FASD were more likely to report illicit substance use than those without, however effects were significantly attenuated after controlling for confounders and confidence intervals were wide due to the low prevalence of prenatal drug use (aOR 1.05 95% CI 0.73 - 1.49).

*Table 14: Descriptive statistics and odds ratios for FASD among children with PAE as a function of different patterns of prenatal substance use using multiply imputed data.*

| | Total sample % (95% CI) N = 9,135 | Not FASD % (95% CI) | FASD % (95% CI) | Unadjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
|---|---|---|---|---|---|
| **Prenatal alcohol exposure (maximum weekly consumption during pregnancy; categorical variable)[a]** | | | | | |
| < 1 glass/week | 63.5 (62.5 - 64.5) | 63.2 (62.0 - 64.4) | 64.6 (62.1 - 67.1) | Reference | Reference |
| 1-6 glasses/week | 31.8 (30.8 - 32.7) | 32.3 (31.2 - 33.5) | 29.6 (27.1 - 32.1) | 0.89 (0.78 - 1.03) | 0.96 (0.83 - 1.11) |
| 7+ glasses/week | 4.8 (4.3 - 5.2) | 4.5 (4.0 - 5.0) | 5.8 (4.6 - 7.0) | 1.27 (0.98 - 1.64) | 1.20 (0.90 - 1.61) |
| **Weekly prenatal alcohol exposure[a]** | | | | | |
| No | 63.5 (62.4 - 64.5) | 63.1 (62.0 - 64.4) | 64.6 (62.1 - 67.1) | Reference | Reference |
| Yes | 36.5 (35.5 - 37.5) | 36.8 (35.6 - 38.0) | 35.4 (32.9 - 37.9) | 0.94 (0.83 - 1.07) | 0.99 (0.86 - 1.13) |
| **Prenatal binge drinking[a]** | | | | | |
| No | 64.3 (63.2 - 65.5) | 66.3 (65.0 - 67.6) | 56.5 (53.7 - 59.3) | Reference | Reference |
| Yes | 35.7 (34.5 - 36.8) | 33.7 (32.4 - 35.0) | 43.5 (40.7 - 46.3) | 1.52 (1.33 - 1.74) | 1.10 (0.95 - 1.28) |
| **Prenatal smoking[b]** | | | | | |
| No | 71.8 (70.9 - 72.7) | 74.9 (73.8 - 75.9) | 59.9 (57.4 - 62.3) | Reference | Reference |
| Yes | 28.2 (27.3 - 29.1) | 25.2 (24.1 - 26.2) | 40.1 (37.7 - 42.6) | 1.99 (1.77 - 2.25) | 1.19 (1.03 - 1.36) |
| **Prenatal illicit drug use[b]** | | | | | |
| No | 96.2 (95.8 - 96.6) | 96.6 (96.1 - 97.1) | 94.6 (93.3 - 95.8) | Reference | Reference |
| Yes | 3.8 (3.4 - 4.2) | 3.4 (2.9 - 3.9) | 5.5 (4.2 - 6.7) | 1.63 (1.20 - 2.22) | 1.05 (0.73 - 1.49) |

[a] Adjusted for physical, sexual or domestic abuse towards the mother, maternal age during pregnancy, maternal ADH genotype (rs1229984), prenatal mental health (anxiety and depression), pre-pregnancy alcohol consumption (categorical glasses per day), maternal impulsivity, maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status, prenatal stress (weighted life events), unplanned pregnancy.

[b] Adjusted for maternal age during pregnancy, prenatal mental health (anxiety and depression), maternal impulsivity, maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status, prenatal stress (weighted life events), unplanned pregnancy.

### 5.3.2 Prenatal nutrition

Results for the effect of prenatal nutrition on FASD are presented in Table 15, showing first the results for associations with prenatal vitamin supplements and then with daily nutrient intake. Among children with PAE, the odds of FASD were lower for those who took folic acid supplements (aOR 0.82 95% CI 0.69 - 0.97). There was also weak evidence of a protective effect of iron supplementation (aOR 0.90 95% CI 0.80 - 1.02). Most point estimates indicated a protective effect of meeting the recommended daily intake for each nutrient during pregnancy. However, following adjustment for potential confounders, all confidence intervals included the null value.

### 5.3.3 Prenatal mental health

Table 16 presents odds ratios for the effect of prenatal mental health symptoms on FASD. Twenty-one percent of mothers who drank alcohol during pregnancy reported high levels of prenatal depression symptoms and 24% reported high levels of prenatal anxiety. Prenatal anxiety and depression increased the odds of FASD by 30% (aOR 1.30 95% CI 1.13 - 1.50) and 32% (aOR 1.32 95% CI 1.12 - 1.55), respectively.

*Table 15 (continued overleaf): Descriptive statistics and odds ratios for FASD among children with prenatal alcohol exposure as a function of prenatal nutrition using multiply imputed data.*

| | Total sample<br>% (95% CI)<br>N = 9,135 | Not FASD<br>% (95% CI) | FASD<br>% (95% CI) | Unadjusted odds ratio<br>(95% CI) | Adjusted odds ratio[a]<br>(95% CI) |
|---|---|---|---|---|---|
| **Prenatal vitamin supplement use[b]** | | | | | |
| Multivitamin supplements | 20.7 (19.8 - 21.5) | 21.0 (20.0 - 21.9) | 19.5 (17.5 - 21.6) | 0.92 (0.79 - 1.06) | 0.97 (0.83 - 1.14) |
| Calcium supplements | 5.8 (5.3 - 6.2) | 5.6 (5.0 - 6.1) | 6.5 (5.2 - 7.7) | 1.17 (0.92 - 1.50) | 1.20 (0.92 - 1.58) |
| Folic acid supplements | 19.4 (18.6 - 20.2) | 20.5 (19.5 - 21.5) | 15.2 (13.4 - 17.0) | 0.70 (0.59 - 0.81) | 0.82 (0.69 - 0.97) |
| Iron supplements | 44.5 (43.5 - 45.5) | 44.8 (43.6 - 46.0) | 43.3 (40.8 - 45.9) | 0.94 (0.84 - 1.06) | 0.90 (0.80 - 1.02) |
| Zinc supplements | 2.1 (1.8 - 2.4) | 2.2 (1.8 - 2.5) | 1.7 (1.0 - 2.5) | 0.79 (0.49 - 1.29) | 0.87 (0.52 - 1.45) |
| **Daily recommended nutrient intake met (RNI)[c]** | | | | | |
| Calories (2303 - 2375 kcal)[d] | 10.0 (9.4 - 10.7) | 9.7 (8.9 - 10.5) | 11.3 (9.5 - 13.2) | 1.19 (0.96 - 1.48) | 1.04 (0.83 - 1.32) |
| Calcium (700 mg) | 81.3 (80.4 - 82.2) | 81.8 (80.8 - 82.8) | 79.2 (76.9 - 81.6) | 0.85 (0.72 - 1.00) | 0.97 (0.82 - 1.16) |
| Folate (300 µg) | 21.1 (20.3 - 22.0) | 21.6 (20.6 - 22.6) | 19.4 (17.2 - 21.6) | 0.88 (0.75 - 1.03) | 1.02 (0.86 - 1.20) |
| Iodine (140 µg) | 56.2 (55.1 - 57.3) | 56.8 (55.5 - 58.1) | 53.7 (51.2 - 56.3) | 0.88 (0.78 - 1.00) | 0.98 (0.86 - 1.11) |
| Iron (14.8 mg) | 9.2 (8.6 - 9.9) | 9.4 (8.7 - 10.2) | 8.5 (6.9 - 10.1) | 0.89 (0.71 - 1.12) | 0.96 (0.75 - 1.22) |
| Magnesium (270 mg) | 36.9 (35.9 - 38.0) | 38.2 (36.9 - 39.4) | 32.0 (29.3 - 34.8) | 0.76 (0.66 - 0.88) | 0.97 (0.83 - 1.13) |
| Niacin (13 mg) | 72.3 (71.3 - 73.2) | 73.9 (72.9 - 75.0) | 65.8 (63.4 - 68.2) | 0.68 (0.60 - 0.77) | 0.96 (0.84 - 1.10) |
| Omega-3 (250 mg)[e] | 18.9 (18.1 - 19.8) | 19.9 (18.9 - 20.9) | 15.2 (13.2 - 17.2) | 0.72 (0.61 - 0.86) | 0.93 (0.77 - 1.12) |
| Phosphorous (550 mg) | 98.5 (98.2 - 98.8) | 98.8 (98.4 - 99.1) | 97.6 (96.7 - 98.5) | 0.52 (0.31 - 0.85) | 0.86 (0.50 - 1.49) |

| | Total sample<br>% (95% CI)<br>N = 9,135 | Not FASD<br>% (95% CI) | FASD<br>% (95% CI) | Unadjusted odds ratio<br>(95% CI) | Adjusted odds ratio[a]<br>(95% CI) |
|---|---|---|---|---|---|
| Potassium (3500 mg) | 20.0 (19.1 - 20.8) | 19.6 (18.6 - 20.6) | 21.4 (19.1 - 23.6) | 1.11 (0.95 - 1.30) | 1.04 (0.89 - 1.22) |
| Riboflavin (1.4 mg) | 71.4 (70.4 - 72.4) | 72.4 (71.3 - 73.5) | 67.4 (65.1 - 69.8) | 0.79 (0.70 - 0.89) | 0.91 (0.80 - 1.04) |
| Selenium (60 µg) | 63.4 (62.3 - 64.4) | 64.6 (63.3 - 65.8) | 58.7 (55.7 - 61.6) | 0.78 (0.68 - 0.90) | 1.01 (0.86 - 1.17) |
| Vitamin B12 (1.5 µg) | 97.7 (97.4 - 98.0) | 97.9 (97.5 - 98.2) | 97.1 (96.0 - 98.1) | 0.73 (0.47 - 1.12) | 1.06 (0.66 - 1.69) |
| Vitamin B6 (1.2 mg) | 91.5 (90.9 - 92.1) | 92.5 (91.8 - 93.1) | 87.7 (85.9 - 89.5) | 0.58 (0.47 - 0.70) | 0.81 (0.65 - 1.01) |
| Vitamin C (50 mg) | 79.5 (78.6 - 80.4) | 81.5 (80.5 - 82.6) | 71.6 (69.3 - 73.9) | 0.57 (0.50 - 0.66) | 0.86 (0.74 - 1.01) |
| Vitamin E (15 mg) | 9.0 (8.3 - 9.6) | 9.3 (8.6 - 10.0) | 7.7 (6.3 - 9.1) | 0.81 (0.66 - 1.01) | 0.95 (0.77 - 1.18) |
| Zinc (7 mg) | 69.5 (68.5 - 70.5) | 70.8 (69.7 - 72.0) | 64.3 (61.7 - 66.9) | 0.74 (0.65 - 0.84) | 0.97 (0.84 - 1.11) |

[a] Adjusted for physical, sexual or domestic abuse towards the mother, prenatal illicit drug use, prenatal mental health (anxiety and depression), prenatal alcohol consumption (categorical glasses per week and binge), pre-pregnancy alcohol consumption (categorical glasses per day), maternal impulsivity, maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status, prenatal smoking, prenatal stress (life events), unplanned pregnancy.

[b] Reference category is not taking the specified prenatal vitamin supplement.

[c] Reference category is not meeting the recommended daily level for the nutrient of interest.

[d] RNI 2375 kcal for pregnant women ≤ age 35 and 2303 kcal for pregnant women > age 35 years.

[e] RDA from Weichselbaum[502] and European Food Safety Authority[501] 250 mg/day of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) plus 100 to 200 mg preformed DHA for women during pregnancy or lactation. ALSPAC reports nutrients from fish intake only, so threshold set as 250mg/day for this analysis.

### 5.3.4 Prenatal stress and social support

Results for the effect of specific prenatal stressful life events and social support on FASD are presented in Table 17. Unadjusted odds ratios suggested a 4% increase in the odds of FASD for each additional prenatal life event among mothers who drank while pregnant (OR 1.04 95% CI 1.03 - 1.05); however, this effect was attenuated in adjusted estimates (aOR 1.01 95% CI 1.00 - 1.03). Mothers who reported having major financial problems in pregnancy had a 24% increase in the odds of having a child with FASD following prenatal alcohol use (aOR 1.24 95% CI 1.05 - 1.46), increasing to 39% among those who reported that they had been very affected by these financial problems (aOR 1.39 95% CI 1.11 - 1.75). In general, across most of the categories, mothers who reported having been very affected by a prenatal life event were more likely to have a child with FASD, compared to those who did not report being very affected. However, with the exception of financial problems, effect estimates were inconclusive following adjustment for potential confounders.

Social support had a protective effect on the odds of FASD among children with PAE (aOR per 5-point increase in prenatal social support score 0.84 (95% CI 0.79 - 0.89).

*Table 16: Descriptive statistics and odds ratios for FASD among children with prenatal alcohol exposure as a function of prenatal mental health using multiply imputed data.*

| | **Total sample % (95% CI) N = 9,135** | **Not FASD % (95% CI)** | **FASD % (95% CI)** | **Unadjusted odds ratio (95% CI)** | **Adjusted odds ratio[a] (95% CI)** |
|---|---|---|---|---|---|
| **Prenatal anxiety** | | | | | |
| No | 76.0 (75.1 -76.9) | 78.1 (77.1 - 79.1) | 67.7 (65.2 - 70.1) | Reference | Reference |
| Yes | 24.0 (23.1 - 24.9) | 21.9 (20.9 - 22.9) | 32.3 (29.9 - 34.8) | 1.70 (1.49 - 1.94) | 1.30 (1.13 - 1.50) |
| **Prenatal depression** | | | | | |
| No | 79.2 (78.4 - 80.1) | 81.5 (80.5 - 82.4) | 70.5 (68.0 - 73.0) | Reference | Reference |
| Yes | 20.8 (19.9 - 21.6) | 18.5 (17.6 - 19.5) | 29.5 (27.0 - 32.0) | 1.84 (1.59 - 2.12) | 1.32 (1.12 - 1.55) |

[a] Adjusted for physical, sexual or domestic abuse towards the mother, maternal grandmother history of alcoholism (as a proxy for maternal FASD), maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status, social support score.

*Table 17: Descriptive statistics and odds ratios for FASD among children with prenatal alcohol exposure as a function of prenatal stressful life events and social support using multiply imputed data.*

| | **Total sample % (95% CI) N = 9,135** | **Not FASD % (95% CI)** | **FASD % (95% CI)** | **Unadjusted odds ratio (95% CI)** | **Adjusted odds ratio (95% CI)** |
|---|---|---|---|---|---|
| **Stressful life events during pregnancy**[a] | | | | | |
| Relationship difficulties (any) | 66.5 (65.5 - 67.5) | 65.7 (64.6 - 66.9) | 69.4 (67.1 - 71.6) | 1.18 (1.05 - 1.33) | 0.90 (0.78 - 1.03) |
| Relationship difficulties (very affected) | 13.2 (12.5 - 13.9) | 11.8 (11.0 - 12.6) | 18.4 (16.2 - 20.5) | 1.67 (1.40 - 2.00) | 1.10 (0.89 - 1.35) |
| Bereavement (any) | 22.2 (21.3 - 23.1) | 22.4 (21.4 - 23.4) | 21.6 (19.5 - 23.6) | 0.95 (0.83 - 1.09) | 0.98 (0.84 - 1.13) |
| Bereavement (very affected) | 5.4 (4.9 - 5.8) | 5.3 (4.8 - 5.8) | 5.6 (4.5 - 6.8) | 1.06 (0.83 - 1.36) | 0.99 (0.76 - 1.29) |
| Major financial problems (any) | 19.9 (19.1 - 20.7) | 18.1 (17.1 - 19.0) | 27.1 (24.7 - 29.6) | 1.69 (1.45 - 1.96) | 1.24 (1.05 - 1.46) |
| Major financial problems (very affected) | 7.2 (6.7 - 7.8) | 6.2 (5.6 - 6.8) | 11.3 (9.6 - 13.0) | 1.92 (1.55 - 2.37) | 1.39 (1.11 - 1.75) |
| Moved house | 19.4 (18.6 - 20.2) | 18.7 (17.7 - 19.7) | 22.2 (19.8 -24.6) | 1.24 (1.05 - 1.46) | 0.99 (0.82 - 1.18) |
| Moved house (very affected) | 4.9 (4.4 - 5.3) | 4.8 (4.2 - 5.3) | 5.4 (4.2 - 6.7) | 1.15 (0.86 - 1.53) | 0.95 (0.69 - 1.31) |
| Accident or illness (any) | 16.9 (16.2 - 17.7) | 16.6 (15.7 - 17.5) | 18.4 (16.4 - 20.4) | 1.13 (0.97 - 1.32) | 1.07 (0.91 - 1.26) |
| Accident or illness (very affected) | 4.6 (4.2 - 5.0) | 4.4 (3.9 - 4.9) | 5.4 (4.3 - 6.5) | 1.25 (0.97 - 1.61) | 1.17 (0.89 - 1.54) |
| **Social support during pregnancy**[b] | **Mean (95% CI)** | **Mean (95% CI)** | **Mean (95% CI)** | | |
| Per 5-point increase in support score | 19.4 (19.3 - 19.5) | 19.7 (19.6 - 19.9) | 18.1 (17.8 - 18.4) | 0.73 (0.68 - 0.77) | 0.84 (0.79 - 0.89) |

[a] Adjusted for physical, sexual or domestic abuse towards the mother, prenatal mental health (anxiety and depression), maternal impulsivity, maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status, social support score. Reference category for categorical variables is not having experienced the specified life event.

[b] Adjusted for marital status, religion, maternal social class, paternal social class, maternal education (highest qualification), paternal education (highest qualification), home ownership status.

## 5.4 Population attributable fractions

I calculated PAFs for risk factors that were statistically significant in the multivariable logistic regression models for FASD. Results are summarised in Table 18.

*Table 18: Population attributable fractions for prenatal exposures and FASD among children with PAE*

| **Prenatal factor** | **Population attributable fraction % (95% CI)[a]** |
|---|---|
| Smoking | 17 (14 - 20) |
| Folic acid supplements | 22 (13 - 30) |
| Anxiety | 11 (8 - 14) |
| Depression | 11 (8 - 14) |
| Financial problems (any) | 9 (6 - 12) |
| Financial problems (very affected) | 4 (3 - 6) |
| Social support (maximum score of 30) | 45 (38 - 52) |
| Social support (score of 25) | 28 (22 - 33) |

[a] Counterfactual scenarios are as follows: PAF for smoking represents the proportion of FASD cases that would be prevented if no mothers smoked during pregnancy; PAF for vitamin supplements represents the proportion of FASD cases that would be prevented if all mothers took the supplement; PAFs for prenatal depression and anxiety represent the proportion of FASD cases that would be prevented if no mothers exceeded the threshold for significant prenatal anxiety or depression symptoms; PAF for financial problems (any) represents the proportion of FASD cases that would be prevented if none of the mothers had experienced major financial problems during pregnancy; PAF for financial problems (very affected) represents the proportion of FASD cases that would be prevented if no mother was 'very affected' by major financial problems during pregnancy; PAF for social support represents the proportion of FASD cases that would be prevented if all mothers had maximum social support (equivalent to a score of 30 on the prenatal social support questionnaire). This method follows the approach of the World Health Organisation who assign PAFs based on the maximum (or minimum) theoretical possibility for continuous exposures.[514] I have also presented a more conservative scenario where the mean social support score is 25. The distribution of all factors, other than the exposure of interest, remains constant when estimating PAFs.

Assuming that the relationships between the hypothesised risk factors in this study were causal, and given the exposure distributions in this sample, population attributable fractions indicated that: interventions for smoking cessation could

reduce the proportion of FASD among children with PAE by approximately 17% (95% CI 14% - 20%), folic acid supplementation by 22% (95% CI 13% - 30%) and interventions that alleviate prenatal anxiety and depression by 11% (95% CI 8% - 14%). Social support interventions showed the greatest potential for FASD prevention. PAFs indicated that 45% of FASD cases may be prevented if prenatal social support was maximal (equivalent to a total score of 30 on the social support questionnaire). Based on a more conservative scenario, where social support is increased to a score of 25 (approximately 5 points above the sample mean), an estimated 28% of FASD cases could be prevented.

# 6 Discussion

## 6.1 Main results

This study sought to investigate risk and protective factors for FASD among children from a general population sample who were exposed to alcohol prenatally. Analyses of the effects of different drinking patterns were inconclusive, as all confidence intervals included the null value. However, results suggested that several factors increased the odds of FASD following PAE. In order of the magnitude of association, these were: being very affected by major financial problems during pregnancy (aOR 1.39; 95% CI 1.11 - 1.75), prenatal depression (aOR 1.32 95% CI 1.12 - 1.55), prenatal anxiety (aOR 1.30 95% CI 1.13 - 1.50) and prenatal smoking (aOR 1.19 95% CI 1.03 - 1.36). Protective factors were: increased prenatal social support (aOR per 5-point increase in prenatal social support score 0.84 95% CI 0.79 - 0.89) and folic acid supplementation (aOR 0.82 95% CI 0.69 - 0.97).

Population attributable fractions (PAFs), based on the exposure distributions in this sample, indicated that social support interventions had the greatest potential to reduce FASD prevalence among children with PAE, with an estimated 45% reduction (95% CI 38% - 52%) for an increase to the maximum social support score and 28% reduction (95% CI 22% - 33% for a 5-point increase in social support score). Prenatal folic acid supplements had the next largest expected impact on FASD prevention (estimated reduction 22%; 95% CI 13% - 30%), followed by smoking cessation (estimated reduction 17%; 95% CI 14% - 20%), mental health interventions (estimated reduction 11%; 95% CI 8% - 14%), and interventions aimed at reducing prenatal financial problems (estimated reduction 4%; 95% CI 3% - 6% for those 'very affected' by financial problems to 9% reduction; 95% CI 6% - 12% for those who experience any major financial problem).

## 6.2 Comparison with the existing literature

I provided a detailed summary of the existing literature on FASD risk factors in Chapter 5. In this section, I highlight how some of the key results in this chapter compare to the existing research and indicate which are the most novel findings from this study.

### 6.2.1 Patterns of prenatal alcohol exposure (PAE)

First, as described previously, the existing literature has provided inconsistent evidence on the relationship between PAE and developmental outcomes, particularly when exposure is in the low to moderate range.[20-22] My analysis does not provide clear evidence on the effects of different levels of PAE on the risk of FASD. There was no evidence of a linear or non-linear association between

continuous measures of prenatal alcohol use and FASD status. Categorical measures of prenatal alcohol use (glasses of alcohol per week) produced weak evidence of a J-shaped relationship between PAE and FASD. Similar relationships have been found in other studies of PAE and child outcomes, including the Millennium Cohort Study.[307] Kelly and colleagues found a J-shaped relationship between prenatal alcohol use and behavioural and cognitive outcomes at age 3.[14] Consistent with findings from my analysis, effects in Kelly et al.'s study were attenuated following adjustment for confounders, however some effects remained statistically significant. Boys with light PAE[p] were less likely to have conduct and hyperactivity problems, and had higher scores on the Bracken School Readiness Assessment than those born to abstainers. Studies from the wider alcohol literature have also found evidence of a protective effect of low to moderate levels of alcohol use and an array of outcomes including: all cause mortality, asthma, colorectal cancer, the common cold, coronary heart disease, type 2 diabetes, hearing loss and liver cirrhosis.[515,516] However, these associations have been deemed spurious by some on the grounds that many of these relationships lack a plausible biological basis. Furthermore, as described in Chapter 5, patterns of alcohol use are highly socially patterned, with light or moderate drinkers typically having more favourable circumstances than heavy drinkers and abstainers. Consistent with proposals that many of the apparent protective effects of alcohol use may be accounted for by methodological shortcomings, the protective effect of low-level alcohol use on mortality has been found to disappear after controlling for

[p] Light drinking was defined as ≤ 1-2 units of alcohol per week or per occasion during pregnancy.

abstainer bias (caused by the inclusion of former-drinkers in the abstainer group) and study characteristics (including greater confounder adjustment and assessment measure quality).[515,517]

With regards to prenatal alcohol use, the biological basis for a potential protective effect of low to moderate PAE is not well supported. Animal studies have provided convincing evidence of alcohol's status as a teratogen and indicate deleterious effects on the developing brain, even at low levels of exposure.[23] In their recent review of light drinking in pregnancy, Mamluk and colleagues summarised the limitations of human studies of PAE.[24] They note that the main limitation of investigations into the effects of PAE is the risk of bias due to residual confounding. Drinking behaviour during pregnancy is strongly associated with socioeconomic status, and socioeconomic status is strongly associated with developmental outcomes. Although studies typically adjust for a range of indicators of socioeconomic status, the potential for uncontrolled confounding remains.[24] Evidence from Mendelian randomisation studies have indicated a detrimental effect of PAE on child IQ and educational attainment, even at low levels, and support the theory that apparent protective effects are due to residual confounding.[26,27]

The point estimates that suggested that that heavy and binge-pattern drinking increase the odds of FASD are in agreement with the vast majority of research findings. Studies in North America and South Africa have consistently found that mothers of children with FASD are less likely to reduce their alcohol intake following pregnancy recognition and report more binge drinking and more drinks

per week, compared to mothers of children without FASD.[89,266,273,274,276-278,325,342] Studies in Europe have been less consistent. In Italian samples, postnatal drinking patterns have been found to be more predictive of FASD than reported prenatal drinking.[83,345] The authors of these studies note that compared to mothers in the South African samples, it was more difficult to engage mothers from Europe in "frank and accurate discussion of drinking during the prenatal period."[83(p. 1572)] This indicates that mothers may underreport their alcohol consumption in the prenatal period.

### 6.2.2 Prenatal smoking

Consistent with previous studies,[111,266] my analysis showed that FASD was more common among children born to mothers who smoked while pregnant. The existing literature indicates a synergistic relationship between PAE and smoking on perinatal outcomes including preterm labour and birthweight.[346] There is a plausible biological basis for the combined adverse effects of PAE and prenatal smoking on FASD-relevant outcomes. Both are known to disrupt folate metabolism and to lead to vasoconstriction of the umbilical cord and placenta, thereby contributing to disrupted DNA synthesis, increased hypoxia and prolonged uterine exposure to ethanol.[8,285,346]

### 6.2.3 Prenatal stress, mental health and social support

Results suggesting that prenatal stressful life events, anxiety and depression increase the risk of FASD are consistent with wider evidence from human and animal studies, which show that offspring exposed to these factors in combination with PAE have poorer outcomes than those exposed to either in isolation.[286,432,433]

Stressful life events have been linked to a range of congenital anomalies including cleft palate,[431] gastroschisis[518] and neural tube defects.[441] To the best of my knowledge this is the first study to demonstrate the impact of stressful life events on FASD risk, and further, to indicate that it is the perceived impact, rather than simply the occurrence of the event that has the strongest association with FASD. Such findings may support the role of a biological stress response in shaping FASD risk. Financial problems were associated with a significant increase in the odds of FASD. This may indicate that financial problems are particularly stressful for mothers, and/or may be an additional marker of social disadvantage, which has been strongly associated with FASD.[111,266]

Evidence for a protective effect of social support is consistent with previous studies, which indicate that social support promotes decreased alcohol consumption.[449,450] Other studies have found low social support to be associated with poorer prenatal mental health and FASD-relevant outcomes including decreased birthweight and length,[519] thus implicating it as an important target for intervention.

### 6.2.4 Prenatal nutrition

As described in Chapter 5, animal studies suggest that nutritional supplementation may protect against the risk of FASD-relevant outcomes, such as facial abnormalities, adverse behavioural outcomes and growth deficiency.[314] Human studies of the effects of pre- and postnatal nutritional supplementation among children with PAE and FASD have been limited to choline and multivitamin supplements and have not provided consistent evidence of benefit.[394-396] The

results of this analysis suggest that folic acid and iron supplements may warrant further investigation as potential candidates for FASD prevention. In rat models, iron deficiency has been found to interact with PAE to produce FASD-relevant outcomes including neurological anomalies and impaired associative learning.[520] The effect of iron supplementation on FASD has not been investigated in human studies.

Folic acid is crucial for fetal development. It contributes to DNA synthesis and protects against neural tube defects.[314,521] Animal studies have shown that folic acid may be particularly important in alcohol-exposed pregnancies. Ethanol disrupts folic acid absorption and folic acid supplementation decreases the risk of congenital anomalies and growth restriction following PAE.[314]

Unlike the results from the vitamin supplementation analyses, dietary intake measures did not indicate protective effects of meeting the recommended daily intake for folate. This may be explained by the fact that folic acid does not occur naturally in food. It is a synthetic form of a range of molecules that are collectively known as folate.[376]

## 6.3 Strengths and limitations

To the best of my knowledge, this study is the largest to date to investigate risk factors for FASD within a population-based birth cohort. The rich dataset provided by the ALSPAC study enabled investigation of a comprehensive range of candidate risk factors. This study further benefitted from having a transparent and systematic framework for confounder selection, based on established principles for causal inference.[304,522]

It is important to note that although the application of causal diagrams can strengthen causal inference by informing analysis strategies, this approach rests on the assumptions of no unmeasured confounding and no measurement bias.[302,523] Furthermore, the causal interpretation of the odds ratios and PAFs that I have presented rests on the assumption that the causal pathways that I have proposed are correct.

As described previously, a fundamental limitation of observational studies of prenatal exposures is the risk of measurement bias due to the use of self-report methods. The ALSPAC sample benefits from repeated prospective measurement of many prenatal exposures, however data are still likely to be subject to measurement error, particularly for exposures such as smoking, drug and alcohol use, which are likely to be underreported due to stigma and social desirability bias.[60-64] Furthermore, adverse exposures are known to cluster together. Mothers who use substances in the prenatal period are more likely to experience a less favourable prenatal environment, including greater stress, greater social disadvantage and poorer nutrition.[524] A key strength of this study was the use of the literature synthesis, causal diagram and complimentary multivariable models to identify and control for potential confounders. Nevertheless, there is the potential for residual confounding due to the multitude of influences on child development and imperfect measurement of identified confounders. In particular, I used proxy indicators for some of the potential confounders. For example, I used reports that the maternal grandmother had alcoholism as a proxy for maternal FASD. This is an imperfect proxy, since it is not clear when the reported alcoholism occurred and

PAE does not necessarily imply FASD. Furthermore, I used reported maternal impulsivity as a partial proxy indicator of a latent variable that I called “risky maternal behaviour,” which in turn was proposed to account for the co-occurrence of adverse prenatal exposures. This too is an imperfect proxy and the factors that influence multiple risk behaviours remain poorly understood and measured.

Potential misreporting of PAE has the most significant implications for the studies in this thesis, as results may be impacted in multiple ways by its misclassification. Inaccurate measurement of PAE may have compromised efforts to determine the effects of different levels of PAE on the risk of FASD, may have led to misclassification of FASD status, and may have resulted in participants with true (unreported) PAE having been wrongfully excluded from the eligible sample in this study. As described in Chapter 3, FASD status was ascertained using an algorithm, which is not equivalent to a gold standard formal diagnosis. Nevertheless, the use of a range of prospective measures of PAE at multiple time points is a strength of the ALSPAC study and is likely to improve the classification of exposure status and developmental functioning, relative to retrospective reporting.

Multiple imputation methods were used to maximise sample size and to minimise the bias caused by missing data. However, some exposures such as prenatal drug use, were relatively uncommon in this sample. This led to sparse data in some analyses and wide confidence intervals. Confidence intervals included the null value for the many of the effect estimates. Due to the large number of factors investigated, it is also possible that some of the significant results are a chance finding.

Finally, the effects of prenatal alcohol use and other exposures are thought to differ according to the timing and duration of exposure.[13,19] In my analyses it was not possible to test for interactions with time due to sparse data in complete case analyses and convergence issues in multiple imputation models, due to model complexity. Therefore, the risk factor analyses that I presented in this chapter represent average effects across the whole of pregnancy. If trimester-specific interactions were present, the total causal effect estimates that I have presented in the preceding analyses may represent a simplification of the true relationship between patterns of PAE and co-occurring exposures.

## 6.4 Implications for research and practice

Since alcohol use in pregnancy is the sole necessary cause of FASD, prevention efforts must focus on reducing PAE. Exposure may occur prior to pregnancy recognition, and knowledge of the risks of PAE and related guidance is generally low.[56] The recent revisions to the UK guidance on PAE, which promote abstinence, are a welcome contribution to efforts to reduce the burden of FASD, to resolve the confusion around guidance, and to bring the public health messaging for PAE in line with the majority of international recommendations.[48] However, as it is recognised that many women may choose to drink some amount of alcohol in pregnancy, and others may have alcohol use disorders, including dependence, it is important to consider other factors that contribute to FASD risk. Research that increases understanding of the factors that influence susceptibility to FASD among children with PAE is also important from an aetiological perspective, to improve knowledge

of mechanisms of harm, and to support efforts for prevention in populations with high levels of PAE, such as the UK.[11]

The PAFs that I have presented indicate potential targets for interventions to reduce FASD prevalence. However, it is important to highlight that these estimates are based on the exposure distributions in this sample, and are not generalisable to populations with a different prevalence of exposure.[525] Furthermore, when considering these potential interventions, is it important to note that these statistics simply indicate the expected reduction of FASD under the counterfactual scenario in which the exposure has been removed. However, in this context, the intervention and corresponding causal effect is poorly defined.[526] PAFs do not indicate what format of intervention may be effective, or any unanticipated adverse effects. For example, although the PAFs suggest that interventions that reduce prenatal depression and anxiety could reduce FASD, management of mental health conditions in pregnancy requires consultation with experienced clinicians, particularly when considering the benefits and risks of pharmacological treatment.[527]

Intensive peer-support interventions have been shown to be feasible and acceptable to women in the UK,[528] and may promote pregnancy-related health behaviours, such as breastfeeding maintenance.[529] Therefore, such interventions may also be of interest for FASD prevention. For other exposures, such as prenatal smoking, cessation initiatives are already routine in the UK,[114] and results from my analyses suggest that these initiatives may have particular benefits for alcohol-exposed pregnancies. Conclusions about whether nutritional supplementation may

be protective, and whether stressful life events increase FASD risk are tentative, however. Confidence intervals indicated uncertainty in the direction of effect for most estimates.

Nevertheless, since adequate nutrition is vital for optimal fetal development and stress is known to adversely affect child outcomes, potential interventions are likely to have benefits for the mother and child that extend beyond FASD prevention. Nutritional supplementation has been of interest at both the prenatal stage, where the focus is to prevent FASD among alcohol-exposed individuals, and postnatally, where the focus is to ameliorate the adverse behavioural and cognitive effects of PAE. As described in Chapter 5, while animal studies have supported nutritional supplementation for FASD, human studies have been inconclusive and limited to a small number of supplements (multivitamins and choline). More research is required to confirm whether nutritional interventions for FASD are effective and, if so, to determine the optimal levels and timing for supplementation, and to investigate the combined effect of multiple supplements.[530]

Studies that evaluate the effect of folic acid supplementation may be an important priority for FASD research, given that folic acid is established as an important protective factor against neural tube defects,[376] and that it is associated with a decreased risk of FASD in this study and in controlled animal experiments.[314] Since observational studies of nutrition in humans may be particularly prone to residual confounding by socioeconomic and lifestyle factors, where feasible, randomised controlled trials of nutritional supplementation are recommended.[531] A large

randomised controlled trial, published in 1991, demonstrated that taking 4mg of folic acid daily before pregnancy and in the first trimester led to a 72% reduction in the risk of neural tube defects. Consequently, over 80 countries have now introduced mandatory fortification of flour with folic acid.[376] Population-level folic acid fortification has not been introduced in the UK, but interventions have been implemented at the individual level. NICE guidelines recommend that women should take folic acid supplements if planning a pregnancy and in the first trimester,[371] and folic acid supplements are available to women with low income in the UK via the Healthy Start Programme.[372] A review, published in 2018,[376] concluded that there are no risks associated with high levels of folic acid intake. Given that mandatory fortification has led to a reduced incidence of neural tube defects, and that there is no evidence of an upper threshold for folic acid intake, the authors argued that mandatory folic acid fortification should be introduced universally.[376] Therefore, if feasible, it may also be informative to conduct a natural experiment to investigate the incidence of FASD in countries that have introduced mandatory folic acid fortification at the population level.

Unlike the nutritional supplements, there was no evidence of a protective effect of meeting the recommended daily intake for a range of micronutrients in this chapter. Within ALSPAC, scores have recently been derived from factor analyses to represent a variety of composite dietary patterns including: 'healthy', 'processed', 'confectionary', 'vegetarian' and 'traditional'.[379,499] Further research that investigates the association between FASD risk and these more holistic

representations of prenatal diet may offer further insights into the role of nutrition in FASD.

# 7 Conclusions

Several factors influence susceptibility to FASD among children with PAE. Prenatal smoking, mental health problems and stress may increase the risk of FASD, while social support and nutritional supplementation may be protective. Findings from this chapter suggests several candidate risk factors that warrant further investigation and suggest that in addition to efforts to reduce PAE, public health interventions that promote smoking cessation and folic acid supplementation may also benefit FASD prevention efforts. Social support interventions may also present promising opportunities for FASD prevention.

# Chapter 7. Summary of main results and conclusions

## 1 Overview

The main aims of this thesis were:

i. To assess the validity of the biological tests that are available to obtain an objective measure of prenatal alcohol exposure (PAE).
ii. To describe the epidemiology of fetal alcohol spectrum disorder (FASD) within a population-based birth cohort in England (ALSPAC).

In this concluding chapter, I summarise the key results from this thesis for each of the research questions that I specified, consider the main strengths and limitations of my analyses, propose avenues for future research, and present the overarching implications and conclusions of this work. A detailed discussion, including comparison with the extant literature, has been provided in each of the results chapters within this thesis.

## 2 Main results

This thesis presented several novel methodological and empirical contributions to further the understanding of the epidemiology of PAE and FASD. In this section, I describe how the results from my research address the research questions that I proposed in the introductory section of this thesis.

## 2.1 Research Question 1: What is the diagnostic accuracy of objective measures of prenatal alcohol use?

I conducted the first systematic review of the validity of objective measures of PAE (Chapter 2) and found that:

- The evidence does not offer strong support for the use of current biomarkers of PAE in practice.
- Tests of the total concentration of four fatty acid ethyl esters (FAEEs; ethyl palmitate, ethyl stearate, ethyl oleate and ethyl linoleate) in meconium and placenta tissue showed the highest levels of sensitivity (82% to 100%), but specificity was variable (13% to 98%).
- Evidence was sparse for most biomarkers and the methodological quality of included studies was low for reasons including the lack of a gold standard reference test.

## 2.2 Research Question 2: What is the prevalence of FASD within the ALSPAC cohort?

I developed and validated a series of novel FASD case ascertainment algorithms that could be applied to identify individuals who met criteria for FASD within the ALSPAC cohort (Chapter 3). I applied these algorithms to provide what is, to the best of my knowledge, the first population-based prevalence estimate of FASD in the UK (Chapter 4). Overall. I found that:

- It was feasible to use case ascertainment algorithms to estimate the prevalence of FASD in an existing population-based dataset (ALSPAC).

- The complete case and single imputation[q] methods that are commonly used in FASD prevalence studies are likely to underestimate FASD prevalence. Analyses with multiply imputed data provide more plausible estimates of FASD prevalence.
- The estimated prevalence of FASD in the UK could be as high as 17%, indicating that FASD may be a significant public health concern.
- Alcohol-related neurodevelopmental disorder (ARND) - the non-dysmorphic subtype of FASD - was the most prevalent subcategory, and classified 15.4% of children in analyses with multiply imputed data. Partial fetal alcohol syndrome (pFAS) and fetal alcohol syndrome (FAS) - the dysmorphic subtypes of FASD - were less common. Across the analyses, a maximum of 1.6% of participants met criteria for pFAS and 0.03% for FAS.
- FASD was more common among children of lower socioeconomic status and those born to mothers who reported that their pregnancy was not planned.

## 2.3 Research Question 3: What are the risk factors for FASD within the ALSPAC cohort?

I presented a novel synthesis of the evidence of FASD risk factors, using causal diagram (DAG) methodology (Chapter 5) and used this to inform statistical models that quantified the effect of hypothesised causal risk factors on FASD among children with PAE (Chapter 6). I found that:

[q] These single imputation methods equate missing PAE data with no exposure, and missing CNS data with no impairment.

- There was no clear dose-response relationship between the amount of alcohol consumed during pregnancy and FASD.
- Among children with PAE, the following factors increased the odds of FASD: prenatal smoking (adjusted odds ratio; aOR 1.19 95% confidence interval; CI 1.03 - 1.36), prenatal anxiety (aOR 1.30 95% CI 1.13 - 1.50) and prenatal depression (aOR 1.32 95% CI 1.12 - 1.55). The odds of FASD were also higher among children born to mothers who experienced major financial problems in the prenatal period (aOR 1.24 95% CI 1.05 - 1.46), and increased further among those born to mothers who reported that they had been very affected by prenatal financial problems (aOR 1.39 95% CI 1.11 - 1.75).
- Factors that decreased the odds of FASD among children with PAE included: prenatal folic acid supplementation (aOR 0.82 95% CI 0.69 - 0.97) and increased prenatal social support (aOR per 5-point increase in prenatal social support score 0.84 95% CI 0.79 - 0.89).
- Population attributable fractions indicated that social support interventions may have the largest impact on reducing FASD prevalence. Based on the exposure distributions in this sample, and assuming that relationships are causal, an increase in social support score of 5 points was associated with an estimated 28% reduction in the prevalence of FASD. Folic acid supplementation had the next largest expected impact (estimated 22% reduction), followed by smoking cessation (estimated 17% reduction), mental health interventions (estimated 11% reduction), and interventions

aimed at reducing the stress caused by prenatal financial problems (estimated 4% to 9% reduction).

# 3 Strengths and limitations of key results

## 3.1 Data considerations

A key strength of this research was the use of existing data from a large-population based birth cohort in England (ALSPAC). This rich dataset enabled me to derive and validate a comprehensive FASD case ascertainment algorithm, using data from multiple informants, standardised tests, and multiple time points to create a detailed profile of each child's prenatal exposures, and physical and neuropsychological profile. Furthermore, to the best of my knowledge, the sample sizes in the prevalence and risk factor studies are the largest in the FASD epidemiological literature to date.

However, as described in detail in previous chapters (Chapters 3, 4 and 6), one important caveat of the data is the likelihood of measurement error due to the use of self-report methods. Most notably, potential misclassification of PAE will have implications for the validity of the PAE and FASD prevalence and effect estimates. Since PAE is likely to be underreported, FASD may be underestimated. Nevertheless, in the absence of a valid objective measure of PAE, the ALSPAC dataset represents a valuable source of PAE data. Compared to other UK-based cohorts (such as the Millennium Cohort Study,[307] which collected information about PAE postnatally), the ALSPAC dataset benefits from prospective and anonymous collection of PAE data at several time points during pregnancy. This reduces the risk of recall bias and counters some of the potential reasons for

underreporting, such as social desirability bias and the fear of repercussions from disclosing PAE.[125] Furthermore, the PAE items included in the ALSPAC questionnaires had a range of formats, included examples of drink sizes, and incorporated dose/frequency and drink type options, which have been shown to improve the accuracy of reporting.[62]

It is also important to re-emphasise that the FASD status outcome that I have used in this thesis is not equivalent to a formal diagnosis. Confidence in this outcome measure is strengthened by the use of a multidisciplinary case conference panel to validate the algorithm (Chapter 3). Nevertheless, the possibility of misclassification of FASD remains.

On reflection, it may have been preferable to use multiple imputation methods to address missing data prior to the case conference panel. However, this was not feasible, given the availability of the expert-panel and the need to progress with the algorithm development within the project timescale.

Finally, participants in the ALSPAC sample tended to be of higher socioeconomic status, and children tended to have better educational outcomes, than those in the general population of the UK. These differences should be considered when attempting to generalise prevalence estimates to the rest of the UK.[228,229]

## 3.2 Statistical methods

As in many cohort studies, missing data were common in the ALSPAC sample. The epidemiological studies that I present in this thesis were strengthened by the use of multiple imputation methods to reduce selection bias and maximise sample size. However, the actual values of the missing data remain unknown and therefore it is

not possible to determine whether this method resulted in an accurate imputed dataset, or whether data were truly missing at random. Nevertheless, investigation of the observed and imputed values offered support for the validity of the imputation model.

The use of causal inference methods (DAGs) to guide the statistical modelling strategy and the interpretation of FASD risk factors is a further strength of this work. This approach enabled me to identify plausible causal risk factors for FASD, and to use a transparent method for covariate selection to minimise bias in effect estimates. The DAG that I created was informed by subject-matter knowledge based on a synthesis of the current available evidence. Using this evidence, I linked all nodes in the DAG for which there was plausible evidence of a connection. This approach is consistent with the view that DAGs should represent a researcher's understanding and beliefs about how the world works for a particular causal question.[305] Another approach to DAG construction is to link all of the variables based on temporal precedence, regardless of whether there is convincing evidence of a causal link.[293,532] This method asserts that in a DAG, all exposures that precede another exposure in time should be linked, unless there is convincing evidence that a causal path does not exist. This approach can lead to large 'forward saturated' models. Due to the complexity of the multiple imputation models, and their failure to converge under specifications that included larger numbers of variables, I favoured the DAG approach that was based on subject matter knowledge, rather than simply temporal precedence. I assumed, therefore, that any extra covariates that might have been suggested by the temporal precedence approach would have had a negligible impact on estimates, after accounting for the key variables

suggested by the DAG that was derived from subject matter knowledge. Further research will help elucidate the optimal method for DAG development.

Although I grounded my analyses in causal inference, it is important to again note the limitations of observational data. The risk factor effect estimates (odds ratios and population attributable fractions) that I have presented are based on measures of association, and will only resemble the true causal effect to the extent to which there is no unmeasured confounding. The assumption of no unmeasured confounding is unlikely to hold for most epidemiological investigations. Therefore, the results I have presented represent my attempt to obtain the most rigorous estimates, given the limitations of the data.

# 4 Implications and future research

## 4.1 Research gaps and extensions

There has been a paucity of evidence on FASD in the UK. Contributors to the All Party Parliamentary Group on FASD argue that this lack of evidence has contributed to uncertainty and mixed messaging around the 'safety' of PAE among health professionals and pregnant women, and inadequate service provision.[108] The British Medical Association further note that accurate data on FASD in the UK are crucial to inform decisions on prevention, health, education, and justice policy.[10] The results that I have presented in this thesis indicate the potential burden of FASD in the UK and suggest that further investigation is warranted. In particular, results point to a need for an active case ascertainment study to further elucidate the true prevalence of FASD in the UK.

Second, clinic-based studies suggest that individuals with FASD have an increased risk of a range of adverse secondary conditions including mental health problems, substance misuse and antisocial behaviour.[90,91,93] However, there are no large population-based studies of the long-term outcomes of people with FASD. Therefore, the needs of these individuals and the impact of this condition on health services are not well understood. My development of a case ascertainment algorithm for FASD in ALSPAC allows for longitudinal analyses of outcomes, including mental and physical health, antisocial behaviour, independent living and substance use. Such research would be useful for estimating the wider impact of FASD and highlighting opportunities for intervention to reduce the adverse outcomes associated with FASD.

Third, results from this thesis indicate potential targets for FASD prevention, among children exposed to alcohol prenatally. Future research is required to test the feasibility and effectiveness of these proposed interventions, which include smoking cessation, nutritional supplementation and social support. For example, randomised studies in Ukraine are investigating the impact of multivitamin and choline supplementation on the outcomes of children with PAE.[394,533] Results from this thesis indicate that evaluation of iron and folic acid supplementation may also be warranted.

Finally, the ALSPAC study has recently enhanced data linkage to NHS records.[248] This provides an opportunity to increase detection of FASD in the cohort and to further validate the FASD algorithm that I have developed. Using this resource, it may be possible to estimate the prevalence of FASD using GP-linked data, to

further evaluate the performance of the FASD algorithm, and to create a pooled dataset of participants' FASD status by combining information from GP codes and the FASD algorithm.

## 4.2 Methodological and statistical recommendations

Results from this thesis indicate that the complete case and single imputation methods that are commonly used to manage missing data in epidemiological studies of FASD are likely to underestimate true prevalence. Therefore, I would suggest that future research should consider multiple imputation methods to address missing data. In particular, it would be interesting to determine how the prevalence estimates from existing active case ascertainment studies, such as those from the Collaboration on FASD Prevalence (CoFASP) research consortium in the USA,[534] compare to estimates with multiply imputed data.

Like the UK, many countries lack population-based estimates of FASD prevalence.[86] The application of a FASD case ascertainment algorithm to existing data mitigated the ethical and resource concerns that have precluded active-case ascertainment studies of FASD to date in the UK.[108] This methodology presents a potential model for similar studies in other countries that have comprehensive datasets on prenatal exposures and children's physical and neurobehavioural outcomes.

# 5 Main conclusions

This thesis identified and addressed gaps in the evidence on PAE and the epidemiology of FASD in the UK. The research in this thesis has highlighted limitations in current objective measures of PAE; presented a case ascertainment

algorithm for FASD that can be applied to existing data; described the aetiological context of FASD using causal diagram methodology; and has presented the first population-based estimates of FASD prevalence and risk factors in the UK.

Taken together, the results from this thesis suggest that PAE and FASD are a significant public health concern in the UK. Children at particular risk include those whose mothers report that pregnancy was unplanned, those of lower socioeconomic status, and those who experience adverse prenatal environments including concurrent exposure to smoking, mental health issues and lower social support. Given the paucity of evidence in this population, further research is required to corroborate prevalence estimates and to investigate risk factors for FASD as potential targets for intervention.

# Appendices

Appendix 1: Medline search string for systematic review

| Search: Objective measurement of alcohol use during pregnancy | |
|---|---|
| Interface: OVID SP | Databases: Medline |
| Limits: Humans, English Language | Date Range: 1990 to August 2015 |

1. (Objectiv* adj (measure* or test* or assess* or screen*)).ti,ab.

2. (Aminotransferase or AST or ALT or Biomarker* or Carbohydrate-deficient transferrin or CDT or Ethyl or (Fatty adj Acid) or FAEE or (Gamma adj glutamyltransferase) or GGT or (Guthrie adj card*) or Mean Corpuscular Volume or MCV or Phosphatidylethanol or PEth).tw.

3. "area under curve"/ or "predictive value of tests"/ or roc curve/ or "sensitivity and specificity"/ or validation studies/

4. exp Biological Markers/ or Fetal Monitoring/ or exp Prenatal Diagnosis/

5. 1 or 2 or 3 or 4

6. exp Alcoholism/ or exp Alcohol drinking/ or exp Alcohol induced disorders/ or exp Alcoholic intoxication/ or exp Binge drinking/ or exp Drunkenness/ or exp Ethanol/

7. (Alcohol* or Binge drinking or ARBD or ARND or Drunk* or Ethanol or FAS or FASD or Intoxicat* or PAE or pFAS).tw.

8. 6 or 7

9. maternal-fetal exchange/ or pregnancy, high-risk/ or exp Infant, Newborn/

10. (Baby or F?etus or F?etal or Gestation* or Infant or In?utero or Matern* or Mother or Newborn or Neonat* or Postnat* or Pregnan* or Antenat* or Prenatal*).tw.

11. 9 or 10

12. 5 and 8 and 11

13. limit 12 to (english language and humans and yr="1990 -Current")

Appendix 2: Coding criteria for methodological quality assessment

| **Quality domain** | **Question** | **Coding criteria** |
|---|---|---|
| Participant selection | Were participants selected in a way that avoided bias? | *Yes:* If the characteristics of the spectrum of patients fulfilled the pre-stated requirements of the study, if inappropriate exclusions, were avoided, if the study avoided a case-control design and the method of recruitment was consecutive or random samples were taken from consecutive series.<br><br>*No:* If the sample does not fit with what was pre-specified within the primary study as acceptable or if groups with and without the target disorder were recruited separately, particularly with healthy controls. If there were systematic differences between those with and without the target condition.<br><br>*Unclear:* If there is insufficient information available to make a judgement either about the spectrum or the method of sampling. |
| Reference standard | Is the reference standard likely to correctly classify the target condition? | *Yes:* The reference standard is likely to correctly classify the target condition.<br><br>*No:* The reference standard is not likely to correctly classify the target condition.<br><br>*Unclear*: It is unclear exactly what reference standard was used/it is unclear whether the reference standard will correctly classify the target condition. |

<table>
<tr><th>Quality domain</th><th>Question</th><th>Coding criteria</th></tr>
<tr><td>Detection window</td><td>Does the window of detection for the index test [i.e. the period in which the biomarker can be detected following alcohol consumption] overlap with the period assessed by the reference standard?</td><td>Yes: If the time between tests was within the window of detection (see guidance below for detection periods), at least for an acceptably high proportion of patients.<br><br>No: If the time between tests was outside of the window of detection for an unacceptably high proportion of patients.<br><br>Unclear: If information on timing of tests is not provided.<br><br>
<table>
<tr><td>Biomarker (abbreviation; matrix)</td><td>Window of detection</td></tr>
<tr><td>Carbohydrate deficient transferrin (CDT; blood)</td><td>2 - 4 weeks</td></tr>
<tr><td>Ethyl sulphate (EtS; urine)</td><td>≤ 30 hours</td></tr>
<tr><td>Ethyl glucuronide (EtG; urine)</td><td>≤ 5 days</td></tr>
<tr><td>Ethyl glucuronide (EtG; hair)</td><td>Months to years depending on the length of hair collected. Human hair grows at approximately 1cm per month.</td></tr>
<tr><td>Fatty acid ethyl esters (FAEE; meconium)</td><td>2nd and 3rd trimester</td></tr>
</table>
</td></tr>
</table>

| Quality domain | Question | Coding criteria | |
|---|---|---|---|
| | | Fatty acid ethyl esters (FAEE; placenta) | Unknown |
| | | Gamma glutamyltransferase (GGT; blood) | ≤ 3 - 4 weeks |
| | | Haemoglobin-acetaldehyde adducts (Hb-Ach; maternal blood) | ≤ 4 weeks |
| | | Mean corpuscular volume (MCV; maternal blood) | ≤ 17 weeks |
| | | Phosphatidylethanol (PEth; maternal and infant blood) | ≤ 3 weeks |
| Partial verification | Did the whole sample or a random selection of the sample, receive verification using the intended reference standard? | *Yes:* If all patients, or a random selection of patients, who received the index test received verification of their disease status using a reference standard, even if the reference standard was not the same for all patients.<br>*No:* If some of the patients who received the index test did not receive verification of their true disease state, and the selection of patients to receive the reference standard was not random. | |

| Quality domain | Question | Coding criteria |
|---|---|---|
| | | *Unclear:* If this information is not reported by the study. |
| Differential verification | Did patients receive the same reference standard? | *Yes*: If the same reference standard was used in all patients.<br><br>*No*: If the choice of reference standard varied between individuals.<br><br>*Unclear*: If it is unclear whether different reference standards were used. |
| Incorporation bias | Was the reference standard independent of the index test [i.e. the index test did not form part of the reference standard]? | *Yes:* If the index test did not form part of the reference standard.<br><br>*No:* If the reference standard included components of the index test.<br><br>*Unclear:* If it is unclear whether the results of the index test were used in the final diagnosis. |
| Uninterpretable results | Were uninterpretable/ intermediate test results reported? | *Yes:* If the number of uninterpretable test results is stated, or if the number of results reported agrees with the number of patients recruited (indicating no uninterpretable test results).<br><br>*No:* If it states that uninterpretable test results occurred or were excluded and does not report how many.<br><br>*Unclear:* If it is not possible to work out whether uninterpretable results occurred. |

| Quality domain | Question | Coding criteria |
|---|---|---|
| Withdrawals | Were withdrawals from the study explained? | *Yes:* If it is clear what happened to all patients who entered the study, for example if a flow diagram of study participants is reported explaining any withdrawals or exclusions, or the numbers recruited match those in the analysis.<br><br>*No:* If it appears that some of the patients who entered the study did not complete the study, i.e. did not receive both the index test and reference standard, and these patients were not accounted for.<br><br>*Unclear:* If it is unclear how many patients entered and hence whether there were any withdrawals. |

| Quality domain | Question | Coding criteria |
|---|---|---|
| Selective outcome reporting | Did the study avoid selective outcome reporting? | *Yes:* If there is no evidence to suggest that the study has omitted key data or outcomes. For example:<br><br>i. If summary statistics including sensitivity, specificity and predictive values and the number of true positive, false positive, true negative and false negative results are reported directly and the study reports the accuracy of all of the index tests that were investigated.<br><br>ii. If summary statistics are reported directly in combination with the number of participants included in analysis and prevalence of the target condition in order to allow calculation of the number of true positive, false positive, true negative and false negative results. The study reports the accuracy of all of the index tests that were investigated.<br><br>iii. If the number of true positive, false positive, true negative and false negative results are reported in a way that enables calculation of diagnostic summary statistics and the study reports the accuracy of all of the index tests that were investigated.<br><br>*No:* If the study does not report summary statistics or raw data, or does not provide sufficient information for each to be calculated. If it appears that the |

| Quality domain | Question | Coding criteria |
|---|---|---|
| | | study has investigated an index test but not reported the findings. If it is not possible to replicate the summary statistics presented in the paper.<br><br>*Unclear:* Insufficient information to permit judgement of 'Yes' or 'No'; for example if a study protocol is not available. |

Appendix 3: Comparison of FASD criteria across diagnostic frameworks.

Note: A description of how these criteria correspond to FASD subcategories is presented within the diagnostic categories row. Bold emphasis denotes key differences in criteria between guidelines.

| | Canadian 2005[72] | Canadian 2016[227] | 4-Digit Code 2004[535] | IOM revised 2005[73] | IOM revised 2016[74] | DSM-5 ND-PAE[212] |
|---|---|---|---|---|---|---|
| **Growth[a] (A)** | Pre- and/or postnatal height or weight ≤ 10th percentile **or low weight-to-height ratio ≤ 10th percentile** | **Not included** | Pre- and/or postnatal height or weight ≤ 10th percentile | Pre- and/or postnatal height or weight ≤ 10th percentile | Pre- and/or postnatal height or weight ≤ 10th percentile | **Not included** |
| **Face[b] (B)** | All 3 facial features: short palpebral fissures ≤ 3rd percentile; smooth philtrum, thin upper lip | All 3 facial features: short palpebral fissures ≤ 3rd percentile; smooth philtrum, thin upper lip | All 3 facial features: short palpebral fissures ≤ 3rd percentile; smooth philtrum, thin upper lip | **≥ 2 facial features:** short palpebral fissures **≤ 10th percentile**; smooth philtrum, thin upper lip | **≥ 2 facial features:** short palpebral fissures **≤ 10th percentile**; smooth philtrum, thin upper lip | **Not included** |
| **Central nervous system (C)** | **Impairment in ≥ 3 subdomains** including: neurological signs; brain structure; CNS functioning | **Impairment in ≥ 3 subdomains** including: neurological signs; brain structure; CNS functioning | **Impairment in ≥ 1 structural or neurological subdomain(s)** including: brain structure; seizures/other neurological signs; or impairment in **≥ 3 domains of CNS functioning** | **Impairment in ≥ 1 structural subdomain** and/or **evidence of a behavioural or cognitive abnormalities inconsistent with developmental level** | **Impairment in ≥ 1 structural or neurological subdomain(s)** including: brain structure; seizures; and/or **global cognitive impairment** and/or **cognitive or behavioural deficit not including adaptive functioning in ≥ 1 subdomain(s)** | **Impairment in ≥ 3 subdomains which must include:** neurocognitive functioning; self-regulation; and adaptive functioning |

| | Canadian 2005[72] | Canadian 2016[227] | 4-Digit Code 2004[535] | IOM revised 2005[73] | IOM revised 2016[74] | DSM-5 ND-PAE[212] |
|---|---|---|---|---|---|---|
| **Alcohol exposure (D)[c]** | Any | **More than specified threshold:**<br>≥ 7 drinks per week, or > 4 drinks per occasion | Any | **More than specified threshold:**<br>Pattern of excessive intake characterized by substantial regular intake or heavy episodic drinking | **More than specified threshold:**<br>≥ 6 drinks/week for ≥ 2 weeks during pregnancy<br>≥ 3 drinks per occasion on ≥ 2 occasions during pregnancy<br>Documentation of alcohol-related social or legal problems in proximity to (before or during) the index pregnancy<br>Documentation of intoxication during pregnancy by blood, breath, or urine alcohol content testing<br>Positive testing with established alcohol-exposure biomarker(s) during pregnancy or at birth (eg, analysis of FAEEs, PEth and/or EtG in maternal hair, nails, urine, blood, | **More than specified threshold:**<br>> 13 drinks per month or more than 2 drinks per occasion |

| | Canadian 2005[72] | Canadian 2016[227] | 4-Digit Code 2004[535] | IOM revised 2005[73] | IOM revised 2016[74] | DSM-5 ND-PAE[212] |
|---|---|---|---|---|---|---|
| | | | | | placenta, or meconium)<br>− Increased prenatal risk associated with drinking during pregnancy as assessed by a validated screening tool of, for example, T-ACE or AUDIT | |
| **Diagnostic categories (criteria)** | **FAS** (A, B, C and D [but external confirmation of PAE not required when facial phenotype is present])<br>**Partial FAS** (2 features from B plus C and D - confirmed exposure only)<br>**ARND** (C and D - confirmed exposure only) | **FASD with sentinel facial features** (B, C and D [but external confirmation of PAE not required when facial phenotype is present])<br>**FASD without sentinel facial features** (C and D - confirmed exposure only) | **FAS** (A, B, C and D [but external confirmation of PAE not required when facial phenotype is present])<br>**Partial FAS** (B with relaxed criteria: ≤ 1 SD below mean for palpebral fissures, or rank 3 for lip/philtrum, C and D - confirmed exposure only)<br>**Static encephalopathy /alcohol-exposed (SE/AE)** C with 1 structural or hard neurological sign of impairment e.g. seizures or 3 | **FAS** (A, B, C [which must include structural impairment] and D [but external confirmation of PAE not required when facial phenotype is present])<br>**Partial FAS** (B and A or C extended to include evidence of behavioural/cognitive abnormalities, and D [but external confirmation of PAE not required when facial phenotype is present])<br>**ARBD** (B and evidence of congenital | **FAS** (A, B, C [which must include structural or neurophysiological impairment with global impairment or cognitive or behavioural impairment in ≥ 1 domain] and D [but external confirmation of PAE not required when facial phenotype is present])<br>**Partial FAS** (A, [required if D is unconfirmed only] B, C [which must include cognitive or behavioural impairment in ≥ 1 | **Neurobehavioral Disorder Due to Prenatal Alcohol Exposure (ND-PAE;** C and D, plus onset must be in childhood and disturbance must cause clinically significant distress or impairment in functioning) |

| | Canadian 2005[72] | Canadian 2016[227] | 4-Digit Code 2004[535] | IOM revised 2005[73] | IOM revised 2016[74] | DSM-5 ND-PAE[212] |
|---|---|---|---|---|---|---|
| | | | functional domains with performance ≥ 2 SDs below the mean, D - confirmed exposure only)<br><br>**Neurobehavioral disorder / alcohol-exposed (ND/AE)** C but with no structural/hard neurological abnormalities and with 1-2 functional domains with performance ≥1.5 SDs below the mean, D - confirmed exposure only) | abnormalities and D - confirmed exposure only)<br><br>**ARND** (C [structural and or behavioural/cognitive abnormalities] and D - confirmed exposure only) | domain] and D [but external confirmation of PAE not required when facial phenotype is present])<br><br>**ARBD** (evidence of congenital abnormalities and D - confirmed exposure only)<br><br>**ARND** (C [global impairment or cognitive or behavioural impairment in ≥ 2 domains] and D - confirmed exposure only) | |

[a] In the UK, the 9th percentile is used as the threshold for growth deficiency, rather than the 10th percentile.[106]

[b] With the exception of the revised IOM 2016 guideline, which is not explicit about which lip-philtrum guide to use (except that it should be 'racially normed' if available), and the ND-PAE criteria, which do not consider the facial phenotype, all other diagnostic frameworks recommend the use of the University of Washington Lip-Philtrum Guide[247] to assess the FAS facial phenotype. Ranks 4/5 correspond to the FAS facial phenotype based on the University of Washington Guide.

[c] Confirmation of PAE by external sources is not required for a diagnosis of FAS (or FASD with sentinel facial features) because the facial phenotype is highly specific to PAE. Relative to other guidelines, the IOM guidelines relax their criteria so that pFAS may also be diagnosed without confirmed PAE.

Appendix 4: Overview of the ALSPAC assessment measures used to derive FASD classifications

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| **Growth (A)** | Weight | Weight | Birth - 9 | ≤ 9th percentile at birth | Standard norms | Excel LMS Growth Add-in based on UK growth norms.[239,240]<br><br>Centiles for pre-term babies < 35 weeks gestation were generated using the Fenton Growth Calculator.[241] |
| | Height | Height | Birth - 9 | ≤ 9th percentile at birth and postnatally (up to puberty) | Standard norms | |
| | Body mass index (BMI) | BMI | Birth - 9 | ≤ 9th percentile at birth and postnatally (up to puberty) | Standard norms | |
| **Face (B)** | Palpebral fissure length | 3D facial scan data | 15.5 | ≤ 2.5th percentile | Standard norms | FAS Diagnostic and Prevention Network Z-Score Calculator for palpebral fissure length.[237,238] |
| | Thin upper lip | | | Equivalent to 4/5 on the lip-philtrum guide | Standard norms | Wilson Scale for Lips.[236] |
| | Smooth philtrum | | | | Standard norms | |
| **CNS (C)** | a) Hard and soft neurologic signs (including sensory-motor) | Movement score | 7 | Top 5% (95th percentile)<br>Score: ≥ 6 for girls<br>≥ 7 for boys | Research literature | ALSPAC coordination test (modified version of Movement Assessment Battery)<br><br>The Movement Assessment Battery for Children was used to test the children's motor ability.[246] It comprises three sections, assessing static and dynamic balance, manual dexterity and ball skills. Because of time constraints, it was not possible to conduct the whole assessment, so specific subtests from each of the three sections were carried out:<br>Manual dexterity: Placing Pegs and Threading lace |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | | | | | Ball skills: Bean bags<br>Balance: Heel to toe walking<br><br>Odd et al. derived a summary score based on the three tasks. 'The top (i.e. indicating worse performance) 5th centile of this summed score was used to define severe motor coordination difficulties as has been used previously in the literature.[235] |
| | | Seizures | 1 - 13 | > 1 seizure not due to fever, breath-holding or response to immunisation (i.e. due to epilepsy) | Expert opinion and FASD Canadian 2005 guidelines | ALSPAC asks the child's caregiver:<br>Has the child ever had a seizure, fit or convulsion?<br>And whether the seizure was due to factors including immunisation, fever or breath-holding, epilepsy. |
| | | Cerebral palsy | NR | Cerebral palsy | Expert opinion | Cerebral palsy reported by mother/carer |
| | b) Brain structure | Head circumference | Birth and 7 | ≤ 2nd percentile | Standard norms | Excel LMS Growth Add-in based on UK growth norms.[239,240] |
| | c) Cognition | WISC-III (short-form) | 8 | Score ≤ 70 on total, verbal or performance IQ<br><br>Discrepancy of at least 1 SD (i.e. 15 points) between the subdomains (i.e. verbal and performance IQ). | Standard norms and FASD Canadian 2005 guidelines | <u>WISC-III (short form)</u><br><br>Alternate WISC items were used for all subtests, except for the coding subtest which was administered in its full form.[536-539]<br>Administered by members of the psychology team.<br>Raw scores were calculated according to the items used in the alternate item form of the WISC, |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | | | | | making the raw scores comparable to those that would have been obtained had the full test been administered.[245,281] |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| d) | Communication: receptive and expressive | WOLD (Wechsler Objective Language Dimensions) | 8 | Score ≤3 for wold_list<br>Score ≤3 for wold_express | Based on the distribution of ALSPAC participant data. | Wechsler Objective Language Dimensions (WOLD; modified):[245]<br><br>Listening comprehension: The listening comprehension subtest of the WOLD is divided into two parts. The first is a single word receptive vocabulary test, similar to the vocabulary subtest of the WISC. This was not therefore used.<br>In the second part of the assessment, the child listens to the tester read aloud a paragraph about a picture, which the child is shown. The child then answers questions on what they have heard.<br>The child has to make inferences about what was read to them and answer the questions verbally. The task was discontinued if the child got three consecutive questions incorrect. Alternate items from the standard test were sampled except where the item had American cultural loading. In those cases, the next item was selected.<br><br>Expressive language: The WOLD has two expressive language subtests. In the second subtest three tasks were performed. Firstly, a picture was shown to the child who was asked to describe the scene, as if to someone who was not present and so could not see the picture.<br>Secondly, the child was shown a map and asked to give directions from one location to another, using the shortest route possible and finally they were asked to explain the steps involved in a sequential task of putting batteries into a torch using pictures to help. |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | | | | | These tasks assess the child's descriptive, narrative and sequencing skills. All responses in this task were recorded on audio tape for later coding on five features, relating to the relevance, accuracy and logicality of the child's responses. In the full WOLD assessment, each task has two examples. Only one of each was used in the ALPSAC tests. |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | Communication (general) | 7 and 10 | 'Yes' indicates reported impairment at any time point | Expert opinion | Teacher-reported speech and language difficulties in school (needing special assistance) |
| | e) Academic achievement | Special needs | 9 to 10<br>10 to 11<br>11 to 12 | 2 (School Action) 3 (School Action plus) and 4 (SEN statement) indicate impairment | Standard norms | Pupil Level Annual School Census (PLASC) recorded SEN: School action, school action plus and statement.[540] |
| | | Academic attainment | 6-7 | "Failing to meet expected level"<br>Key stage 1: Level 1 or W | Standard norms | |
| | | | 10-11 | "Failing to meet expected level"<br>Key Stage 2: < Level 4 | Standard norms | |

| Key Stage | Range of levels within which most children will work | Target that most children reach by the end of the key stage | Further information about Key Stage levels (Source: *ALSPAC SATS Doc* and *ALSPAC Key Stage 2 File)* |
|---|---|---|---|
| 1 | 1 - 3 | 2 | W = Code W ("working towards level 1") means that the child was assessed but didn't achieve level 1. |
| 2 | 2 - 5 | 4 | The basic scale consists of levels 1, 2, 3 and 4+, with |

| Criterion | | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|---|
| | | | | | | | grades A, B and C within level 2.<br>Point Score - All Subjects<br>4+ = 27<br>3 = 21<br>2A = 17<br>2B = 15<br>2C = 13<br>1 = 9 |
| | f) | Memory | Short term memory | 8 | Score ≤ 3 | Based on the distribution of ALSPAC participant data. | WISC-III forward digit span[245,281,541,542]<br><br>Children repeated lists of digits in order. |
| | | | | 8 | Score ≤ 2 | Based on the distribution of ALSPAC participant data. | Modified Non-word Repetition Test[245,543]<br><br>Twelve nonsense words, four each of 3, 4 and 5 syllables and conforming to English rules for sound combinations.<br>The child was asked to listen to each word via an audio cassette recorder and then repeat each item. |
| | g) | Executive functioning and abstract reasoning | Working memory | 10 | Score ≤ 2 | Based on the distribution of ALSPAC participant data. | Counting Span Task[544-546]<br><br>The child was presented with red and blue dots on a white screen. The child was asked to point to and count the number of red dots out loud (the processing component).<br>The children were shown:<br>• Two practice sets of two screens<br>• Three sets of two screens |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | | | | | • Three sets of three screens<br>• Three sets of four screens<br>• Three sets of five screens<br>After each set, the child was asked to recall the number of red dots seen on each screen in the order they were presented within that set (the storage component). |
| | | Working memory | 8 | ≤ 2 correct responses | Based on the distribution of ALSPAC participant data. | WISC-III (short form) Backwards Digit Span.[245,281,541,542]<br><br>Children repeated lists of digits in reverse order. |
| | | Inhibition | 10 | ≤ 12 correct responses | Based on the distribution of ALSPAC participant data. | Stop-signal paradigm[545-547]<br><br>This task observes the child's ability to inhibit a body movement that has already been requested using a computerized measure of impulsivity. When a 'stop signal cue' (bleep) was not heard the child was asked to press the corresponding button according to what was presented on screen. When the bleep was sounded the child was told to refrain from pressing the response button, therefore inhibiting the stimulus response. |
| | | Opposite Worlds Task | 8 | Time (secs)<br>≥ 28 for males aged 7 - <9<br>≥ 24.5 for males aged 9 - 11<br><br>≥ 26 for females aged 7 - <9<br>≥ 24 for females aged 9 - 11 | Based on the distribution of ALSPAC participant data. | TEACh- Opposite Worlds Task[245,548]<br><br>A Stroop task, where the child is required to give a verbal response that contradicts the visual information he or she is given. The child is shown a trail made up of the numbers 1 and 2. In the |

| Criterion | | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|---|
| | | | | | | | 'opposite world' condition, the child must call out 'two' when he or she reaches a 1 and 'one' when he or she reaches a 2. |
| | h) | Attention deficit/hyperactivity | Selective attention | 8 | ≥ 11 for males aged 7 - <9<br>≥ 9 for males aged 9 - 11<br><br>≥ 8 for females aged 7 - 11 | Based on the distribution of ALSPAC participant data. | TEA-Ch Sky Search Task[245,548]<br><br>The child was asked to circle identical pairs of spaceships as quickly as possible but not missing any out. The child was asked to tick a box on the sheet to indicate that he/she had circled all the identical pairs he/she could find. |
| | | | DAWBA ADHD | 7.5 | DAWBA ADHD | Clinical diagnosis by ALSPAC team | Development and Well-Being Assessment (DAWBA)[549]<br><br>DAWBA diagnoses were classified by psychiatrists. For ADHD and oppositional/conduct disorders, the diagnostic procedure considers the teacher report in addition to the parent report. Full DSM-IV diagnoses were only made for children for whom the parent report had been completed. |
| | | | SDQ Hyperactivity Score | 7 to 11 years | High SDQ<br>Score ≥ 8 | Standard norms | Strengths and difficulties questionnaire (SDQ)[550] |
| | i) | Adaptive behaviour, social skills, social communication | SDQ Peer Problems Score | | High SDQ<br>Score ≥ 5 for teacher-rated<br>Score ≥ 4 for parent-rated | Standard norms | |
| | | | SDQ Conduct Problems Score | | High SDQ<br>Score ≥ 4 | Standard norms | |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | DAWBA Oppositional-conduct disorder | 7.5 | DAWBA Oppositional-conduct disorder | Clinical diagnosis by ALSPAC team | Development and Well-Being Assessment (DAWBA)[549]<br><br>DAWBA diagnoses were classified by psychiatrists. For ADHD and oppositional/conduct disorders, the diagnostic procedure considers the teacher report in addition to the parent report.<br>Full DSM-IV diagnoses were only made for children for whom the parent report had been completed. |
| | | Diagnostic Analysis of Non-Verbal Accuracy (DANVA) | 8 | ≥ 7 errors | Research literature | Diagnostic Analysis of Non-Verbal Accuracy (DANVA)[551]<br><br>The DANVA faces subtest comprises 24 photos of child faces, with each face showing one of four emotions: happiness, sadness, anger or fear. The photos are presented to the child for two seconds each and he or she must respond as to whether the person in the photo is happy, sad, angry or afraid. |
| | | Social cognition | 7.5 and 11 | Score ≥ 9 | Standard norms | Social Communication Disorders Checklist (SCDC)[552-554]<br><br>Measure of social-cognitive dysfunction. |
| | | Autism spectrum disorder | Up to age 11 | Any autism spectrum disorder | Clinical diagnosis recorded in NHS or PLASC | Autism identified by NHS or PLASC records.[204] |

| Criterion | Domain | ALSPAC variable | Age assessed (years) | Suggested threshold for impairment | Method for deriving threshold for impairment | Test details |
|---|---|---|---|---|---|---|
| | | Teacher reported emotional or behavioural difficulties | 7 and 10 | 'Yes' indicates reported impairment at any time point | Expert opinion | Teacher-reported emotional or behavioural difficulties at school |
| **Prenatal alcohol exposure (D)** | Prenatal alcohol exposure (PAE) | Prenatal alcohol exposure | Prenatal (reported for each trimester): Data collected at approximately 8, 18 and 32 weeks gestation and 8 weeks postpartum. | N/A | Self-reported alcohol consumption during pregnancy including information about dose, frequency and timing.<br><br>**Timing**: First, second or third trimester<br>**Duration**: None, one trimester, two trimesters, all trimesters<br>**Dose/frequency**: <1 glass per week<br>At least one glass per week<br>1-2 glasses daily<br>3-9 glasses daily<br>>9 glasses daily<br>The ALSPAC questionnaire defined a glass of alcohol as equivalent to a pub measure of spirits, ½ pint lager/beer, wine glass of wine etc.<br><br>**'Binge' drinking in the last month:** 1-2 days<br>3-4 days<br>5-10 days<br>>10 days<br>everyday<br>'Binge drinking' defined as the consumption of 2 pints of beer, 4 glasses of wine, 4 pub measures of spirits or equivalent on a single occasion. Information on first trimester binge drinking was not available.<br>**Dose (units):** UK standard units |

Appendix 5: Factors for differential diagnosis in the ALSPAC sample

| Domain | ALSPAC variable | Details | Age assessed | Details | Action[a] |
|---|---|---|---|---|---|
| **Genetic** | Genetic anomalies dataset<br>Down Syndrome | Genetic anomalies | NA | Exclude Ps with **any** recorded genetic disorders. Microarray not available. | Exclude |
| **Perinatal trauma/complications** | Eclamptic convulsions<br>Umbilical cord around neck or prolapse during labour<br>Blood transfusion in labour<br>Resuscitation of baby<br>Premature delivery ≤ 34 weeks<br>Apnoeic attacks in first 14 days postnatally<br>Admitted to SCBU | Perinatal trauma | NA | Any of the complications listed may account for neurological abnormalities, particularly if related to hypoxia. | Consider |
| **Prematurity** | Gestational age at delivery | Prematurity | NA | Consider prematurity but note that prematurity may be caused by PAE as well as influencing later outcomes. | Consider |
| **Abuse/neglect** | Postnatal abuse/neglect | Physical or sexual abuse | 6 months to 8 years | Any reported physical or sexual abuse. Some variables list 'how affected' the child was, however I have coded to indicate any abuse, regardless of perceived impact, as is it reasonable to assume that the child is likely to have been adversely affected, even if the caregiver reports otherwise. | Consider |

| Domain | ALSPAC variable | Details | Age assessed | Details | Action[a] |
|---|---|---|---|---|---|
| **Taken into care** | Taken into care after 6 months old | Child taken into care | 6 months to 6 years | Children taken into care aged < 6 months generally have better outcomes than those aged > 6 months (shorter duration of exposure to neglectful environment) | Consider |
| **Illicit substances during pregnancy** | Cannabis<br>Amphetamine<br>Barbiturate<br>Crack<br>Cocaine<br>Heroin<br>Methadone<br>Ecstasy<br>Other | Illicit substance use in pregnancy | Prenatal | Consider associated adverse effects | Consider |
| **Smoking in pregnancy** | Tobacco | Smoking in pregnancy | Prenatal | Consider associated adverse effects | Consider |
| **Postnatal injury** | Head injury causing loss of consciousness | Head injury | 0 to 12 years | Consider timing of injury with time of CNS assessment. I.e. if head injury occurs after CNS impairment then not a plausible alternative diagnosis. | Consider |
| **Postnatal illness** | Readmitted to hospital<br><br>Health of child | Postnatal illness | 4 weeks to 18 months | Coded to indicate severe postnatal illness as only severe illness may be a plausible alternative cause for the extensive range of impairments associated with FASD.<br>e.g. child 'mostly unwell' at any time point, or child readmitted to hospital. | Consider |
| **Postnatal nutrition** | Weak Sucking<br>Feeding difficulty<br>Number of solid meals a day | Postnatal nutrition | 4 weeks to 6 months | Not informative in terms of providing adequate information about alternative explanations for growth deficiency. Also birth weight will not be affected by postnatal nutrition and since evidence of consistent growth impairment is needed this should | Not relevant - accounted for in criteria that requires evidence of |

| Domain | ALSPAC variable | Details | Age assessed | Details | Action[a] |
|---|---|---|---|---|---|
| | | | | exclude postnatal nutrition as an alternative explanation. | consistent growth deficiency as per 4 Digit Code |
| **Parental mental health** | Parental learning disabilities/ADHD | N/A | N/A | N/A | Not available |
| **Child's main language** | Language child speaks<br><br>English is main language spoken by study child | Child's main language | Age ~3 and ~5 years | Variables describe whether English is the child's main (or equal) language or not. This is a consideration when considering whether CNS test performance reflects genuine ability or language capability. | Exclude children for whom English is not the main language |
| **Physical and sensory disability** | Child has physical disabilities<br><br>Childs needs special arrangements at school for a physical problem<br><br>PLASC SEN areas | Physical disability | Age 7 to 11 years | Consider whether physical or sensory disabilities may have affected testing outcomes<br><br>In general, physical and sensory disabilities should not be a plausible explanation for impairment across the diverse range of domains included in the FASD case definition. In addition, the ALSPAC team considered children's special needs to minimise problems with test procedures as described in the following excerpt:<br>"It is envisaged that some children with special needs will find some of the tests difficult at any Focus visit. All parents are asked if they think their child will have difficulties with any of the activities. If so, they are telephoned by a member of staff with responsibility for families with special needs to discuss whether a visit to the clinic is feasible; if not, then other possibilities for assessment are discussed. If they would like to come, modifications to the visit or to particular measures are discussed. If necessary extra staff or specialists such as signers for the deaf are brought in for the visit." (F10 file). | Consider |

| Domain | ALSPAC variable | Details | Age assessed | Details | Action[a] |
|---|---|---|---|---|---|
| | | | | PLASC SEN codes group sensory and physical needs so it is not clear which of these is present for child. Also, consider timing of disability and whether this coincides with time of testing. Teacher reports indicate whether disability is past or current. | |
| **Sensory impairment** | **Hearing**<br><br>Hearing Sensorineural hearing loss<br><br>Hearing Impairment<br><br>High frequency hearing loss<br><br>Child has hearing problems requiring special arrangements at school<br><br>PLASC SEN areas<br><br>**Vision**<br><br>Observable abnormality<br><br>Child has eyesight problems requiring special arrangements at school | Sensory impairment (vision and/or hearing) | 7 to 11 | Note: PLASC SEN codes group sensory and physical so not clear which of these is present for child. Also, consider timing of disability and whether this coincides with time of testing. Teacher reports identify disability as past or current.<br><br>Relevance of impairment for differential diagnosis depends on CNS test type (i.e. could sensory disability be a plausible explanation for poor performance?). Also check test arrangements on built files to see if adaptations made for Ps with sensory problems. | |

| Domain | ALSPAC variable | Details | Age assessed | Details | Action[a] |
|---|---|---|---|---|---|
| | Child has sensory impairment (visual) | | | | |
| **Parental height** | **Height of mother and father** | Parental height | N/A | Consider genetic potential for growth. If mother and/or father have significantly short stature then child may not truly be growth deficient | Not adjusted for. Substantial amount of missing information on father's height (71% missing data) |

a 'Consider' means that this factor should be considered as an alternative explanation for a child's presentation but this does not rule out the possibility that alcohol has been an important causal factor in influencing this child's outcome.

Appendix 6: Example participant profile for case conference panel

# Case conference file

# Participant ID: 297

## General information

Sex: Female

Ethnicity: White

Gestational age at delivery: 36 weeks

Maternal age at delivery: 29 years

## DOMAIN D

## Prenatal alcohol exposure history (maternal self-report)

Duration

This mother reported drinking in the first trimester only

Dose/frequency

First trimester: Less than one glass of alcohol per week

Units of alcohol per week

First trimester: 0

Binge drinking

No

## DOMAIN B

### FAS Facial Phenotype

Normal palpebral fissure length, **smooth philtrum** and normal upper lip

## DOMAIN A

## Growth centiles

Normal

| Age | Weight | Height | BMI |
|---|---|---|---|
| Birth | 85 | 59 | 95 |
| 7 | 98 | 98 | 96 |
| 8 | 98 | 98 | 94 |
| 9 | Missing | Missing | Missing |

# DOMAIN C

# Central Nervous System

## a) Hard and soft neurologic signs (including sensory-motor)

**EVIDENCE OF IMPAIRMENT**

**Seizures**: No evidence of multiple seizures

**ALSPAC Coordination Test**: Poor motor coordination

## b) Brain structure

**NORMAL**

**Head circumference**

Birth centile: 53

Age 7 centile: 30

## c) Cognition (IQ)

**EVIDENCE OF IMPAIRMENT**

**WISC-III (short form)**

Full Scale IQ = 68 (exceptionally low)

Performance IQ = 67 (exceptionally low)

Verbal IQ = 75 (low)

Significant difference between IQ subdomains (≥ 1 SD): No

## d) Communication: receptive and expressive

**EVIDENCE OF IMPAIRMENT**

**Wechsler Objective Language Dimension (WOLD)**

Normal listening comprehension (score = 6)

Missing expressive language

**Teacher-reported speech and language problems requiring special assistance at school**

Yes

## e) Academic achievement

**EVIDENCE OF IMPAIRMENT**

**Special Educational Needs (SEN)**

SEN age 9-10: Missing

SEN age 10-11: SEN Statement

SEN age 11-12: SEN Statement

Primary reason for SEN: Other needs

Secondary reason for SEN: Missing

**Key Stage attainment**

| | Key Stage 1 | | | | Key Stage 2 | | |
|---|---|---|---|---|---|---|---|
| **Subject** | **Reading** | **Writing** | **Spelling** | **Maths** | **English** | **Maths** | **Science** |
| **Level** | **Low** | | | **Low** | **Low** | **Low** | **Normal** |
| | 2C | w | Missing | w | N | N | 4 |

## f) Memory

**EVIDENCE OF IMPAIRMENT**

**WISC-III - Forward Digit Span**

Normal (score = 6)

**Non-Word Repetition Task**

Low non-word repetition (score = 1)

## g) Executive functioning and abstract reasoning

**EVIDENCE OF IMPAIRMENT**

**Working Memory tasks:**

Counting Span Task: Missing

Backwards Digit Span: Low backwards digit span (score = 2)

**Inhibition tasks:**

Stop Signal Task: Missing

Opposite Worlds Task: Slow Opposite Worlds (score = 34)

## h) Attention deficit/hyperactivity

**EVIDENCE OF IMPAIRMENT**

**Selective Attention:** Normal (score = 6)

**Development and Well-Being Assessment - ADHD:** Missing

**Strengths and Difficulties Questionnaire Hyperactivity**: Yes (parent-rated)

SDQ parent-rated hyperactivity score:

Very high at age 7

Missing at age 9, 11 and 13

SDQ teacher-rated hyperactivity score:

Close to average at age 7 and 10

## i) Adaptive behaviour, social skills, social communication

**EVIDENCE OF IMPAIRMENT**

**Strengths and Difficulties Questionnaire Peer Problems:** Yes (parent and teacher-rated)

SDQ parent-rated peer-problems score:

High at age 7

Missing at age 9, 11 and 13

SDQ teacher-rated peer-problems score:

Very high at age 7 and 10

**Strengths and Difficulties Questionnaire Conduct Problems:** Yes (parent-rated)

SDQ parent-rated conduct problems score:

High at age 7

Missing at age 9, 11 and 13

SDQ teacher-rated conduct problems score:

Close to average at age 7 and 10

**DAWBA Conduct/Oppositional Defiant Disorder (CD/ODD):** Missing

**Autism:** No

**Diagnostic Analysis of Non-Verbal Accuracy (DANVA):** Significantly low

**Social Communication Disorder Checklist (SCDC):** Missing

**Teacher-reported emotional or behavioural difficulties:**

Emotional/behavioural problems in Year 3: Yes

Emotional/behavioural problems in Year 6: No

## Other pre- and postnatal exposures (factors for differential diagnosis)

**Any considerations for differential diagnosis**: Yes

Perinatal trauma/complications: Umbilical cord wrapped around neck and baby resuscitated at delivery.

Physical disability: Teacher-completed questionnaires suggest that this child had physical problems requiring special support at school, but that these problems had resolved by age 7. Parent reports are missing.

Participant number: 297

*Tick box if evidence of significant impairment for each domain*

| FASD Domain | Definition of significant impairment |
|---|---|
| **Growth (A)** ☐ | Pre- and/or postnatal height or weight ≤ 9th percentile or disproportionately low weight-to-height ratio ≤ 9th percentile |
| **Face (B)** ☐ | 3 facial features: short palpebral fissures ≤ 3rd percentile; smooth/flattened philtrum, thin upper lip |
| **Central nervous system (C)** ☐ | Impairment in ≥ 3 of the below subdomains:<br>❑ Hard and soft neurologic signs<br>❑ Brain structure<br>❑ Cognition (IQ).<br>❑ Communication: receptive and expressive.<br>❑ Academic achievement.<br>❑ Memory.<br>❑ Executive functioning and abstract reasoning.<br>❑ Attention deficit/hyperactivity.<br>❑ Adaptive behaviour, social skills, social communication.<br>A domain is considered "impaired" when on a standardized measure:<br>Scores are 2 standard deviations or more below the mean (or ≤ 3rd percentile), or there is a discrepancy of at least 1 standard deviation between subdomains.<br>In areas where standardized measurements are not available, a clinical judgment of "significant dysfunction" is made, taking into consideration that important variables, including the child's age, mental health factors, socioeconomic factors and disrupted family or home environment (e.g., multiple foster placements, history of abuse and neglect), may affect development but do not indicate brain damage. |
| **Alcohol exposure (D)** ☐ | Prenatal alcohol exposure requires confirmation of alcohol consumption by the mother during the index pregnancy based on reliable clinical observation, self-report, reports by a reliable source or medical records documenting positive blood alcohol, alcohol treatment or other social, legal or medical problems related to drinking during the pregnancy. |

**FASD definitions:**

**Fetal alcohol syndrome (FAS)** *(A, B, C and D [confirmed or unconfirmed PAE])*
**Partial FAS** *(2 features from B plus C and D - confirmed exposure only)*
**Alcohol-related neurodevelopmental disorder (ARND)** *(C and D - confirmed exposure only)*

## Final decision

☐ **FASD** ☐ **Not FASD**

## Comments

Appendix 7: Qualitative summary of the discussions relevant to FASD classification decisions from the case conference panel

| Participant | Discussion notes | Panel decision | | Most stringent FASD algorithm classification |
|---|---|---|---|---|
| | | FASD | NOT FASD | |
| **1.** | Insufficient information to determine FASD although general agreement of a disorder/difficulty. Low level of alcohol exposure and unlikely to cause significant problems. Parents education 'CSE' level and social class 4 (manual unskilled). A clinical setting would diagnose a 'possible' case and further information would be requested. | | ✓ | Strict CNS/Any PAE |
| **2.** | Low level of alcohol exposure. High level key stage 2 attainment. Huge difference in verbal cognition IQ. Does not meet criteria. | | ✓ | Liberal CNS/Any PAE |
| **3.** | Very difficult case to determine. Some alcohol/some smoking/small baby. Mother's height 157m - which may account for short stature. Some difficulties which improved with age when the opposite is expected. Hyperactivity reported by teacher and parents. | | ✓ | Mid CNS/Strict PAE |
| **4.** | Possible case but based on the information provided there is insufficient evidence to confirm FASD. In a clinical setting this case would be asked to return to the clinic and complete the missing data. Agreement that there is some problem/disorder present. | | ✓ | Mid CNS/Strict PAE |
| **5.** | Low level of alcohol exposure, short in stature. Some risk as the mother drank alcohol in 2nd and 3rd trimester. Cognition appears to be fine although a question mark over cognitive dysfunction. | | ✓ | Liberal CNS/Mid PAE |
| **6.** | Parents degree educated and social class 2 (managerial).<br>Mother's height normal 170m. IQ is high. ADHD result interesting. SEN result - average but underachieved based on IQ - possible functional issue. Definitely something to investigate in more detail. Cognitive domain is limited. More detailed sensitive testing required. | | ✓ | Strict CNS/Mid PAE |
| **7.** | Insufficient information. Good progress at key stage 2 level. | | ✓ | Mid CNS/Mid PAE |

| Participant | Discussion notes | Panel decision | | Most stringent FASD algorithm classification |
|---|---|---|---|---|
| | | FASD | NOT FASD | |
| **8.** | Some risk due to alcohol intake during all trimesters. Nothing else to note. No functional issues. | | ✓ | No FASD |
| **9.** | Cognition details missing. Low risk due to alcohol intake. A possible case but more information required. | | ✓ | Mid CNS/Any PAE |
| **10.** | Low level of alcohol exposure. No functional deficit so no disorder. | | ✓ | Liberal CNS/Strict PAE |
| **11.** | Evidence of alcohol exposure. Academic functioning is fine. Meets adaptive and educational criteria. ODD at 7½ years. Clearly a problem and highest risk so far. Confirmed as FASD but information is missing to be confident with this diagnosis. | ✓ | | Strict CNS/Strict PAE |
| **12.** | No discussion required. | | ✓ | No FASD |
| **13.** | Parents educational level missing. Educational attainment is low but not at a level to indicate a problem and may be comparable to parents if this was available. Science level satisfactory. Not FASD based on information provided. | | ✓ | Liberal CNS/Mid PAE |
| **14.** | Parents - vocational qualifications and social class 3. Smooth philtrum - 2 features which suggest partial FASD. Low key stage 1 and low English key stage 2 attainment. Out of kilter with expectation.<br>Evidence of hyperactivity in teenage years. Discrepancy on WISC. No adaptive deficits. A low possible case. | | ✓ | Mid CNS/Mid PAE |
| **15.** | Resuscitated immediately after birth but no re-admittance to hospital. Question over neurological difficulties. Probable case based on low growth, low IQ, co-ordination problems - which are more classic symptoms, difficulties with education and impairment with memory. | ✓ | | Strict CNS/Any PAE |
| **16.** | Unconfirmed hearing difficulties which could be the cause for sensory problems. Symptoms worsen with age. Data to prove alcohol intake is missing. Possible case but not enough information to make a reliable decision | | ✓ | Liberal CNS/Strict PAE |
| **17.** | High risk of exposure. Short and small. Issues at school. School action plus then downgraded to school action. Meets 4 domains but not adaptive. | ✓ | | Strict CNS/Strict PAE |

| Participant | Discussion notes | Panel decision | | Most stringent FASD algorithm classification |
|---|---|---|---|---|
| | | FASD | NOT FASD | |
| **18.** | Question over whether measurement was correct at birth. Use of cannabis throughout pregnancy but there is no evidence that cannabis causes long standing disability. Parents 'O' level education, social class 3 (skilled non-manual). Abnormal at age 7, small head circumference, growth issues, alcohol exposure is low, executive function is low, memory low. | ✓ | | Mid CNS/Any PAE |
| **19.** | Parents 'O' and 'A' level educated; social class 3 and 5.<br>Below predicted genetic potential, significantly low for DANVA, social cognitive dysfunction and ODD. Attention deficit high. Fairly low for alcohol intake but older maternal age increases risk. | ✓ | | Strict CNS/Mid PAE |
| **20.** | Parents 'A' level and degree educated. Social class 2. Discrepancy on reported alcohol dose frequency. Data inconsistent, non-verbal difficulties, peer problems, ADHD traits. Possible/probable case. | ✓ | | Mid CNS/Strict PAE |
| **21.** | Missing information. Parents reported hyperactivity. Low attainment at one time point. | | ✓ | Liberal CNS/Any PAE |
| **22.** | Parents 'O' level and 'CSE' educated. Social class 3 (skilled non-manual).<br>Low IQ, significantly low non-verbal accuracy. Low key stage impairment but consistent with genetic level. Social communication deficit, low risk of alcohol, adaptive functioning. Not enough evidence and only meets 2 domains. | | ✓ | Liberal CNS/Strict PAE |
| **23.** | SEN action plus but achieved normal attainment. Suspect SEN for functional and ADHD behavioural issues. Early problems addressed. Three domains reduced to 2 if SEN due to behavioural problems. | | ✓ | Mid CNS/Strict PAE |
| **24.** | Parents 'A' level and degree educated. Social class 1 and 2. Not enough information to make a positive diagnosis. | | ✓ | No FASD |
| **25.** | Head injury and loss of consciousness but evidence of improvement following head injury. ADHD reported by parents.<br>Parents 'A' level, social class 2 and 3. Education impairment, adaptive behaviour, social skills and motor impairment poor. SEN after head injury therefore cannot be associated with alcohol intake. | | ✓ | Strict CNS/Strict PAE |

| Participant | Discussion notes | Panel decision | | Most stringent FASD algorithm classification |
|---|---|---|---|---|
| | | FASD | NOT FASD | |
| **26.** | Parents 'CSE' educated, social class 3 and 5. Genetically linked to IQ from parents. Low level of exposure, low educational attainment can be linked to parents. Although this case is hitting the indicators it was agreed that it is an unlikely case due to differential diagnosis. | | ✓ | Strict CNS/Any PAE |
| **27.** | Parents 'O' and 'A' level educated. Social class 3. Meets 5 domains. Alcohol exposure in 1st and 3rd trimester. Some facial evidence. Dysfunction not what would be expected. | ✓ | | Strict CNS/Mid PAE |
| **28.** | Parents 'O' level and 'CSE' educated, social class 3. Not enough information to make a diagnosis. Speech reported problems by teacher, contradictory information on school action plus, SDQ problems and some social issues. Unsafe diagnosis. | | ✓ | Liberal CNS/Any PAE |
| **29.** | Parents degree and 'O' level education, social class 1 and 3. Inconsistencies in units of alcohol reported. Average IQ - should have achieved higher at key stage 1. Key stage 2 missing which would have helped with diagnosis. Meets 3 domains - attention, adaptive and executive function, but these could also suggest ADHD. Certain risk of alcohol but no physical features. Meets criteria and enough information to support further investigation. | ✓ | | Liberal CNS/Mid PAE |
| **30.** | Parents 'A' level education. Social class 3 and 5. Head circumference small but smoking could be responsible. Missing information but agreed on FASD due to academic achievement, query hyperactivity, adaptive problems, peer conduct problems. | ✓ | | Mid CNS/Mid PAE |
| **31.** | Parents vocational and 'A' level educated. Some facial evidence. No evidence of ADHD from teachers and true hyperactivity would be evident in all environments. DANVA significantly low and social cognitive dysfunction. Using criteria this would be FASD. | ✓ | | Mid CNS/Any PAE |

Appendix 8: Qualitative summary of case conference panel recommendations for refinements to the FASD case ascertainment algorithm

| Discussion point | Relevant domain | Action point | Method | Decision |
|---|---|---|---|---|
| A child's level of functioning must be compared to their expected capability to determine whether there is likely to be a true impairment<br><br>A discrepancy between WISC subdomains is likely to indicate impairment, expect in cases where child has very high IQ in one domain and high in another. | CNS: c) Cognition | Compare child cognitive ability to educational attainment to see if both are consistent. If both are low then only count as evidence of impairment in one distinct area to avoid 'double counting' of this impairment. | Given evidence of impaired educational attainment, recode educational attainment as not impaired if IQ low/borderline. Education remains as impaired if ≥ average IQ. | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| | | | If WISC is missing use parental attainment to estimate child's genetic potential.<br><br>Lisa Hurt [LH] subsequently queried this - parental attainment may not be good indicator of child potential. | Not actioned: Parental attainment reflects both genetic potential and social factors and is an imperfect proxy for child potential. |
| | CNS: e) Educational attainment | Compare cognition and educational attainment to genetic potential - using parental social class and educational attainment as proxies. | Recode parental attainment as low if highest educational level is equivalent to CSE or below for one or both parents or if highest parental social class is unskilled.<br><br>In further discussion with LH we considered the limitations of this approach, which implies that children may not be able to succeed if their parents have low attainment/social class. Parental attainment may not be a good | |

| Discussion point | Relevant domain | Action point | Method | Decision |
|---|---|---|---|---|
| | | | substitute for genetic potential, as it will reflect a mixture of cognitive ability and social opportunities. Also, parents with alcohol problems may be less likely to have high achievement so low attainment among participants whose parents were low attainment/social class does not rule out the possible influence of prenatal alcohol exposure. | |
| | CNS: c) Cognition | Explore level of WISC functioning among discrepant cases (≥ 1 SD between subdomains) | Recode c) Cognition as not impaired if discrepancy between subdomains but high IQ. | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| There must be convergent evidence of hyperactivity from more than one source and/or across time points. In this dataset hyperactivity would be expected to be stable, as medication for ADHD was much less common in the 1990s. | CNS: h) Attention deficit hyperactivity | Look for convergent evidence in CNS domain h) | Recode hyperactivity so that it is only classed as impaired if convergent evidence across raters and/or time points. | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| There should be convergent evidence between measures within the adaptive functioning domain. For example, a child with | CNS: i) Adaptive functioning | Look for convergent evidence in domain i) | Recode i) Adaptive functioning so only impaired if convergent evidence across measures. | Actioned: Incorporated in Revised CNS case ascertainment algorithm |

| Discussion point | Relevant domain | Action point | Method | Decision |
|---|---|---|---|---|
| low DANVA or SCDC would be expected to have peer problems. | | | | |
| Functional deficits should be considered a core feature of FASD diagnosis. | CNS: i) Adaptive functioning<br>CNS: e) Educational attainment | Look for evidence of functional impairment to classify a participant as FASD. | Recode final FASD categorisation so that a FASD classification is given only if there is evidence of impairment in ≥ CNS domains and CNS domains i) and/or e) are classed as impaired. | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| Special educational needs may indicate a range of different impairments. Need to reclassify so that SEN categories are placed in the correct domain. | CNS: e) Educational attainment<br>CNS: i) Adaptive functioning<br>CNS: d) Communication | Look for SEN reason and reclassify under relevant CNS domains where necessary | If SEN code:<br>1. Cognition and Learning - keep in domain e)<br>2. Behavioural/emotional - move to domain i)<br>3. Communication and interaction - move to domain d)<br>If SEN reason missing then keep SEN in domain e) | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| School attainment should remain low across Key Stages 1 and 2 if indicative of a true, stable impairment | CNS: e) Educational attainment | Look for stable impairment | Recode education so that only low if evidence of impairment across time points i.e. low grades across time points | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| Head circumference ≤2nd percentile at any point should be classed as deficient | CNS: a) Head circumference | Consider small OFC at all time points | Recode a) as impaired if small OFC at birth or age 7 | Actioned: Incorporated in Revised CNS case ascertainment algorithm |
| Consider parental height when assessing whether child's height is deficient | Growth | Add parental height to prevalence considerations | Recode growth as not deficient for children with short mother or | Not actioned: Substantial amount of missing information on |

| Discussion point | Relevant domain | Action point | Method | Decision |
|---|---|---|---|---|
| | | | biological father <=9$^{th}$ centile, if BMI not also deficient | father's height (71% missing). Lack of adjustment for parental height will not affect prevalence estimates for total FASD. It could affect prevalence estimates for FAS and descriptive characteristics of clinical features. |
| WISC score seems pivotal for establishing a child's baseline cognitive ability. Drop participants from the dataset if they have a missing WISC and no indicator of genetic potential based on parental ability | CNS: c) Cognition and participant selection | Check for missingness of WISC and parental attainment | Drop participants with missing WISC and missing parental educational attainment as it makes it difficult to determine their potential | Not actioned: This would lead to a significant reduction in sample size and the absence of a WISC score does not preclude the assignment of FASD status, as it is still possible to obtain evidence of impairment in ≥ 3 distinct domains even if WISC score is missing. |
| Partial FAS facial features and low reported PAE make the reported levels of PAE questionable, as facial features are highly specific to heavy PAE | Prenatal alcohol exposure | Be aware that participants with the partial FAS facial phenotype are likely to have had heavy PAE | If we decide to apply a threshold for FASD classification based on reported PAE then consider lowering the threshold for participants with the partial FAS facial phenotype | Not actioned: PAE threshold already systematically varied across case ascertainment algorithms and subcategories include assignment of FAS |

| Discussion point | Relevant domain | Action point | Method | Decision |
|---|---|---|---|---|
| | | | | category if the full FAS facial phenotype is present, even in the absence of confirmed PAE. Only the full facial phenotype is highly specific to PAE. |
| The inclusion of seizures as a criterion was not discussed explicitly at case conference, as none of the participant profile examples had seizures. The main diagnostic frameworks (Canadian, 4-Digit) suggest seizures as a marker of neurological impairment for domain a) so I think it is reasonable to include this for reasons of consistency with the chosen Canadian diagnostic framework. | CNS: a) Sensory-motor | Add seizures to domain a) | Add multiple seizures not due to postnatal insult as criterion for CNS domain a) for mid and strict CNS classifications (already included in liberal CNS) | Actioned: Incorporated in Revised CNS case ascertainment algorithm |

Abbreviations: CNS; central nervous system; CSE, Certificate of Secondary Education; DANVA; Diagnostic Analysis of Non-Verbal Accuracy; IQ, intelligence quotient; OFC, occipital frontal head circumference; SD, standard deviation; SCDC, Social Communication Disorders Checklist; SEN, special educational needs; SES, socioeconomic status; WISC, Weschler Intelligence Scale for Children.

Appendix 9: Multiple imputation model specification and missing data frequencies

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **Variables included in multiple imputation model and featured in prevalence and/or risk factor analyses (exposures, confounding variables and clinical characteristics that contribute to FASD status)** | | | | | | | |
| **dosefreq** | Alcohol dose/frequency during pregnancy | None/<1 glass per week/1-6 glasses per week/7+ glasses per week | 12,947 | 4 | 9,045 | 1 | ologit |
| **bingeb** | Binge drinking during pregnancy (Questionnaire B) | No/Yes | 12,519 | 7 | 8,847 | 3 | logit |
| **bingec** | Binge drinking during pregnancy (Questionnaire C) | No/Yes | 8,411 | 38 | 5,957 | 35 | logit |
| **b720** | Alcohol consumption pre-pregnancy | Never/<1 glass per wk/1+ glasses per week/1-2 glasses daily/3-9 glasses daily/10+ glasses daily | 12,602 | 7 | 8,899 | 3 | ologit |
| **pregdrinkchange** | Alcohol drinking change during pregnancy | No evidence of continued drinking/Had more or No change | 11,621 | 14 | 8,100 | 11 | logit |
| **a261** | Measures of alcohol per week (Questionnaire A) | Continuous | 11,375 | 16 | 7,790 | 15 | regress |
| **c373** | Measures of alcohol per week (Questionnaire C) | Continuous | 6,619 | 51 | 4,586 | 50 | regress |
| **shortpfl** | Short palpebral fissure length | No/Yes | 4,370 | 68 | 3,214 | 65 | logit |
| **Philtrum4Digit** | Philtrum shape | Smooth/Indentation near nose/Indentation in middle/Indentation near vermillion border/Deep groove from nose to vermillion border/Deep groove to Cupid's bow | 4,370 | 68 | 3,213 | 65 | ologit |
| **UpperLipFullness** | Upper lip fullness | Thin/Medium/Thick | 4,370 | 68 | 3,213 | 65 | ologit |
| **coordination** | Low coordination | No/Yes | 6,520 | 52 | 4,774 | 48 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **seizmulti** | More than one seizure not due to postnatal insult | No/Yes | 11,646 | 14 | 8,208 | 10 | logit |
| **ofcbirthLMS** | Small head circumference (OFC) at birth | No/Yes | 8,340 | 38 | 5,749 | 37 | logit |
| **ofc7LMS** | Small head circumference (OFC) at age 7 | No/Yes | 7,449 | 45 | 5,453 | 40 | logit |
| **f8ws110** | Verbal IQ | Continuous | 6,830 | 49 | 5,005 | 45 | regress |
| **f8ws111** | Performance IQ | Continuous | 6,821 | 49 | 4,994 | 45 | regress |
| **f8ws112** | Full-scale IQ | Continuous | 6,800 | 50 | 4,979 | 45 | regress |
| **wold_list** | Low listening comprehension | No/Yes | 6,821 | 49 | 5,003 | 45 | logit |
| **wold_express** | Low expressive language | No/Yes | 4,493 | 67 | 3,315 | 64 | logit |
| **comm_schoolany2** | Communication problems at school | No/Yes | 4,100 | 70 | 2,795 | 69 | logit |
| **sen** | Special educational needs | No/Yes | 11,312 | 16 | 7,633 | 16 | logit |
| **lowedu_all3** | Low academic attainment at KS1 and KS2 | No/Yes | 11,824 | 12 | 8,032 | 12 | logit |
| **nonwordrep** | Low non-word repetition | No/Yes | 6,809 | 50 | 4,995 | 45 | logit |
| **digitspanf** | Low forward digit span | No/Yes | 6,702 | 50 | 4,909 | 46 | logit |
| **oppworldfinal** | Low opposite world task | No/Yes | 6,657 | 51 | 4,890 | 46 | logit |
| **countspan** | Low counting span | No/Yes | 6,455 | 52 | 4,706 | 48 | logit |
| **stopsigfinal** | Low stop signal | No/Yes | 6,426 | 52 | 4,668 | 49 | logit |
| **digitspanb** | Low backwards digit span | No/Yes | 6,683 | 50 | 4,900 | 46 | logit |
| **adhd** | ADHD | No/Yes | 7,952 | 41 | 5,772 | 37 | logit |
| **sdq_hypparent** | SDQ hyperactivity (parent-rated) | No/Yes | 9,402 | 30 | 6,759 | 26 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **sdq_hypteacher** | SDQ hyperactivity (teacher-rated) | No/Yes | 9,145 | 32 | 6,251 | 32 | logit |
| **selectattfinal** | Low selective attention | No/Yes | 6,638 | 51 | 4,875 | 47 | logit |
| **ODD_CD** | Oppositional-conduct disorder | No/Yes | 7,952 | 41 | 5,772 | 37 | logit |
| **sdq_peerparent** | SDQ peer problems (parent-rated) | No/Yes | 9,414 | 30 | 6,771 | 26 | logit |
| **sdq_peerteacher** | SDQ peer problems (teacher-rated) | No/Yes | 9,145 | 32 | 6,251 | 32 | logit |
| **sdq_condparent** | SDQ conduct problems (parent-rated) | No/Yes | 9,412 | 30 | 6,769 | 26 | logit |
| **sdq_condteacher** | SDQ conduct problems (teacher-rated) | No/Yes | 9,137 | 32 | 6,245 | 32 | logit |
| **sigsoccog** | Significant social cognitive dysfunction | No/Yes | 8,678 | 36 | 6,270 | 31 | logit |
| **emobeh_schoolany** | Emotional/behavioural problems at school | No/Yes | 9,088 | 33 | 6,204 | 32 | logit |
| **autism** | Autism | No/Yes | 13,495 | 0 | 4,614 | 49 | - |
| **danva** | DANVA ≥ 7 errors | No/Yes | 6,304 | 53 | 9,086 | 1 | logit |
| **lowgrowth** | Growth impairment | No/Yes | 13,419 | 1 | 9,013 | 1 | logit |
| **drugpreg** | Illicit drug use during pregnancy | No/Yes | 12,886 | 5 | 9,133 | 0 | logit |
| **smokepreg** | Smoking during pregnancy | No/Yes | 13,304 | 1 | 9,005 | 1 | logit |
| **multivitaminsupp** | Multivitamin supplements during pregnancy | No/Yes | 12,898 | 4 | 9,014 | 1 | logit |
| **ironsupp** | Iron supplements during pregnancy | No/Yes | 12,907 | 4 | 9,014 | 1 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **zincsupp** | Zinc supplements during pregnancy | No/Yes | 12,906 | 4 | 9,013 | 1 | logit |
| **calciumsupp** | Calcium supplements during pregnancy | No/Yes | 12,905 | 4 | 9,014 | 1 | logit |
| **folicacidsupp** | Folic acid supplements during pregnancy | No/Yes | 12,903 | 4 | 9,015 | 1 | logit |
| **vitaminsupp** | Any vitamin supplements during pregnancy | No/Yes | 12,908 | 4 | 6,270 | 31 | logit |
| **calories** | Daily calorie intake during pregnancy | Continuous | 11,660 | 14 | 8,242 | 10 | regress |
| **retinol** | Retinol (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **calcium** | Calcium(RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **omega3** | Omega-3 (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **folate** | Folate (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **riboflavin** | Riboflavin (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **selenium** | Selenium (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **vitb12** | Vitamin B12 (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **vitc** | Vitamin C (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **vite** | Vitamin E (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **zinc** | Zinc (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **iodine** | Iodine (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **iron** | Iron (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **magnesium** | Magnesium (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **niacin** | Niacin (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **phosphorus** | Phosphorous (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **potassium** | Potassium (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **sodium** | Sodium RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **thiamin** | Thiamin (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **vitb6** | Vitamin B6 (RNI met) | No/Yes | 11,660 | 14 | 8,242 | 10 | logit |
| **married** | Marital status | Married/Not married | 12,646 | 6 | 8,800 | 4 | logit |
| **home** | Home ownership | Mortgaged or owned/Council or housing assoc./Rented (private)/Other | 12,595 | 7 | 8,763 | 4 | mlogit |
| **socialclassm** | Maternal social class | Professional/Mangerial or technical/Skilled non-manual/Skilled manual/Partly skilled/unskilled | 9,718 | 28 | 7,004 | 23 | ologit |
| **socialclassp** | Paternal social class | Professional/Mangerial or technical/Skilled non-manual/Skilled manual/Partly skilled/unskilled | 10,590 | 22 | 7,570 | 17 | ologit |
| **mumedu** | Highest educational qualification (maternal) | CSE/Vocational/O Level/A Level/Degree | 11,988 | 11 | 8,449 | 8 | ologit |
| **partneredu** | Highest educational qualification (paternal) | CSE/Vocational/O Level/A Level/Degree | 11,516 | 15 | 8,125 | 11 | ologit |
| **agecat2** | Maternal age (years) | < 20/20-29/30+ | 13,495 | 0 | 8,294 | 9 | - |
| **relig** | Religion (maternal) | None/Christian/Other | 11,783 | 13 | 8,887 | 3 | mlogit |
| **unplanned** | Unplanned pregnancy | No/Yes | 12,620 | 6 | 7,335 | 20 | logit |
| **totallifeevents** | Total life events during pregnancy | Continuous | 10,204 | 24 | 7,335 | 20 | regress |
| **weightedlifeevents** | Weighted total life events during pregnancy | Continuous | 10,204 | 24 | 8,949 | 2 | regress |
| **relationdiff** | Relationship problems during pregnancy | No/Yes | 12,801 | 5 | 8,949 | 2 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **relationdiffimpact** | Very affected by relationship problems during pregnancy | No/Yes | 12,801 | 5 | 8,940 | 2 | logit |
| **bereavement** | Bereavement during pregnancy (any) | No/Yes | 12,781 | 5 | 8,940 | 2 | logit |
| **bereavementimpact** | Very affected by bereavement during pregnancy | No/Yes | 12,781 | 5 | 8,933 | 2 | logit |
| **financeprob** | Major financial problem during pregnancy | No/Yes | 12,763 | 5 | 8,933 | 2 | logit |
| **financeprobimpact** | Very affected by major financial problems during pregnancy | No/Yes | 12,763 | 5 | 9,033 | 1 | logit |
| **housemove** | Moved house during pregnancy | No/Yes | 12,984 | 4 | 8,800 | 4 | logit |
| **housemoveimpact** | Very affected by house move during pregnancy | No/Yes | 12,769 | 5 | 8,936 | 2 | logit |
| **ill** | Very affected by illness or accident during pregnancy | No/Yes | 12,783 | 5 | 8,943 | 2 | logit |
| **illimpact** | Illness or accident during pregnancy | No/Yes | 12,783 | 5 | 8,943 | 2 | logit |
| **partnerill** | Partner ill during pregnancy | No/Yes | 12,765 | 5 | 8,932 | 2 | logit |
| **partnerillimpact** | Very affected by partner illness during pregnancy | No/Yes | 12,765 | 5 | 8,932 | 2 | logit |
| **childill** | Child ill during pregnancy | No/Yes | 12,762 | 5 | 8,931 | 2 | logit |
| **childillimpact** | Very affected by child illness during pregnancy | No/Yes | 12,762 | 5 | 8,931 | 2 | logit |

| | | | Prevalence analyses (Chapter 4) N eligible = 13,495 | | Risk factor analyses (Chapter 6) N eligible = 9,135 | | |
|---|---|---|---|---|---|---|---|
| **Variable name** | **Variable description** | **Categories** | **N available** | **Missing (%)** | **N available** | **Missing (%)** | **Imputation command** |
| **depressany** | Depression during pregnancy | No/Yes | 12,551 | 7 | 8,774 | 4 | logit |
| **anxany** | Anxiety during pregnancy | No/Yes | 12,469 | 8 | 8,714 | 5 | logit |
| **d800** | Social support during pregnancy | Continuous | 11,050 | 18 | 7,831 | 14 | regress |
| **maternalabuse** | Any abuse (mother) | No/Yes | 12,902 | 4 | 8,979 | 2 | logit |
| **grandmotheralc** | Maternal grandmother had alcoholism | No or Don't Know/Yes | 11,716 | 13 | 8,257 | 10 | logit |
| **rs1229984m** | Maternal rs1229984 genotype (>= 1 rare allele) | No/Yes | 7,712 | 43 | 5,438 | 40 | logit |
| **riskybeh** | Maternal impulsivity | No/Yes | 7,149 | 47 | 5,219 | 43 | logit |
| **Auxiliary variables** | | | | | | | |
| **pregcomp** | Perinatal trauma/complications | No/Yes | 12,665 | 6 | 8,727 | 4 | logit |
| **binge8w** | Postnatal binge drinking (8 weeks) | No/Yes | 8,214 | 39 | 5,843 | 36 | logit |
| **alcprobpostall** | Postnatal alcohol problems | No/Yes | 9,680 | 28 | 6,956 | 24 | logit |
| **parity** | Parity | 0/1/2/>2 | 12,487 | 7 | 8,777 | 4 | ologit |
| **bestgest** | Gestational age at delivery | Continuous | 13,495 | 0 | 9,135 | 0 | - |
| **miscarriage** | Previous miscarriage | 0/1/≥2 | 12,539 | 7 | 8,807 | 4 | ologit |
| **ultrasound** | Ultrasound at any point in pregnancy | No/Yes | 10,858 | 20 | 7,747 | 15 | logit |
| **bmi** | Maternal BMI (pre-pregnancy) | Underweight/Normal/Overweight/Obese | 11,140 | 17 | 7,861 | 14 | ologit |
| **sex** | Child gender | Male/Female | 13,495 | 0 | 9,135 | 0 | - |
| **ethnic** | Maternal ethnicity | White/Non-White | 11,904 | 12 | 8,413 | 8 | logit |

## Appendix 10: Missing data patterns

*Table 19: Comparison of the distribution of sociodemographic factors, prenatal exposures and FASD outcome variables among participants with complete data, compared to those who had missing data for one or more of the measures required to ascertain FASD status*

| | **Eligible sample N = 13,495 N (%)[a]** | **Participants with complete data N = 223 (N [%])** | **Participants with missing data for one or more of the measures required to ascertain FASD status N = 13,272 (N [%])** |
|---|---|---|---|
| **SOCIODEMOGRAPHIC FACTORS** | | | |
| **Maternal age (years)** | | | |
| <20 | 641 (4.8) | 0 (0) | 641 (4.8) |
| 20-29 | 7,839 (58.1) | 105 (47.1) | 7,734 (58.3) |
| 30+ | 5,015 (37.2) | 118 (52.9) | 4,897 (36.9) |
| **Maternal ethnicity** | | | |
| White | 11,600 (97.5) | 219 (98.2) | 11,381 (97.4) |
| Non-White | 304 (2.6) | 4 (1.8) | 300 (2.6) |
| **Marital status** | | | |
| Not married | 3,163 (25.0) | 28 (12.6) | 3,135 (25.3) |
| Married | 9,483 (75.0) | 194 (87.4) | 9,289 (74.7) |
| **Maternal social class** | | | |
| Professional | 573 (5.9) | 12 (5.7) | 561 (5.9) |
| Managerial/technical | 3,060 (31.5) | 74 (35.4) | 2,986 (31.4) |
| Skilled non-manual | 4,153 (42.7) | 96 (45.9) | 4,057 (42.7) |
| Skilled manual | 763 (7.9) | 13 (6.2) | 750 (7.9) |
| Partly skilled/unskilled | 1,169 (12.0) | 14 (6.7) | 1,155 (12.2) |
| **Paternal social class** | | | |
| Professional | 1,160 (11.0) | 40 (18.5) | 1,120 (10.8) |
| Managerial/technical | 3,596 (34.0) | 74 (34.3) | 3,522 (34.0) |
| Skilled non-manual | 1,156 (10.9) | 33 (15.3) | 1,123 (10.8) |
| Skilled manual | 3,333 (31.5) | 53 (24.5) | 3,280 (31.6) |
| Partly skilled/unskilled | 1,345 (12.7) | 16 (7.4) | 1,329 (12.8) |
| **Maternal education** | | | |
| CSE | 2,416 (20.2) | 19 (8.5) | 2,397 (20.4) |
| Vocational | 1,182 (9.9) | 13 (5.8) | 1,169 (9.9) |
| O Level | 4,149 (34.6) | 77 (34.5) | 4,072 (34.6) |
| A Level | 2,706 (22.6) | 76 (34.1) | 2,630 (22.4) |
| Degree | 1,535 (12.8) | 38 (17.0) | 1,497 (12.7) |
| **Paternal education** | | | |
| CSE | 3,013 (26.2) | 25 (11.3) | 2,988 (26.5) |
| Vocational | 975 (8.5) | 21 (9.5) | 954 (8.5) |
| O Level | 2,436 (21.2) | 49 (22.2) | 2,387 (21.1) |
| A Level | 3,004 (26.1) | 68 (30.8) | 2,936 (26.0) |
| Degree | 2,088 (18.1) | 58 (26.2) | 2,030 (18.0) |
| **Home ownership status** | | | |
| Mortgaged/owned | 9,240 (73.4) | 205 (92.3) | 9,035 (73.0) |
| Council/housing association | 2,016 (16.0) | 5 (2.3) | 2,011 (16.3) |
| Rented (private) | 902 (7.2) | 5 (2.3) | 897 (7.3) |
| Other | 437 (3.5) | 7 (3.2) | 430 (3.5) |

| | Eligible sample N = 13,495 N (%)[a] | Participants with complete data N = 223 (N [%]) | Participants with missing data for one or more of the measures required to ascertain FASD status N = 13,272 (N [%]) |
|---|---|---|---|
| **PRENATAL EXPOSURES** | | | |
| **Prenatal alcohol use (any)** | | | |
| No | 4,132 (31.1) | 61 (27.4) | 4,071 (31.2) |
| Yes | 9,135 (68.9) | 162 (72.7) | 8,973 (68.8) |
| **Prenatal alcohol exposure (max dose/frequency during pregnancy)** | | | |
| None | 4,171 (32.2) | 63 (28.3) | 4,108 (32.3) |
| <1 glass per week | 5,480 (42.3) | 103 (46.2) | 5,377 (42.3) |
| 1-6 glasses per week | 2,872 (22.2) | 49 (22.0) | 2,823 (22.2) |
| 7+ glasses per week | 424 (3.3) | 8 (3.6) | 416 (3.3) |
| **Prenatal binge drinking** | | | |
| No | 9,927 (77.8) | 180 (80.7) | 9,747 (77.7) |
| Yes | 2,839 (22.2) | 43 (19.3) | 2,796 (22.3) |
| **Alcohol use before pregnancy** | | | |
| None | 1,048 (8.3) | 5 (2.2) | 1,043 (8.4) |
| ≤ 1 - 6 glasses per week | 10,144 (80.5) | 191 (85.7) | 9,953 (80.4) |
| 7 - 14 glasses per week | 1,200 (9.5) | 23 (10.3) | 1,177 (9.5) |
| > 14 glasses per week | 210 (1.7) | 4 (1.8) | 206 (1.7) |
| **Prenatal smoking** | | | |
| No | 9,601 (72.2) | 192 (86.1) | 9,409 (71.9) |
| Yes | 3,703 (27.8) | 31 (13.9) | 3,672 (28.1) |
| **Prenatal illicit drug use** | | | |
| No | 12,464 (96.7) | 220 (98.7) | 12,244 (96.7) |
| Yes | 422 (3.3) | 3 (1.4) | 419 (3.3) |
| **Prenatal vitamin supplement use (any)** | | | |
| No | 5,938 (46.0) | 95 (42.6) | 5,843 (46.1) |
| Yes | 6,970 (54.0) | 128 (57.4) | 6,842 (53.9) |
| **Prenatal stressful life events** | | | |
| Mean (SD) | 7 (4) | 6 (4) | 7 (4) |
| **Social support score** | | | |
| Mean (SD) | 20 (5) | 21 (4) | 20 (5) |
| **Prenatal anxiety** | | | |
| No | 9,545 (76.6) | 184 (82.5) | 9,361 (76.4) |
| Yes | 2,924 (23.4) | 39 (17.5) | 2,885 (23.6) |
| **Prenatal depression** | | | |
| No | 9,940 (79.2) | 194 (87.0) | 9,746 (79.1) |
| Yes | 2,611 (20.8) | 29 (13.0) | 2,582 (20.9) |
| **Unplanned pregnancy** | | | |
| No | 8,722 (69.1) | 187 (83.9) | 8,535 (68.9) |
| Yes | 3,898 (30.9) | 36 (16.1) | 3,862 (31.2) |
| **CLINICAL CHARACTERISTICS** | | | |
| **Facial phenotype** | | | |
| **Short palpebral fissure length** | | | |
| No | 3,390 (77.6) | 174 (78.0) | 3,216 (77.6) |
| Yes | 980 (22.4) | 49 (22.0) | 931 (22.5) |
| **Smooth philtrum** | | | |
| No | 3,461 (79.2) | 166 (74.4) | 3,295 (79.5) |

| | Eligible sample N = 13,495 N (%)[a] | Participants with complete data N = 223 (N [%]) | Participants with missing data for one or more of the measures required to ascertain FASD status N = 13,272 (N [%]) |
|---|---|---|---|
| Yes | 909 (20.8) | 57 (25.6) | 852 (20.5) |
| **Thin upper lip** | | | |
| No | 3,951 (90.4) | 193 (86.6) | 3,758 (90.6) |
| Yes | 419 (9.6) | 30 (13.5) | 389 (9.4) |
| **CENTRAL NERVOUS SYSTEM** | | | |
| **Coordination test** | | | |
| Normal | 6,204 (95.1) | 218 (97.8) | 5,986 (95.1) |
| Poor motor coordination | 316 (4.9) | 5 (2.2) | 311 (4.9) |
| **Head circumference at birth** | | | |
| Normal | 8,229 (98.7) | 222 (99.6) | 8,007 (98.6) |
| Small (<2$^{nd}$ percentile) | 111 (1.3) | 1 (0.5) | 110 (1.4) |
| **Head circumference at age 7** | | | |
| Normal | 6,841 (91.8) | 200 (89.7) | 6,641 (91.9) |
| Small (<2$^{nd}$ percentile) | 608 (8.2) | 23 (10.3) | 585 (8.1) |
| **Full scale IQ** | | | |
| Mean (SD) | 104 (17) | 109 (15) | 104 (17) |
| **Verbal IQ** | | | |
| Mean (SD) | 107 (17) | 111 (15) | 107 (17) |
| **Performance IQ** | | | |
| Mean (SD) | 100 (17) | 102 (17) | 100 (17) |
| **Significant difference between IQ subdomains** | | | |
| No | 3,772 (55.5) | 119 (53.4) | 3,653 (55.5) |
| Yes | 3,028 (44.5) | 104 (46.6) | 2,924 (44.5) |
| **WOLD listening comprehension task** | | | |
| Normal | 6,627 (97.2) | 216 (96.9) | 6,411 (97.2) |
| Low performance | 194 (2.8) | 7 (3.1) | 187 (2.8) |
| **WOLD expressive language** | | | |
| Normal | 4,324 (96.2) | 223 (100.0) | 4,101 (96.0) |
| Low performance | 169 (3.8) | 0 (0.0) | 169 (4.0) |
| **Speech and language problems at school** | | | |
| No | 3,552 (86.6) | 217 (97.3) | 3,335 (86.0) |
| Yes | 548 (13.4) | 6 (2.7) | 542 (14.0) |
| **Special educational needs** | | | |
| No | 8,867 (78.4) | 195 (87.4) | 8,672 (78.2) |
| Yes | 2,445 (21.6) | 28 (12.6) | 2,417 (21.8) |
| **Low academic attainment at Key Stage 1 and 2** | | | |
| No | 10,174 (86.1) | 209 (93.7) | 9,965 (85.9) |
| Yes | 1,650 (14.0) | 14 (6.3) | 1,636 (14.1) |
| **Non-word repetition task** | | | |
| Normal | 6,565 (96.4) | 212 (95.1) | 6,353 (96.5) |
| Low performance | 244 (3.6) | 11 (4.9) | 233 (3.5) |
| **Forward digit span task** | | | |
| Normal | 6,557 (97.8) | 214 (96.0) | 6,343 (97.9) |
| Low performance | 145 (2.2) | 9 (4.0) | 136 (2.1) |
| **Opposite worlds task** | | | |
| Normal | 6,460 (97.0) | 221 (99.1) | 6,239 (97.0) |

| | Eligible sample N = 13,495 N (%)[a] | Participants with complete data N = 223 (N [%]) | Participants with missing data for one or more of the measures required to ascertain FASD status N = 13,272 (N [%]) |
|---|---|---|---|
| Low performance | 197 (3.0) | 2 (0.9) | 195 (3.0) |
| **Counting span task** | | | |
| Normal | 6,166 (95.5) | 218 (97.8) | 5,948 (95.4) |
| Low performance | 290 (4.5) | 5 (2.2) | 284 (4.6) |
| **Stop signal task** | | | |
| Normal | 6,214 (96.7) | 215 (96.4) | 5,999 (96.7) |
| Low performance | 212 (3.3) | 8 (3.6) | 204 (3.3) |
| **Backwards digit span task** | | | |
| Normal | 6,182 (92.5) | 204 (91.5) | 5,978 (92.5) |
| Low performance | 501 (7.5) | 19 (8.5) | 482 (7.5) |
| **ADHD** | | | |
| No | 7,786 (97.9) | 215 (96.4) | 7,571 (98.0) |
| Yes | 166 (2.1) | 8 (3.6) | 158 (2.0) |
| **SDQ hyperactivity** | | | |
| No | 10,174 (86.4) | 195 (87.4) | 9,979 (86.4) |
| Yes | 1,604 (13.7) | 28 (12.6) | 1,576 (13.6) |
| **Oppositional-conduct disorder** | | | |
| No | 7,703 (96.9) | 215 (96.4) | 7,488 (96.9) |
| Yes | 249 (3.1) | 8 (3.6) | 241 (3.1) |
| **SDQ peer problems** | | | |
| No | 9,594 (81.4) | 186 (83.4) | 9,408 (81.4) |
| Yes | 2,189 (18.6) | 37 (16.6) | 2,152 (18.6) |
| **SDQ conduct problems** | | | |
| No | 9,530 (80.9) | 189 (84.8) | 9,341 (80.8) |
| Yes | 2,249 (19.1) | 34 (15.3) | 2,215 (19.2) |
| **SCDC social communication problems** | | | |
| No | 7,820 (90.1) | 206 (92.4) | 7,614 (90.1) |
| Yes | 858 (9.9) | 17 (7.6) | 841 (10.0) |
| **Emotional or behavioural problems at school** | | | |
| No | 8,245 (90.7) | 210 (94.2) | 8,035 (90.6) |
| Yes | 843 (9.3) | 13 (5.8) | 830 (9.4) |
| **Autism** | | | |
| No | 13,414 (99.4) | 221 (99.1) | 13,193 (99.4) |
| Yes | 81 (0.6) | 2 (0.9) | 79 (0.6) |
| **DANVA task** | | | |
| Normal | 4,891 (77.6) | 177 (79.4) | 4,714 (77.5) |
| Low performance | 1,413 (22.4) | 46 (20.6) | 1,367 (22.5) |
| **GROWTH** | | | |
| **Growth deficiency** | | | |
| No | 12,310 (91.7) | 214 (96.0) | 12,096 (91.7) |
| Yes | 1,109 (8.3) | 9 (4.0) | 1,100 (8.3) |
| **Auxiliary variables** | | | |
| **Pregnancy/perinatal complications** | | | |
| No | 8,881 (70.1) | 157 (70.7) | 8,724 (70.1) |
| Yes | 3,784 (29.9) | 65 (29.3) | 3,719 (29.9) |
| **Binge drinking (8 weeks postpartum)** | | | |
| No | 4,973 (60.5) | 152 (69.1) | 4,821 (60.3) |

| | Eligible sample N = 13,495 N (%)[a] | Participants with complete data N = 223 (N [%]) | Participants with missing data for one or more of the measures required to ascertain FASD status N = 13,272 (N [%]) |
|---|---|---|---|
| Yes | 3,241 (39.5) | 68 (30.9) | 3,173 (39.7) |
| **Postnatal alcohol problems (maternal self-report and AUDIT 5 to 18 years postpartum)** | | | |
| No | 7,900 (81.6) | 162 (72.7) | 7,738 (81.8) |
| Yes | 1,780 (18.4) | 61 (27.4) | 1,719 (18.2) |
| **Parity** | | | |
| 0 | 5,597 (44.8) | 109 (49.8) | 5,488 (44.7) |
| 1 | 4,369 (35.0) | 72 (32.9) | 4,297 (35.0) |
| 2 | 1,781 (14.3) | 26 (11.9) | 1,755 (14.3) |
| >2 | 740 (5.9) | 12 (5.5) | 728 (5.9) |
| **Gestational age at delivery (weeks)** | | | |
| Mean (SD) | 39.5 (1.8) | 39.5 (1.5) | 39.5 (1.9) |
| **Previous miscarriage** | | | |
| 0 | 9,861 (78.6) | 169 (76.5) | 9,692 (78.7) |
| 1 | 2,017 (16.1) | 45 (20.4) | 1,972 (16.0) |
| ≥2 | 661 (5.3) | 7 (3.2) | 654 (5.3) |
| **Ultrasound scan during pregnancy** | | | |
| No | 533 (4.9) | 9 (4.0) | 524 (4.9) |
| Yes | 10,035 (95.1) | 214 (96.0) | 10,111 (95.1) |
| **Maternal BMI (pre-pregnancy)** | | | |
| Underweight | 558 (5.0) | 9 (4.2) | 549 (5.0) |
| Normal | 8,280 (74.3) | 162 (75.4) | 8,118 (74.3) |
| Overweight | 1,684 (15.1) | 33 (15.4) | 1,651 (15.1) |
| Obese | 618 (5.6) | 11 (5.1) | 607 (5.6) |
| **Child sex** | | | |
| Female | 6,541 (48.5) | 101 (45.3) | 6,853 (51.6) |
| Male | 6,954 (51.5) | 122 (54.7) | 6,419 (48.4) |

[a] Sample size for each variable differs from eligible sample due to missing data. Percentages do not always sum to 100 due to rounding.

Abbreviations: BMI, body mass index; CSE, Certificate of secondary education; DANVA, Diagnostic Analysis of Nonverbal Accuracy; SCDC, Social Communication Disorder Checklist; SD, standard deviation; SDQ, Strengths and Difficulties Questionnaire; WOLD, Weschler Objective Language Dimensions.

Appendix 11: Sociodemographic and pregnancy characteristics of participants by FASD status based on multiply imputed data

| | Total sample N = 13,495 % (95% CI) | Not FASD[a] % (95% CI) | FASD[a] % (95% CI) |
|---|---|---|---|
| **Sociodemographic factors** | | | |
| **Child sex** | | | |
| Female | 48.5 (47.6 - 49.3) | 51.5 (50.5 - 52.4) | 33.9 (31.5- 36.2) |
| Male | 51.5 (50.7 - 52.4) | 48.5 (47.6 - 49.5) | 66.1 (63.8 - 68.5) |
| **Maternal ethnicity** | | | |
| White | 97.1 (96.8 - 97.5) | 97.3 (96.9 - 97.6) | 96.5 (95.5 - 97.6) |
| Non-White | 2.9 (2.5 - 3.2) | 2.7 (2.4 - 3.1) | 3.5 (2.4 - 4.6) |
| **Maternal age at pregnancy (years)** | | | |
| <20 | 4.7 (4.4 - 5.1) | 4.2 (3.8 - 4.7) | 7.2 (5.9 - 8.6) |
| 20-29 | 58.1 (57.3 - 58.9) | 57.4 (56.4 - 58.3) | 61.5 (59.3 - 63.8) |
| 30+ | 37.2 (36.3 - 38.0) | 38.4 (37.5 - 39.3) | 31.2 (29.2 - 33.3) |
| **Home ownership** | | | |
| Mortgaged/owned | 72.4 (71.6 - 73.2) | 75.6 (74.6 - 76.5) | 57.0 (54.3 - 59.6) |
| Council/housing association | 16.6 (15.9 - 17.3) | 14.1 (13.3 - 14.8) | 29.0 (26.7 - 31.3) |
| Rented (private) | 7.4 (6.9 - 7.8) | 6.9 (6.4 - 7.4) | 9.6 (8.0 - 11.3) |
| Other | 3.7 (3.3 - 4.0) | 3.5 (3.1 - 3.9) | 4.4 (3.3 - 5.5) |
| **Maternal highest educational qualification** | | | |
| CSE | 21.5 (20.8 - 22.3) | 19.0 (18.2 - 19.8) | 33.8 (31.3 - 36.3) |
| Vocational | 10.1 (9.5 - 10.7) | 9.8 (9.2 - 10.3) | 11.7 (10.0 - 13.3) |
| O level | 34.3 (33.5 - 35.2) | 35.1 (34.1 - 36.0) | 30.9 (28.3 - 33.4) |
| A level | 21.9 (21.2 - 22.6) | 22.9 (22.1 - 23.8) | 17.0 (15.0 - 18.9) |
| Degree | 12.1 (11.6 - 12.7) | 13.2 (12.6 - 13.9) | 6.7 (5.4 - 8.0) |
| **Paternal highest educational qualification** | | | |
| CSE | 28.6 (27.8 - 29.4) | 25.7 (24.8 - 26.5) | 42.9 (40.4 - 45.5) |
| Vocational | 8.7 (8.2 - 9.2) | 8.6 (8.0 - 9.2) | 9.3 (7.7 - 10.9) |
| O level | 20.9 (20.2 - 21.6) | 21.3 (20.5 - 22.2) | 18.9 (16.8 - 20.9) |
| A level | 25.0 (24.3 - 25.8) | 26.1 (25.2 - 26.9) | 19.9 (18.0 - 21.9) |
| Degree | 16.7 (16.1 - 17.4) | 18.3 (17.5 - 19.1) | 9.0 (7.3 - 10.6) |
| **Maternal social class** | | | |
| Professional | 5.0 (4.6 - 5.4) | 5.6 (5.1 - 6.1) | 2.1 (1.4 - 2.8) |
| Managerial/technical | 28.4 (27.5 - 29.3) | 29.6 (28.7 - 30.6) | 22.3 (20.3 - 24.3) |
| Skilled non-manual | 42.9 (41.9 - 43.8) | 43.0 (42.0 - 44.0) | 42.2 (39.8 - 44.6) |
| Skilled manual | 8.8 (8.2 - 9.4) | 8.3 (7.7 - 9.0) | 11.1 (9.5 - 12.6) |
| Partly skilled/unskilled | 15.0 (14.2 - 15.7) | 13.5 (12.6 - 14.3) | 22.3 (20.0 - 24.6) |
| **Paternal social class** | | | |
| Professional | 9.8 (9.3 - 10.4) | 10.8 (10.1 - 11.4) | 5.3 (4.1 - 6.5) |
| Managerial/technical | 31.6 (30.8 - 32.5) | 32.9 (31.8 - 33.9) | 25.6 (23.4 - 27.9) |
| Skilled non-manual | 10.7 (10.1 - 11.3) | 11.0 (10.4 - 11.6) | 9.2 (7.6 - 10.7) |
| Skilled manual | 32.8 (31.9 - 33.7) | 31.8 (30.8 - 32.8) | 37.4 (35.2 - 39.6) |
| Partly skilled/unskilled | 15.1 (14.3 - 15.8) | 13.5 (12.8 - 14.3) | 22.5 (20.2 - 24.8) |
| **Marital status** | | | |
| Not married | 26.0 (25.2 - 26.7) | 23.8 (23.0 - 24.7) | 36.6 (34.3 - 38.8) |
| Married | 74.0 (73.3 - 74.8) | 76.2 (75.3 - 77.0) | 63.4 (61.2 - 65.7) |

| | Total sample N = 13,495 % (95% CI) | Not FASD[a] % (95% CI) | FASD[a] % (95% CI) |
|---|---|---|---|
| **Pregnancy factors** | | | |
| **Parity** | | | |
| 0 | 44.9 (44.0 - 45.7) | 45.7 (44.7 - 46.7) | 40.9 (38.5 - 43.3) |
| 1 | 34.9 (34.0 - 35.7) | 35.0 (34.1 - 35.9) | 34.3 (31.8 - 36.7) |
| 2 | 14.3 (13.7 - 14.9) | 13.9 (13.2 - 14.5) | 16.2 (14.6 - 17.9) |
| > 2 | 6.0 (5.6 - 6.4) | 5.5 (5.0 - 5.9) | 8.6 (7.1 - 10.1) |
| **Preterm delivery (< 37 weeks)** | | | |
| Yes | 5.0 (4.6 - 5.3) | 4.6 (4.2 - 5.0) | 6.8 (5.7 - 8.0) |
| No | 95.0 (94.7 - 95.4) | 95.4 (95.0 - 95.8) | 93.2 (92.0 - 94.3) |
| **Unplanned pregnancy** | | | |
| Yes | 31.4 (30.6 - 32.3) | 29.8 (28.9 - 30.7) | 39.3 (36.8 - 41.8) |
| No | 68.6 (67.8 - 69.4) | 70.2 (69.3 - 71.1) | 60.7 (58.2 - 63.3) |

[a] Total sample size for multiply imputed data = 13,495. The number of participants with and without FASD varies for each imputation set.

Abbreviations: CI, confidence interval; CSE, Certificate of Secondary Education; FASD, fetal alcohol spectrum disorder; N, sample size.

Appendix 12: Prenatal alcohol exposure and clinical characteristics by FASD status based on multiply imputed data

| | Total sample N = 13,495 % (95% CI) | Not FASD[a] % (95% CI) | FASD[a] % (95% CI) |
|---|---|---|---|
| **PRENATAL ALCOHOL EXPOSURE** | | | |
| **Prenatal alcohol exposure (any)** | | | |
| No | 21.3 (20.5 - 22.0) | 25.6 (24.8 - 26.4) | 0.03 (0.00 - 0.09) |
| Yes | 78.7 (78.0 - 79.5) | 74.4 (73.6 - 75.2) | 99.97 (99.90 - 100.00) |
| **Prenatal binge drinking** | | | |
| No | 74.7 (73.7 - 75.6) | 77.2 (76.2 - 78.3) | 62.2 (59.4 - 64.9) |
| Yes | 25.3 (24.4 - 26.3) | 22.8 (21.7 - 23.8) | 37.8 (35.1 - 40.6) |
| **Prenatal alcohol exposure (max dose/frequency during pregnancy)[b]** | | | |
| None | 32.4 (31.6 - 33.2) | 35.2 (34.3 - 36.1) | 19.0 (16.8 - 21.1) |
| <1 glass per week | 42.2 (41.3 - 43.0) | 40.4 (39.4 - 41.3) | 51.1 (48.7 - 53.5) |
| 1-6 glasses per week | 22.1 (21.4 - 22.8) | 21.5 (20.7 - 22.3) | 25.1 (22.9 - 27.3) |
| 7+ glasses per week | 3.3 (3.0 - 3.6) | 3.0 (2.6 - 3.3) | 4.8 (3.9 - 5.8) |
| **FACIAL PHENOTYPE** | | | |
| **FAS facial phenotype** | | | |
| No | 99.5 (99.3 - 99.7) | 99.5 (99.4 - 99.7) | 99.3 (98.7 - 99.9) |
| Yes | 0.5 (0.3 - 0.7) | 0.5 (0.3 - 0.6) | 0.7 (0.1 - 1.3) |
| **Partial FAS facial phenotype** | | | |
| No | 91.5 (90.4 - 92.7) | 91.7 (90.7 - 92.7) | 90.5 (87.6 - 93.5) |
| Yes | 8.5 (7.3 - 9.6) | 8.3 (7.3 - 9.3) | 9.5 (6.5 - 12.4) |
| **GROWTH** | | | |
| **Growth impairment (< 9$^{th}$ percentile)** | | | |
| No | 91.7 (91.3 - 92.2) | 92.5 (91.9 - 93.0) | 88.3 (86.6 - 90.0) |
| Yes | 8.3 (7.8 - 8.7) | 7.6 (7.0 - 8.1) | 11.7 (10.0 - 13.4) |
| **CENTRAL NERVOUS SYSTEM** | | | |
| **CNS impairment in ≥ 3 domains** | | | |
| No | 78.3 (77.3 - 79.2) | 94.3 (93.7 - 94.8) | 0.0 (0.0 - 0.0)[c] |
| Yes | 21.7 (20.8 - 22.7) | 5.7 (5.2 - 6.3) | 100.0 (100.0 - 100.0)[c] |
| **Impaired CNS domain a) Hard and soft neurologic signs** | | | |
| No | 93.0 (92.2 - 93.8) | 95.8 (95.2 - 96.4) | 79.2 (76.4 - 82.0) |
| Yes | 7.0 (6.2 - 7.8) | 4.2 (3.6 - 4.8) | 20.8 (18.0 - 23.6) |
| **Impaired CNS domain b) Brain structure** | | | |
| No | 99.4 (99.1 - 99.6) | 99.6 (99.4 - 99.7) | 98.4 (97.5 - 99.2) |
| Yes | 0.6 (0.4 - 0.9) | 0.4 (0.3 - 0.6) | 1.6 (0.8 - 2.5) |
| **Impaired CNS domain c) Cognition** | | | |
| No | 44.1 (43.0 - 45.2) | 48.4 (47.2 - 49.5) | 23.2 (20.8 - 25.5) |
| Yes | 55.9 (54.8 - 57.0) | 51.6 (50.5 - 52.8) | 76.8 (74.5 - 79.2) |
| **Impaired CNS domain d) Communication** | | | |
| No | 97.0 (96.4 - 97.6) | 98.7 (98.3 - 99.0) | 89.0 (86.4 - 91.5) |
| Yes | 3.0 (2.4 - 3.6) | 1.3 (1.0 - 1.7) | 11.0 (8.5 - 13.6) |

| | Total sample<br>N = 13,495<br>% (95% CI) | Not FASD[a]<br>% (95% CI) | FASD[a]<br>% (95% CI) |
|---|---|---|---|
| **Impaired CNS domain e) Education** | | | |
| No | 75.0 (74.3 - 75.8) | 85.1 (84.2 - 85.9) | 26.1 (23.8 - 28.3) |
| Yes | 25.0 (24.2 - 25.8) | 14.9 (14.1 - 15.8) | 73.9 (71.7 - 76.2) |
| **Impaired CNS domain f) Memory** | | | |
| No | 91.1 (90.0 - 92.2) | 94.7 (94.0 - 95.4) | 73.7 (69.7 - 77.7) |
| Yes | 8.9 (7.8 - 10.0) | 5.3 (4.6 - 6.0) | 26.3 (22.3 - 30.3) |
| **Impaired CNS domain g) Executive functioning** | | | |
| No | 96.1 (95.5 - 96.7) | 98.1 (97.7 - 98.5) | 86.1 (83.6 - 88.7) |
| Yes | 3.9 (3.3 - 4.5) | 1.9 (1.5 - 2.3) | 13.9 (11.3 - 16.4) |
| **Impaired CNS domain h) Attention deficit/hyperactivity** | | | |
| No | 81.7 (81.0 - 82.5) | 90.3 (89.7 - 91.0) | 39.7 (37.1 - 42.3) |
| Yes | 18.3 (17.5 - 19.0) | 9.7 (9.0 - 10.3) | 60.3 (57.7 - 62.9) |
| **Impaired CNS domain i) Adaptive behaviour** | | | |
| No | 61.3 (60.3 - 62.3) | 70.8 (69.8 - 71.7) | 15.2 (13.1 - 17.3) |
| Yes | 38.7 (37.7 - 39.7) | 29.2 (28.3 - 30.2) | 84.8 (82.7 - 86.9) |

[a] Total sample size for multiply imputed data = 13,495. The number of participants with and without FASD varies for each imputation set.

[b] Participants who reported 'none' for alcohol consumption using the dose/frequency measure may still have reported PAE on other measures of alcohol consumption (such as binge drinking, unit-based measures or continuation of pre-pregnancy drinking patterns).

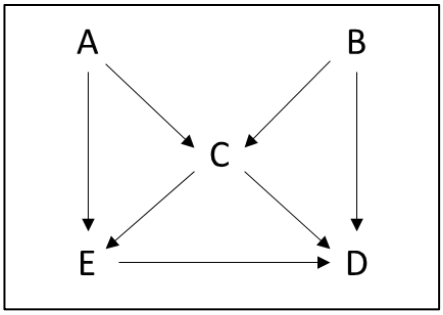
[c] By definition all participants who meet criteria for FASD must have CNS impairment in ≥ 3 domains. Therefore, confidence intervals were constrained to reflect this (i.e. for participants with FASD, the value in the true population can only be 0% for 'no impairment' and 100% for 'impairment in ≥ 3 domains').

# Appendix 13: Causal diagram theory (directed acyclic graphs; DAGs)

## Causal diagram language

Causal diagram theory presents its own language, which uses ancestry (family tree) terminology. This terminology is presented below, with an example based on Figure 21, derived from the work of Greenland and colleagues.[522]

*Figure 21: Causal diagram example*




In Figure 21, the variables depicted by letters (A, B, C, D, E) are called *nodes*. Arrows represent cause-effect relationships and are called *arcs* or *edges.* A *path* is any unbroken route that follows the arcs (regardless of direction) between adjacent nodes. *Causal* or *directed paths* are those which follow a sequence of arcs in a tail-to-head route, such as A → E → D. Any path which is not directed, is *undirected.* In particular, a path which starts with a head-to-tail arc is known as a *backdoor path*. In Figure 21, all paths from E to D except E → D (e.g. E ← C → D) are backdoor paths.

A path is *blocked* at the point at which two arrowheads meet. The variable at which the arrowheads meet is called a *collider*. For example, the path A → C ← B → D is blocked by collider C. The arc from C to D represents a direct causal effect of C on D, as it is not intercepted by any of the other variables included in the diagram. In contrast, the causal path from A → C → D from A to D is indirect, as the effect is mediated by C. The absence of an arc, or any other open path, between A and B implies independence.

*Descendants* of a variable X are those that are affected directly or indirectly by X. For example, E and D are descendants of A in the path A → E → D. More specifically, c*hildren* of a variable X are those that follow a single directed arc. D is a

child of E in the path E → D. *Ancestors* or *causes* are variables that affect other variables directly or indirectly and, more specifically, *parents* are ancestor variables that are adjacent to the affected variable. In the path A → E → D, A and E are both ancestors of D, and E is also a parent of D.

DAGs are directed, as the arcs connecting variables suggest a direction of effect, they are acyclic as they do not contain feedback loops[r] and they are causal as they include all common causes of a pair of variables.[522,523]

**Graphical properties and rules**

Causal diagrams have several properties that are important to their interpretation. First, causal diagrams are qualitative and do not provide information about the strength or nature of association between two variables. For example, causal diagrams do not convey whether variables are categorical or continuous, whether dose-response relationships are linear or non-linear, whether causes are necessary or sufficient, whether there is effect modification, or whether effects are harmful or protective.[305,522,523] These properties must be determined by statistical investigation.

Second, the *parent-child* and *direct/indirect* relationships implied by causal graphs are not inherent properties of the biologic relationship between two variables. This terminology simply reflects the level of detail that is captured in the diagram.[522] If we consider the relationship PAE → FASD, PAE is represented as a direct cause of FASD only because the specific intermediate causal mechanisms between PAE and FASD remain unknown and/or unmeasured.[10]

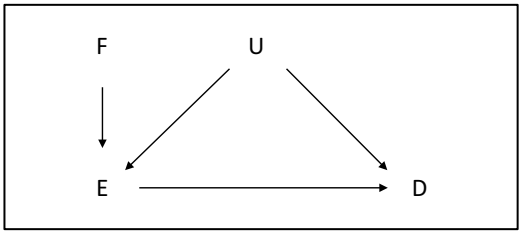
The presence of an arc between two nodes indicates the possibility of a direct causal effect. If there is inconclusive evidence about whether there is a causal link between two variables, it is appropriate to include an arc between them (and in the direction deemed most plausible), since the presence of an arc indicates the

[r] The absence of feedback loops means that a variable cannot be an ancestor or descendant of itself such that X causes Y and Y simultaneously causes X. If the value of X affects Y and then Y affects a later value of X this temporal sequence must be represented in separate variables (e.g. $X_0 \rightarrow Y_0 \rightarrow X_1$). 305. Glymour MM, Greenland S. Causal Diagrams. In: Rothman KJ, Greenland, S., & Lash, T. L., ed. *Modern epidemiology*. Philadelphia: Lippincott Williams & Wilkins; 2008:183-209.

*possibility* of an effect in the depicted direction whereas the absence of an arc or node indicates the stronger assumption of *no effect in either direction*, or at least the belief that the effect has a negligible impact given all other factors in the graph.[290]

Finally, it is not necessary to include all causes of a variable within a causal diagram. However, if two variables share a common cause then this must be represented.[305] If this common cause is unmeasured then it must be included and can be represented graphically as shown by node U in Figure 22. Although not necessary, it is possible to include other variables that are not a common cause of two other variables,[290] as in the case of node F in Figure 22. For the purposes of the DAG developed in this chapter I will include some variables that are not common causes of two other variables to ensure complete coverage of the risk factors that have been described to date in the FASD literature. Sometimes, such variables are important in the analysis (e.g. to improve precision, to investigate effect modification/ mediation), even if they are not important for reducing bias in the total causal effect estimate.

*Figure 22: Causal diagram with unmeasured confounder (U) and additional variable (F)*




Two key concepts are integral to the interpretation and manipulation of DAGs. They are the *d-Separation* criteria and *Causal Markovian Condition* described by Pearl[304] and presented in an epidemiological context by Glymour and colleagues.[305]

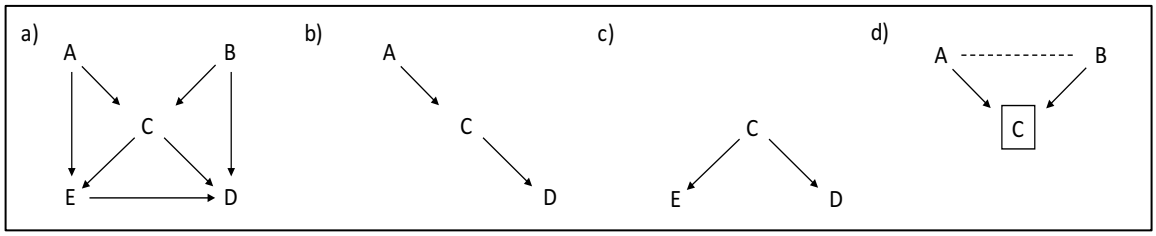
The d-Separation criteria are graphical rules that can be used to infer independencies between variables. D-separation can be either unconditional or conditional. Unconditional d-separation occurs when there is no open path between two nodes. For example, in Figure 23a, variables A and B are unconditionally d-separated, as the only paths between them are blocked by colliders C, D and E. Since variables A and B are d-separated, the graph predicts

that, statistically, they will be marginally independent. In other words, causal diagram theory states that only open paths will create associations.[305]

Conditioning has different implications for d-separation according to whether the node that is conditioned on is a collider or not. Non-colliders may be mediating variables, as in Figure 23b, where C is the mediator; or non-colliders may be a common cause of two variables as in Figure 23c, where C is a confounder. Conditioning a non-collider blocks the flow of statistical dependence along that path and (providing that there are no other open paths) creates d-separation and, therefore, statistical independence between E and D. Conversely, conditioning on a collider can *create* associations between two marginally independent variables. For example, in Figure 23d the path between A and B is unconditionally blocked by collider C and so (in the absence of other paths from A to B) there is no marginal association between A and B; however, adjusting for C opens that path and creates a conditional association between A and B.[304,305] Figure 23d provides a graphical representation of how an open path is created by conditioning on a collider. The dashed line in this figure is a non-directional arc that indicates that A and B are associated for reasons other than influencing each other or sharing a common cause.[522] This dashed line is not formally a part of the DAG, but is often added informally to highlight the conditional associations that may be induced. Collider bias is explained more fully, with an intuitive example, in the next section.

A set of variables (S) is said to block the path between E and D if the path is closed after conditioning on this set. The set (S) unblocks the path if the path is open after conditioning. If there was no open path between E and D to begin with then the empty set is said to separate them.[305]

*Figure 23: Causal diagrams for illustration of the d-separation criteria. Figure a) presents the full diagram, where colliders C, E and D create unconditional separation between A and B. Figure b) presents a causal pathway, where the causal effect between A and D is mediated by C, Figure c) presents a biasing pathway between E and D due to not adjusting for confounder C and Figure d) shows the introduction of a non-causal relationship between variables A and B (depicted by dashed line), due to inappropriate adjustment (depicted by square) on collider C.*




**Bias**

As well as facilitating expression of complex causal networks,[304] DAGs have been advocated as useful tools for informing strategies for bias reduction.[305] Bias is present when the chosen measure of association differs from the true causal effect.[298] Under these circumstances, different exposure groups differ in their probability of the outcome for reasons other than the effect of the exposure. This section will compare traditional and graphical approaches to confounding, selection, and information bias.

Confounding has been defined as a bias of the estimated effect of an exposure on an outcome due to the presence of a common cause of the exposure and the outcome.[555] In epidemiology, confounding variables have commonly been defined by the following criteria:[301]

i. The variable must be associated with both the exposure and the outcome.

ii. The variable must predict the outcome, independent of its association with the exposure.

iii. The variable must not lie on the causal pathway between the exposure and the outcome (i.e. it should not be a mediator).

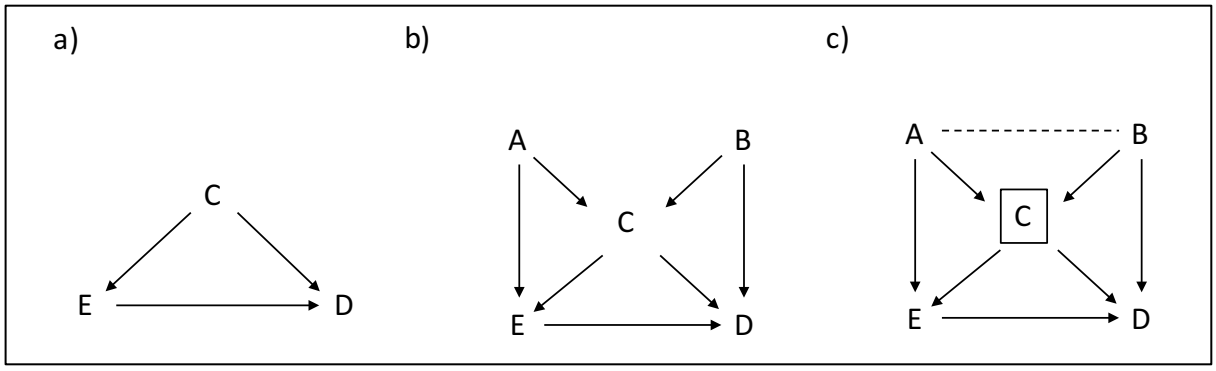
Selection bias may also lead to misleading effect estimates within epidemiological studies. Selection bias occurs when the study population does not represent the target population[556] or, more formally, when:

> "the association between exposure and disease includes a non-causal component attributable to restricting the analysis to certain level(s) of a common effect of exposure and disease or, more generally, to conditioning on a common effect of variables correlated with exposure and disease."[557(p. 182)]

In contrast to epidemiological approaches, graphical methods offer a more precise definition by expressing bias as any unblocked backdoor path between the exposure and outcome. Although in many instances, the traditional epidemiological and DAG definitions coincide, examples can be found in which the traditional definition would dictate that confounding is present, but the graphical definition would not, and vice versa. For example, in Figure 23a, all paths, except for the direct path from E → D are backdoor paths. Therefore, the estimate for the total effect of E on D will be partly due to the direct causal effect and partly due to the remaining biasing paths.[522] Some argue that because graphical rules are sufficient to identify structural sources of bias there is no need to distinguish between confounding and selection bias.[305] Nevertheless, for the purposes of unifying graphical and epidemiological definitions of bias it is useful to indicate how these concepts overlap.[305] Also, knowing whether the source of bias is due to confounding or selection issues may suggest approaches for minimising bias by study design.

Graphical methods depict confounding as an open backdoor path formed by a common cause of two variables. In many instances, application of the traditional and graphical criteria for confounding identify the same variables as confounders and would recommend similar strategies for control of this bias.

*Figure 24: Partial a) and full version b) of a causal diagram to illustrate complexities when adjusting for confounders, including over-adjustment when C is both a confounder and collider c).*




For example, Figure 24a is a sub-section of Figure 24b and demonstrates that the effect estimate for the relationship between E and D is partly confounded by C. If we take Figure 24a to be a complete DAG (i.e. assume that there are no further common causes that are not represented in the graph), then the traditional and graphical approaches would both identify C as a confounder and would both suggest C as a covariate that should be controlled for to gain an unbiased estimate of the effect of E on D. However, the traditional and graphical approaches to confounding sometimes diverge. Greenland and colleagues[522] presented the full DAG (Figure 24b) to colleagues and asked them identify which variables would be sufficient to adjust for in order to create an unbiased estimate of the effect of E on D. Most suggested that adjusting for A or B only would not be sufficient, but that adjusting for C alone would be sufficient. This choice was based on the reasoning that adjustment for only A and B would not resolve the confounding by C. Adjustment for C, however, would block the pathway between A and D, given E, and would leave B unassociated with E. Therefore, A and B would no longer meet the traditional criteria for confounding, as they would not be associated with both the exposure and the outcome. However, the graphical criteria for confounding indicate that controlling for C alone would not be sufficient to eliminate bias. This is because C is a collider (a common effect of A and B, on the pathway A → C ← B). As previously described, conditioning on a collider creates an association between its parents. This, in turn, creates a new backdoor pathway, which, if uncontrolled, will lead to a biased effect estimate between E and D (see Figure 24c). Therefore, to

create an unbiased estimate of E on D, it is also necessary to control for A or B, in addition to C.[522]

Collider bias arises because observing information about one of the common causes of an effect, makes the other cause more or less likely given the occurrence of that effect, even if these causes were previously independent.[304] Hernan provides the following example.[557] Suppose that dieting (E) and a particular form of cancer (D) are statistically independent and, therefore, knowing that someone was on a diet does not change their risk of cancer. A common effect of dieting and cancer is weight loss (C). Given that we know that someone had lost weight (i.e. we condition on C), cancer and diet no longer remain independent. This is because knowing that someone had lost weight and was not on a diet increases the probability that this person has cancer (intuitively: if they lost weight but weren't on a diet then it must have been something else [e.g. cancer] that caused them to lose weight). Therefore, within categories of weight loss, dieting and cancer become inversely associated. In these circumstances the crude effect estimate of the relationship between E and D is unbiased and conditioning on collider C creates a spurious association between E and D, thus leading to a biased adjusted estimate.

Collider bias is a form of selection bias. Of particular relevance to this thesis, which explores the epidemiology of FASD within the ALSPAC cohort, is consideration of loss to follow up/missing data. Selection bias due to loss to follow-up and missing data can also be represented graphically.[558] These forms of bias occur when study drop-out or completeness of data are associated with both the exposure and the outcome.[301] Restriction of analysis to participants with complete data represents a form of conditioning on the common effect of exposure and outcome - data availability.

In summary, DAGs can assist with the identification and representation of bias as well as assisting with the choice of adjustment variables that will help to remove or reduce that bias. Graphical methods can be applied to complex causal networks in situations where traditional criteria for confounder identification may fail.[522] Bias due to confounding can typically be reduced via multivariable regression modelling, using the method outlined above to identify suitable covariates. Selection bias can

be minimised by avoiding harmful adjustment on common effects of the exposure and outcome, or by using appropriate methods to account for missing data. In this thesis, I used multiple imputation, as described in Chapter 4, to reduce the impact of selection bias due to missing data.

## Appendix 14: DAGitty code (compatible with html version)

```
dag {
"Antenatal care" [pos="0.945,1.014"]
"Current alcohol use" [pos="0.367,0.996"]
"Differential diagnosis" [latent,pos="0.880,0.596"]
"Drug use" [pos="0.823,0.446"]
"FASD classification" [outcome,pos="0.880,0.688"]
"FASD diagnostic framework/detection" [pos="0.885,0.480"]
"Genotype (maternal)" [pos="0.417,0.791"]
"Genotype_(infant)" [pos="0.543,0.742"]
"Having another child with FASD" [pos="0.562,0.982"]
"Marital status" [pos="0.092,0.417"]
"Maternal FASD" [pos="0.018,0.327"]
"Maternal knowledge/attitudes towards PAE" [latent,pos="0.086,0.800"]
"Maternal prenatal alcohol consumption" [latent,pos="0.193,0.883"]
"Mental health" [pos="0.559,0.430"]
"Other unmeasured exposures" [latent,pos="0.956,0.326"]
"PAE guidance" [latent,pos="0.019,0.466"]
"Pre-pregnancy alcohol use" [pos="0.143,0.299"]
"Professional knowledge/guidance on PAE" [latent,pos="0.074,0.680"]
"Reasons for PAE measurement error" [latent,pos="0.266,1.102"]
"Risky behaviour" [pos="0.816,0.210"]
"Substance use of friends/family" [pos="0.079,0.190"]
"True FASD" [latent,pos="0.882,0.884"]
"Unplanned pregnancy" [pos="0.774,0.283"]
Abuse [pos="0.356,0.295"]
Age [pos="0.200,0.533"]
BMI [pos="0.686,0.651"]
Nutrition [pos="0.561,0.590"]
Parity [pos="0.065,0.548"]
Preg_comp [pos="0.027,1.056"]
Religion [pos="0.224,0.256"]
```

Reported_PAE [pos="0.196,0.987"]

SES [pos="0.303,0.376"]

Smoking [pos="0.669,0.526"]

Stress [pos="0.243,0.650"]

Support [pos="0.427,0.450"]

"Antenatal care" -> Nutrition [pos="0.785,0.551"]

"Antenatal care" -> Preg_comp [pos="0.905,1.106"]

"Differential diagnosis" -> "FASD classification"

"Differential diagnosis" -> BMI

"Drug use" -> "Antenatal care" [pos="0.966,0.558"]

"Drug use" -> "Differential diagnosis"

"Drug use" -> "True FASD" [pos="0.803,0.689"]

"Drug use" -> Nutrition [pos="0.788,0.580"]

"Drug use" -> Preg_comp [pos="-0.028,0.208"]

"FASD diagnostic framework/detection" -> "Differential diagnosis"

"Genotype (maternal)" -> "Current alcohol use"

"Genotype (maternal)" -> "Genotype_(infant)"

"Genotype (maternal)" -> "Maternal prenatal alcohol consumption"

"Genotype (maternal)" -> "Pre-pregnancy alcohol use" [pos="0.004,0.425"]

"Genotype (maternal)" -> "True FASD"

"Genotype_(infant)" -> "True FASD"

"Marital status" -> "Unplanned pregnancy"

"Marital status" -> SES

"Marital status" -> Support

"Maternal FASD" -> "Maternal prenatal alcohol consumption" [pos="-0.030,0.659"]

"Maternal FASD" -> "Mental health"

"Maternal FASD" -> "Pre-pregnancy alcohol use"

"Maternal knowledge/attitudes towards PAE" -> "Maternal prenatal alcohol consumption"

"Maternal prenatal alcohol consumption" -> "Antenatal care" [pos="0.597,0.921"]

"Maternal prenatal alcohol consumption" -> "Current alcohol use"

"Maternal prenatal alcohol consumption" -> "FASD classification"

"Maternal prenatal alcohol consumption" -> "Having another child with FASD" [pos="0.450,0.927"]

"Maternal prenatal alcohol consumption" -> "True FASD"

"Maternal prenatal alcohol consumption" -> Nutrition [pos="0.035,0.530"]

"Maternal prenatal alcohol consumption" -> Preg_comp

"Maternal prenatal alcohol consumption" -> Reported_PAE

"Mental health" -> "Differential diagnosis"

"Mental health" -> "Drug use"

"Mental health" -> "Maternal prenatal alcohol consumption" [pos="-0.011,0.114"]

"Mental health" -> "True FASD" [pos="0.585,0.686"]

"Mental health" -> Nutrition

"Mental health" -> Smoking

"Mental health" -> Stress

"Other unmeasured exposures" -> "Differential diagnosis"

"PAE guidance" -> "Maternal knowledge/attitudes towards PAE"

"PAE guidance" -> "Professional knowledge/guidance on PAE"

"Pre-pregnancy alcohol use" -> "Current alcohol use" [pos="0.400,0.812"]

"Pre-pregnancy alcohol use" -> "Maternal prenatal alcohol consumption" [pos="-0.023,0.339"]

"Pre-pregnancy alcohol use" -> "Unplanned pregnancy" [pos="0.519,0.362"]

"Pre-pregnancy alcohol use" -> Nutrition [pos="0.569,0.369"]

"Professional knowledge/guidance on PAE" -> "Maternal knowledge/attitudes towards PAE"

"Reasons for PAE measurement error" -> Reported_PAE

"Risky behaviour" -> "Differential diagnosis"

"Risky behaviour" -> "Drug use"

"Risky behaviour" -> "Maternal prenatal alcohol consumption" [pos="-0.016,0.266"]

"Risky behaviour" -> "Other unmeasured exposures"

"Risky behaviour" -> "Pre-pregnancy alcohol use" [pos="0.333,0.249"]

"Risky behaviour" -> "Substance use of friends/family"

"Risky behaviour" -> "Unplanned pregnancy"

"Risky behaviour" -> Nutrition [pos="0.613,0.221"]

"Risky behaviour" -> Smoking [pos="0.640,0.289"]

"Risky behaviour" -> Stress [pos="0.423,0.260"]

"Substance use of friends/family" -> "Maternal FASD"

"Substance use of friends/family" -> "Maternal knowledge/attitudes towards PAE"

"Substance use of friends/family" -> "Maternal prenatal alcohol consumption" [pos="-0.011,0.474"]

"Substance use of friends/family" -> "Pre-pregnancy alcohol use"

"True FASD" -> "FASD classification"

"Unplanned pregnancy" -> "Antenatal care" [pos="0.987,0.439"]

"Unplanned pregnancy" -> "Drug use"

"Unplanned pregnancy" -> "Maternal prenatal alcohol consumption" [pos="0.442,0.381"]

"Unplanned pregnancy" -> Nutrition [pos="0.785,0.560"]

"Unplanned pregnancy" -> Smoking

Abuse -> "Antenatal care" [pos="0.975,0.247"]

Abuse -> "Mental health"

Abuse -> "Unplanned pregnancy" [pos="0.562,0.254"]

Abuse -> Stress [pos="0.010,0.344"]

Age -> "Drug use" [pos="0.211,0.344"]

Age -> "Maternal prenatal alcohol consumption" [pos="-0.007,0.629"]

Age -> "Pre-pregnancy alcohol use"

Age -> "True FASD"

Age -> Parity [pos="0.104,0.543"]

Age -> Smoking [pos="0.359,0.474"]

BMI -> "True FASD"

Nutrition -> "Differential diagnosis" [pos="0.662,0.618"]

Nutrition -> "True FASD" [pos="0.626,0.703"]

Nutrition -> BMI

Nutrition -> Preg_comp [pos="0.038,0.380"]

Religion -> "Current alcohol use"

Religion -> "Marital status" [pos="0.249,0.354"]

Religion -> "Maternal prenatal alcohol consumption" [pos="-0.031,0.231"]

Religion -> "Pre-pregnancy alcohol use"

Religion -> "Unplanned pregnancy" [pos="0.551,0.230"]

Religion -> Support

Reported_PAE -> "FASD classification"

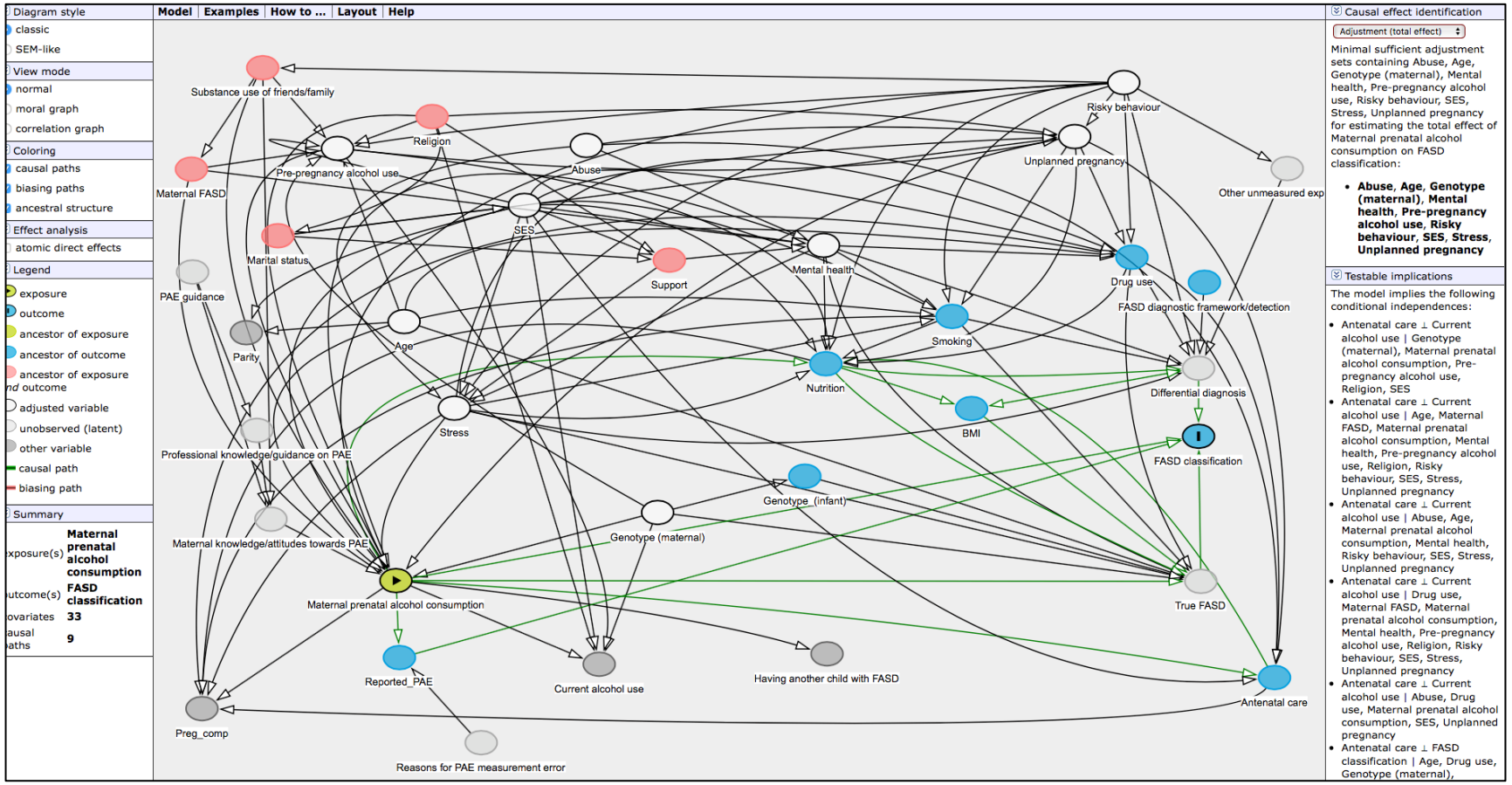
SES -> "Antenatal care" [pos="0.626,1.067"]

```
SES -> "Current alcohol use"
SES -> "Drug use" [pos="0.337,0.267"]
SES -> "Maternal prenatal alcohol consumption" [pos="-0.021,0.498"]
SES -> "Mental health"
SES -> "Pre-pregnancy alcohol use" [pos="-0.008,0.260"]
SES -> "Unplanned pregnancy" [pos="0.376,0.309"]
SES -> Nutrition [pos="0.467,0.366"]
SES -> Parity [pos="0.084,0.458"]
SES -> Smoking [pos="0.432,0.349"]
SES -> Stress
SES -> Support
Smoking -> "Differential diagnosis"
Smoking -> "True FASD"
Smoking -> Nutrition
Smoking -> Preg_comp [pos="0.036,0.523"]
Stress -> "Differential diagnosis" [pos="0.548,0.761"]
Stress -> "Drug use" [pos="0.292,0.114"]
Stress -> "Maternal prenatal alcohol consumption" [pos="0.165,0.799"]
Stress -> "True FASD"
Stress -> Nutrition [pos="0.429,0.691"]
Stress -> Preg_comp [pos="0.085,0.802"]
Stress -> Smoking
Support -> "Mental health"
Support -> Stress
}
```

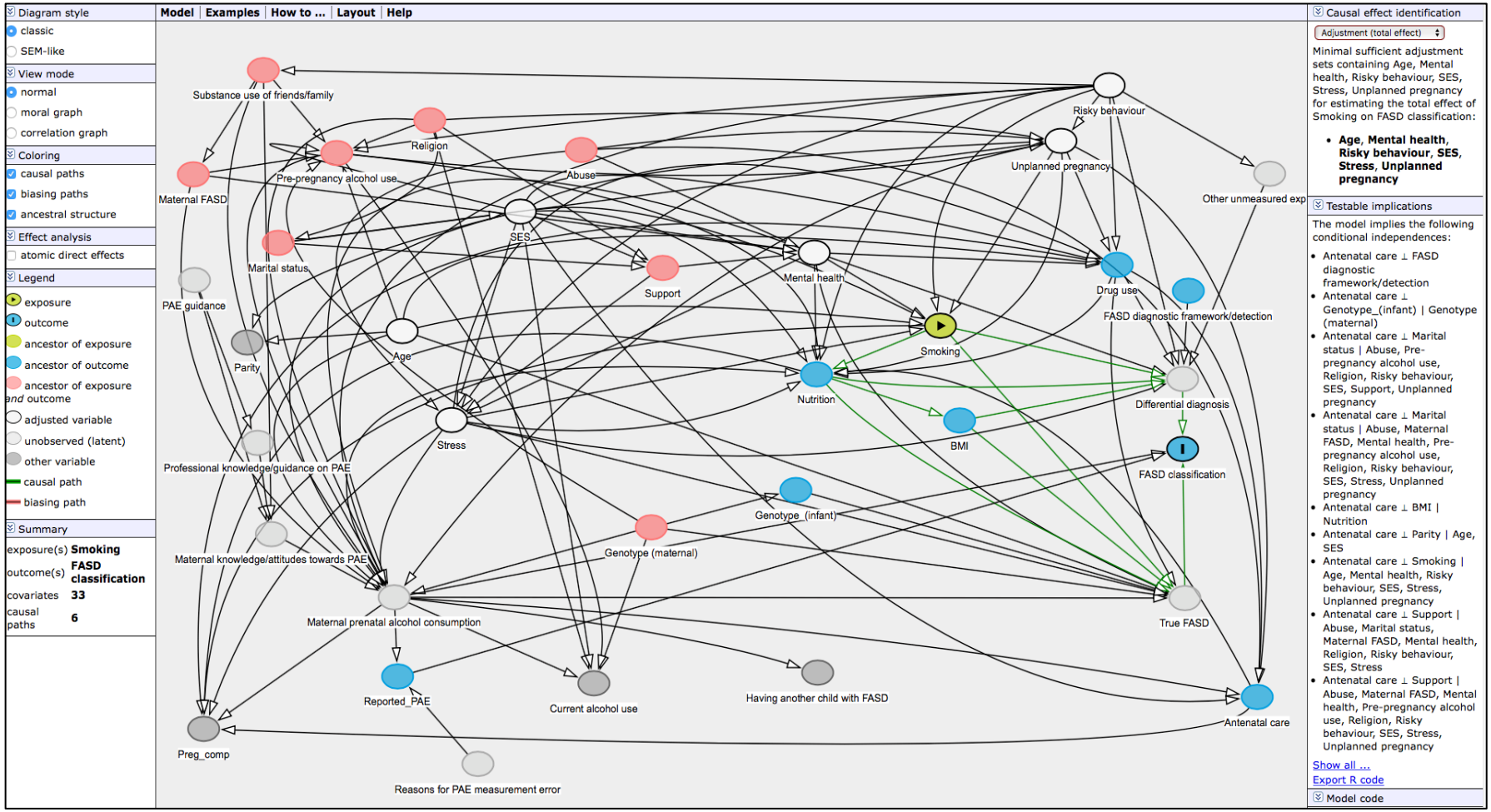
Appendix 15: Causal diagrams for multivariable FASD risk factor analyses (DAGitty output)

## *Prenatal alcohol exposure (PAE) dose/pattern*

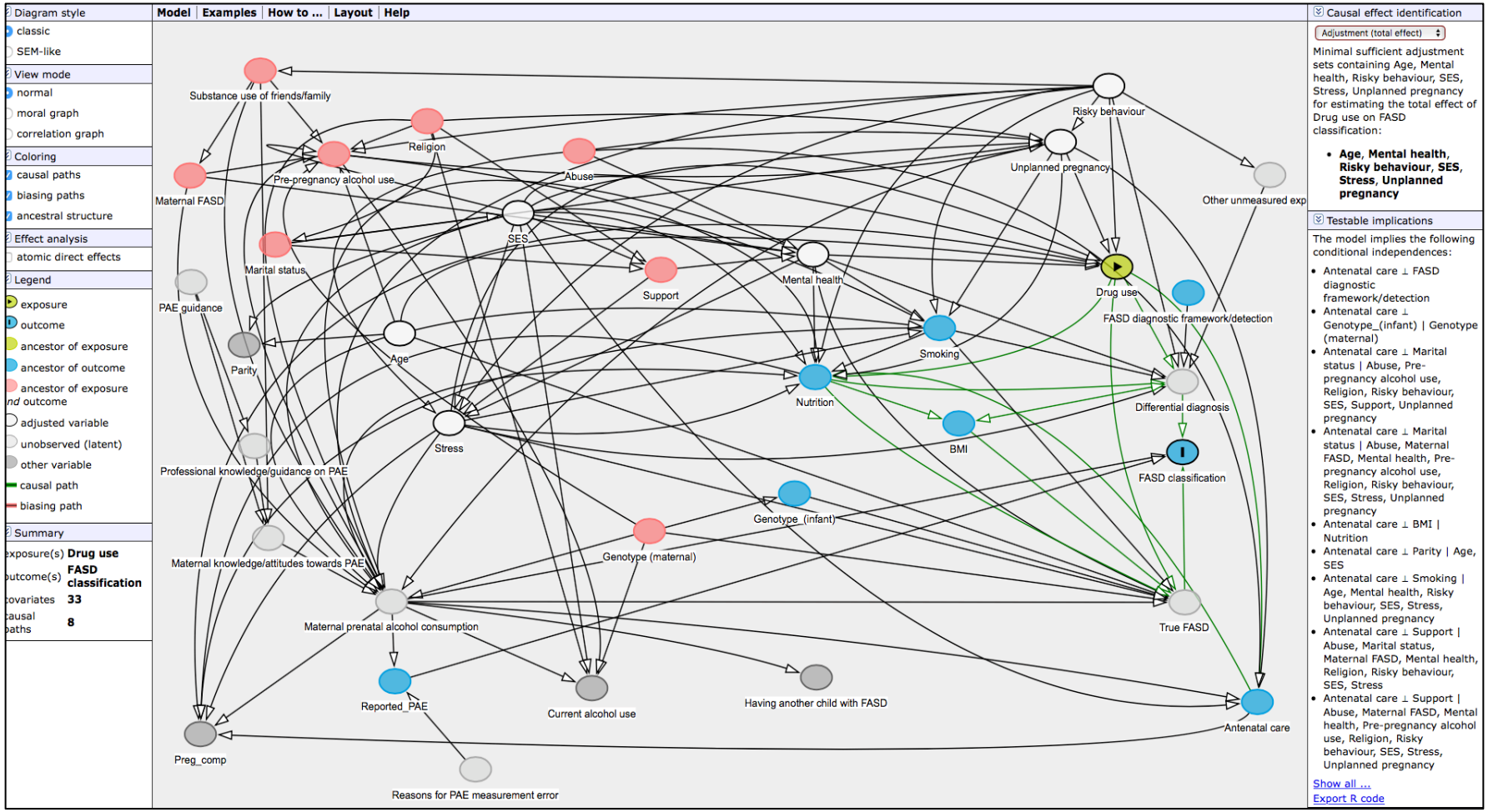
Note: In this DAG, I assumed that true PAE was observed (assumed that it was equivalent to reported PAE) to enable identification of the covariate set.



*Prenatal smoking*

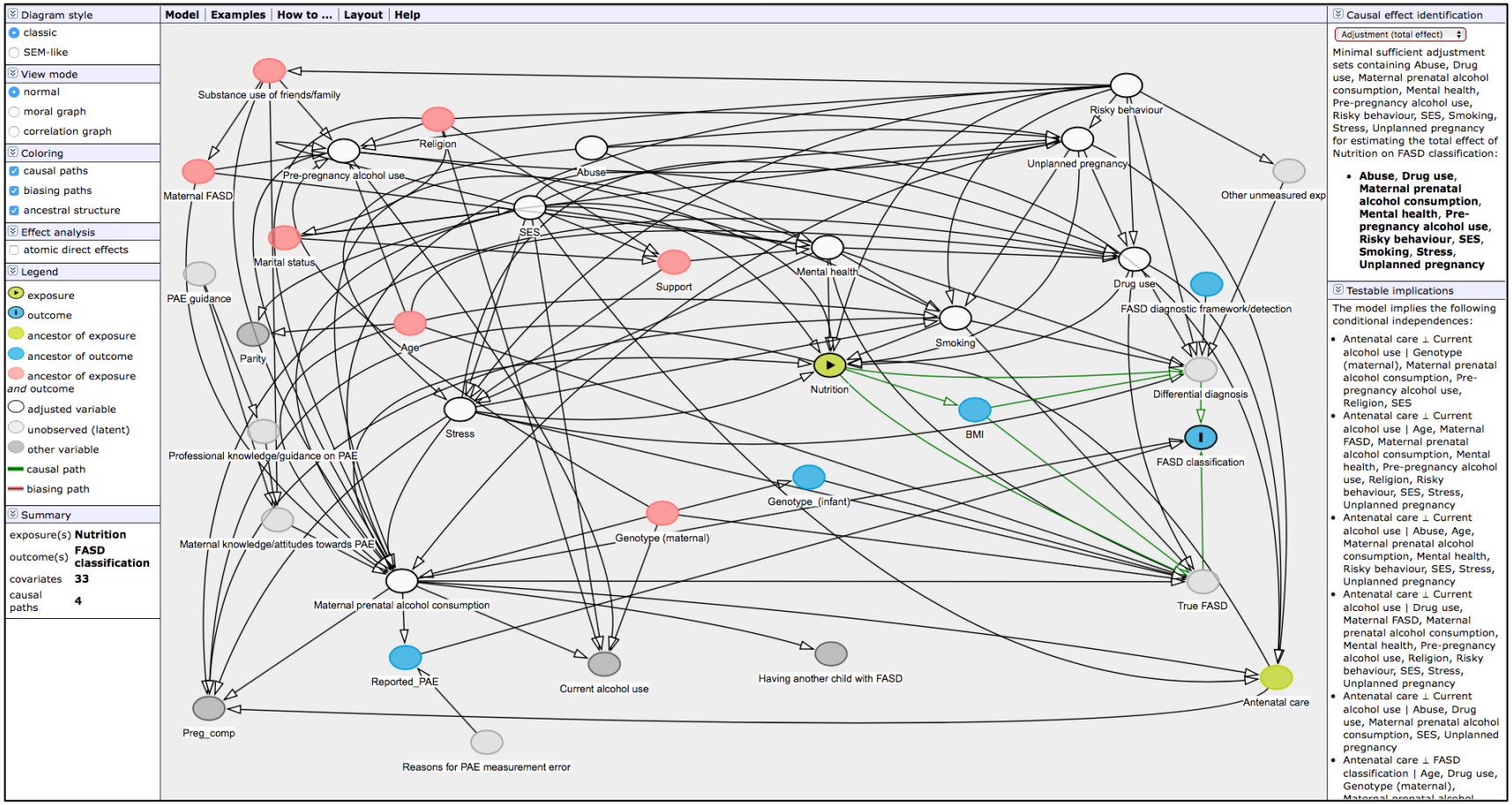


*Prenatal illicit drug use*

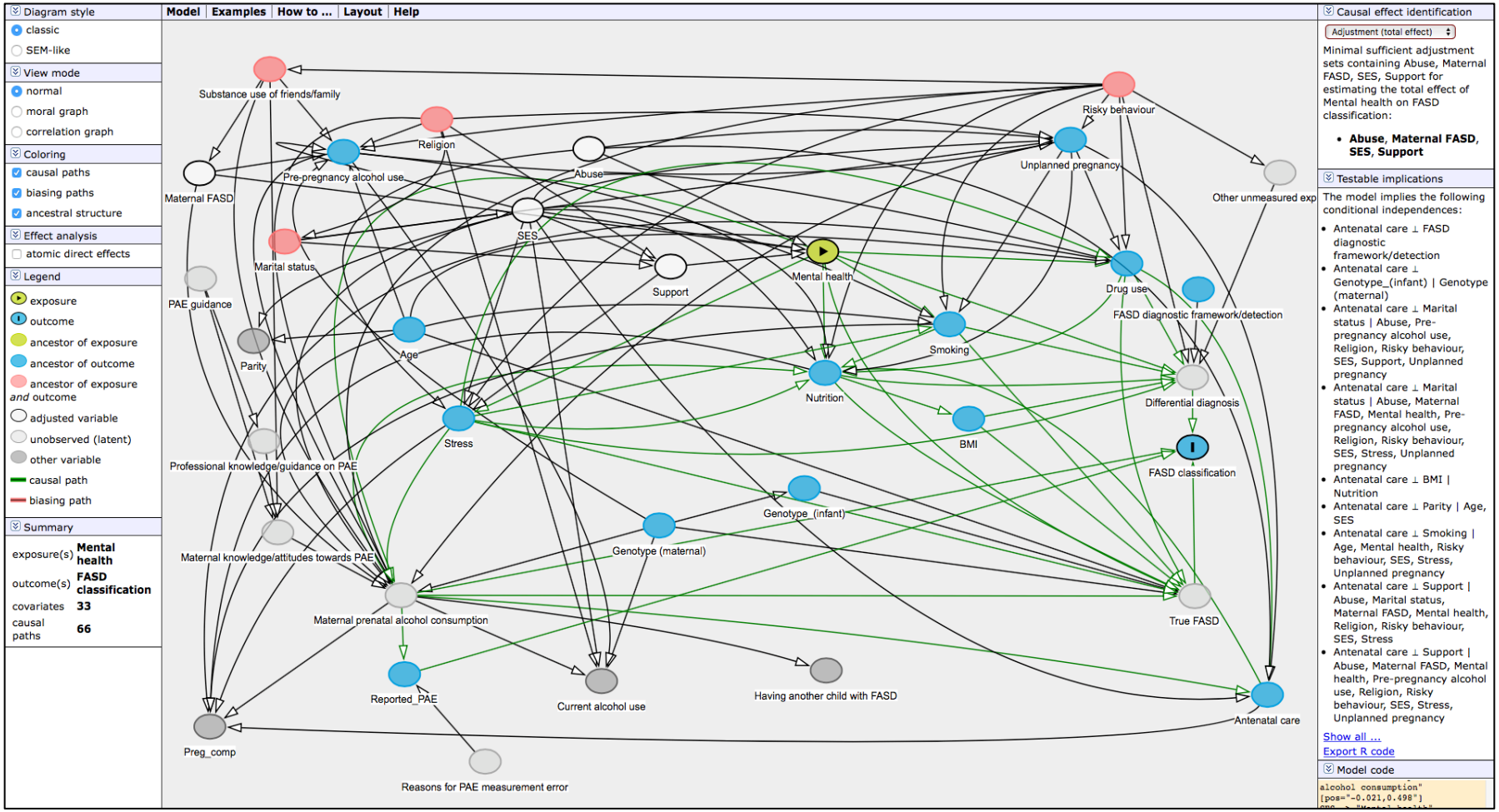


## *Prenatal nutrition*

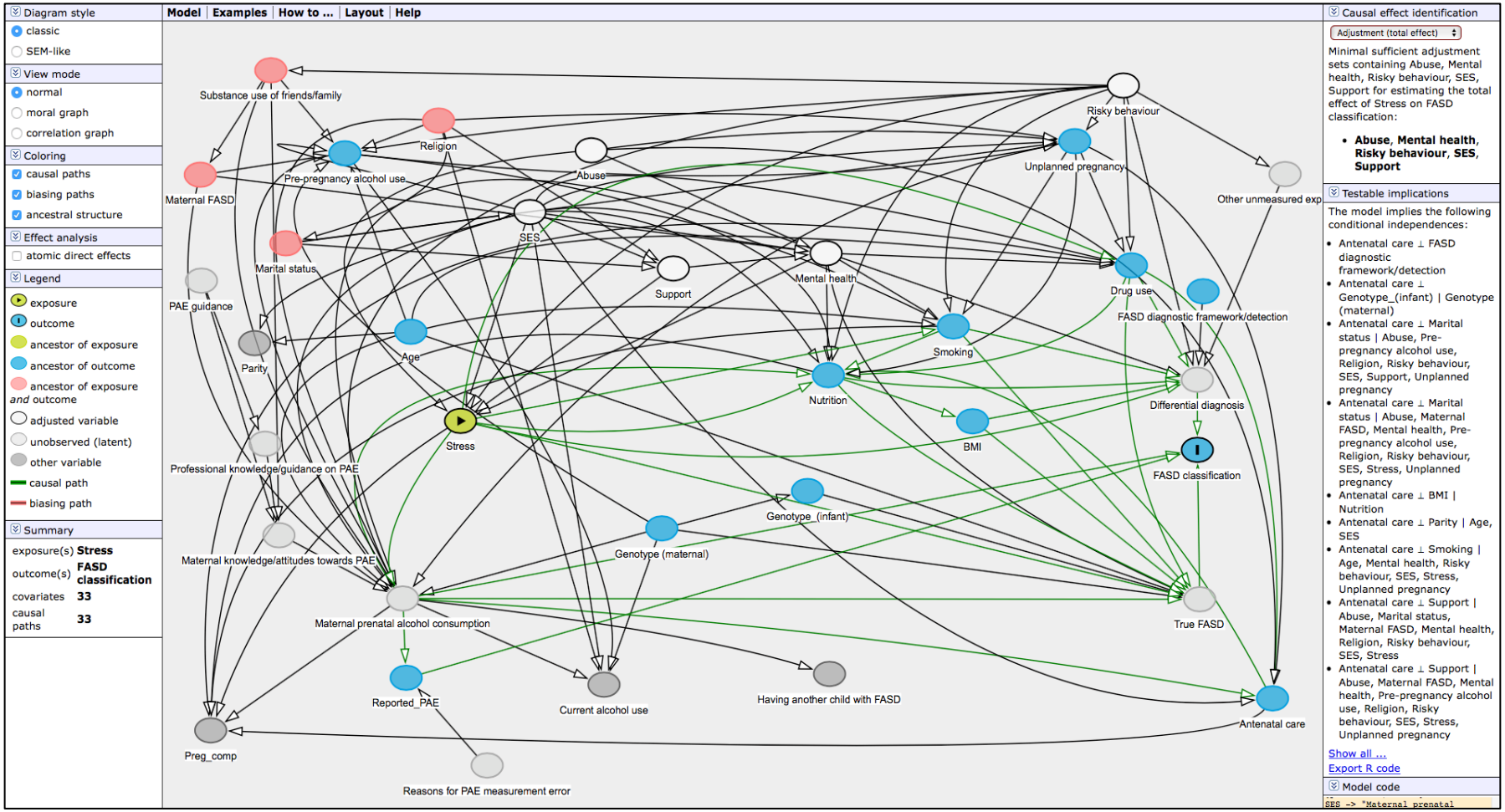
Note: In this this DAG, I assumed that true PAE was observed (assumed that it was equivalent to reported PAE) to enable identification of the covariate set.



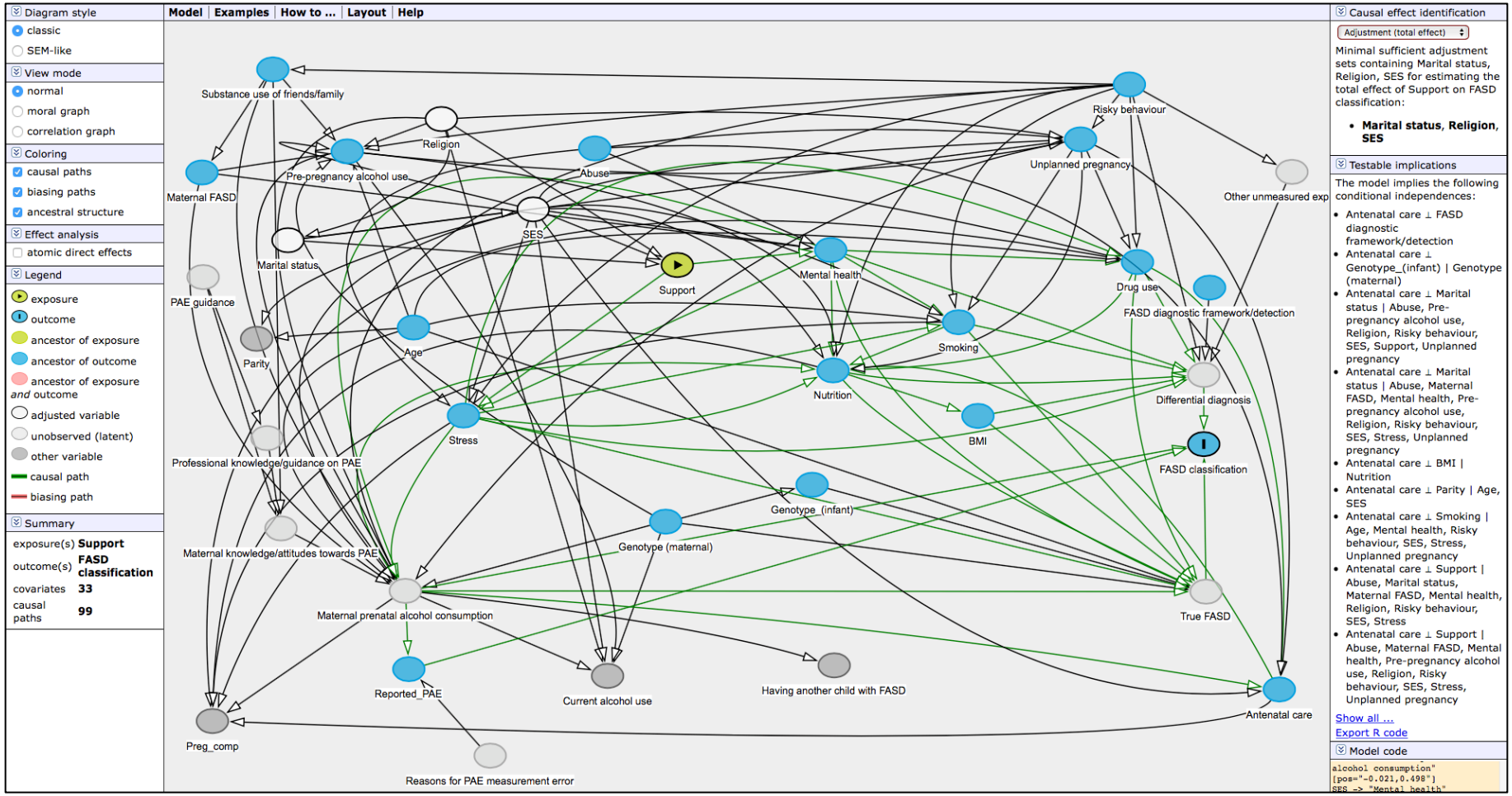
*Prenatal mental health*



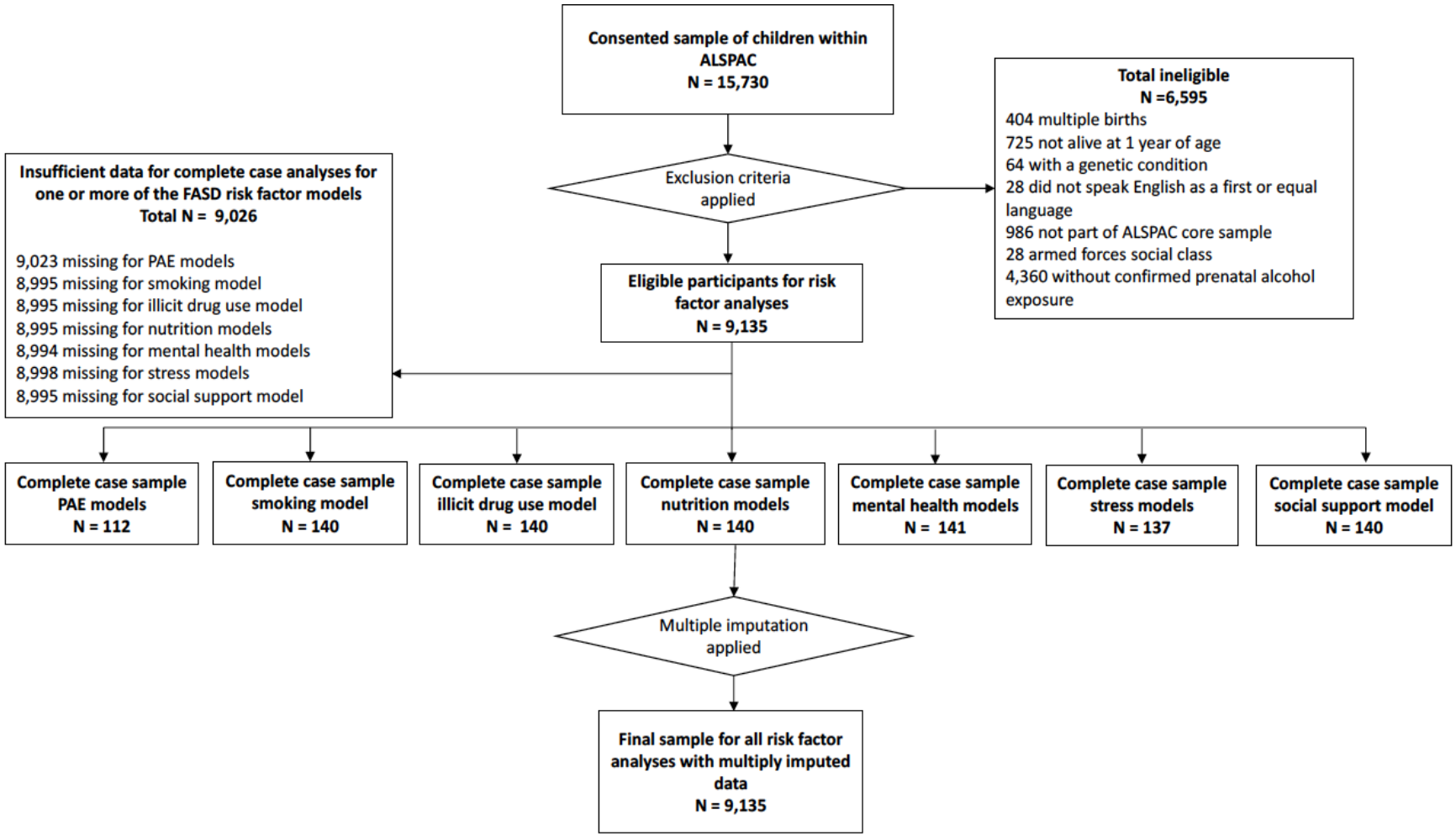
*Prenatal stress*



*Prenatal social support*



Appendix 16: Sample flow diagram showing the number of participants at each stage of the FASD risk factor analyses



Appendix 17: Comparison of the characteristics of participants who had complete versus incomplete data for one or more of the risk factor models prior to multiple imputation

| | Total N = 9,135 N (%)[a] | Included in all risk factor analyses N = 109 (N [%]) | Missing from one or more risk factor analyses[b] N = 9,026 (N [%]) |
|---|---|---|---|
| **Sociodemographic factors** | | | |
| **Maternal age at delivery (years)** | | | |
| <20 | 338 (3.7) | 0 (0.0) | 338 (3.7) |
| 20-29 | 5,055 (55.3) | 53 (48.6) | 5,002 (55.4) |
| 30+ | 3,742 (41.0) | 56 (51.4) | 3,686 (40.8) |
| **Maternal ethnicity** | | | |
| White | 8,261 (98.2) | 109 (100.0) | 8,152 (98.1) |
| Non-White | 152 (1.8) | 0 (0.0) | 152 (1.8) |
| **Marital status** | | | |
| Not married | 2,158 (24.5) | 13 (11.9) | 2,145 (24.7) |
| Married | 6,642 (75.5) | 96 (88.1) | 6,546 (75.3) |
| **Maternal social class** | | | |
| Professional | 453 (6.5) | 4 (3.7) | 449 (6.5) |
| Managerial/technical | 2,354 (33.6) | 46 (42.2) | 2,308 (33.5) |
| Skilled non-manual | 2,924 (41.8) | 49 (45.0) | 2,875 (41.7) |
| Skilled manual | 502 (7.2) | 5 (4.6) | 497 (7.2) |
| Partly skilled/unskilled | 771 (11.0) | 5 (4.6) | 766 (11.1) |
| **Paternal social class** | | | |
| Professional | 892 (11.8) | 23 (21.1) | 869 (11.7) |
| Managerial/technical | 2,680 (35.4) | 40 (36.7) | 2,640 (35.4) |
| Skilled non-manual | 851 (11.2) | 9 (8.3) | 842 (11.3) |
| Skilled manual | 2,250 (29.7) | 32 (29.4) | 2,218 (29.7) |
| Partly skilled/unskilled | 897 (11.9) | 5 (4.6) | 892 (12.0) |
| **Maternal education** | | | |
| CSE | 1,484 (17.6) | 9 (8.3) | 1,475 (17.7) |
| Vocational | 783 (9.3) | 5 (4.6) | 778 (9.3) |
| O Level | 2,894 (34.3) | 38 (34.9) | 2,856 (34.2) |
| A Level | 2,051 (24.3) | 38 (34.9) | 2,013 (24.1) |
| Degree | 1,237 (14.6) | 19 (17.4) | 1,218 (14.6) |
| **Paternal education** | | | |
| CSE | 1,931 (23.8) | 14 (12.8) | 1,917 (23.9) |
| Vocational | 663 (8.2) | 8 (7.3) | 655 (8.2) |
| O Level | 1,703 (21.0) | 27 (24.8) | 1,676 (20.9) |
| A Level | 2,180 (26.8) | 33 (30.3) | 2,147 (26.8) |
| Degree | 1,648 (20.3) | 27 (24.7) | 1,621 (20.2) |
| **Home ownership status** | | | |
| Mortgaged/owned | 6,644 (75.8) | 102 (93.6) | 6,542 (75.6) |
| Council/housing association | 1,199 (13.7) | 3 (2.8) | 1,196 (13.8) |
| Rented (private) | 629 (7.2) | 2 (1.8) | 627 (7.3) |

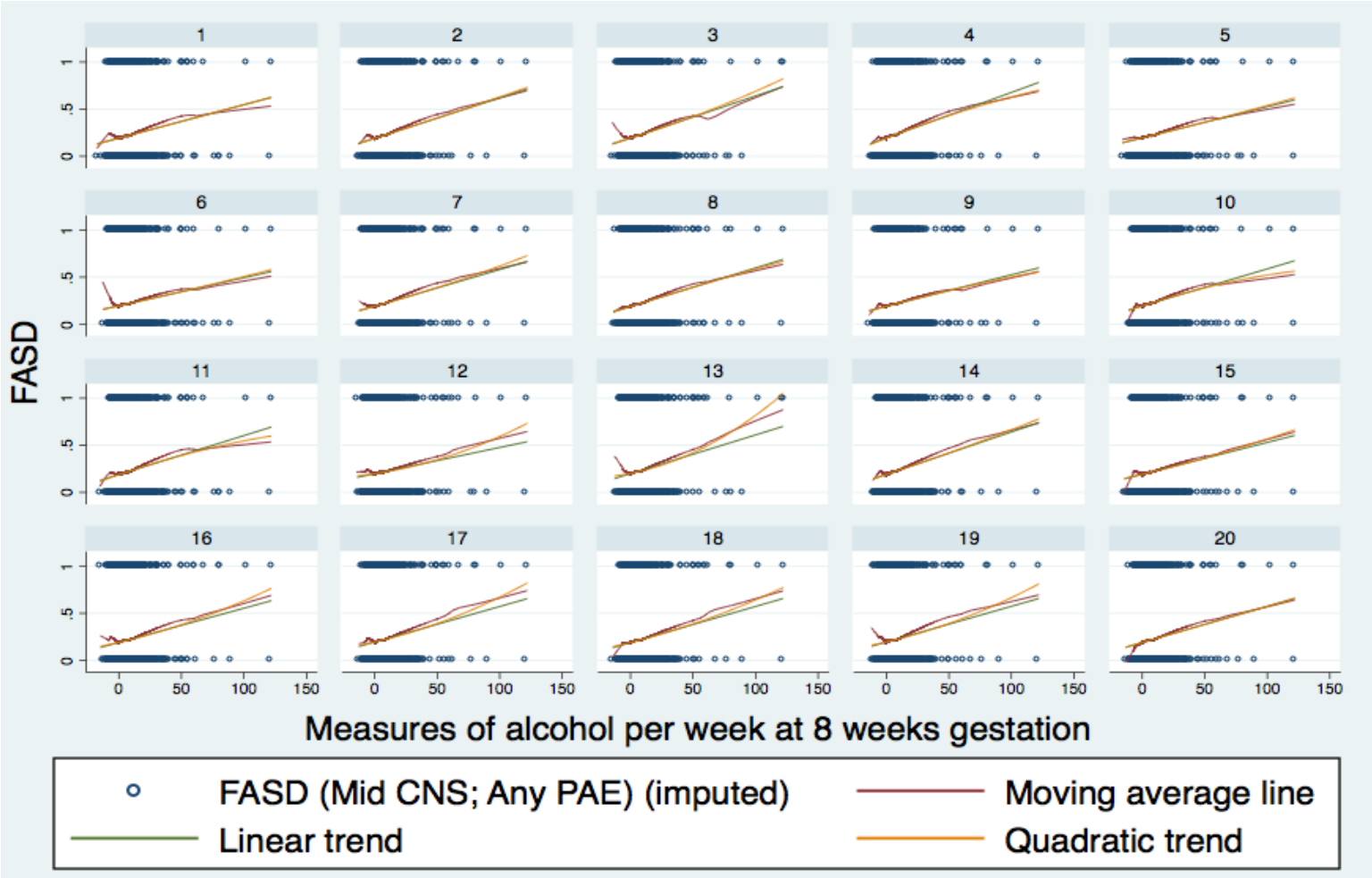
| | Total N = 9,135 N (%)[a] | Included in all risk factor analyses N = 109 (N [%]) | Missing from one or more risk factor analyses[b] N = 9,026 (N [%]) |
|---|---|---|---|
| Other | 291 (3.3) | 2 (1.8) | 289 (3.3) |
| **Exposures** | | | |
| **Prenatal alcohol exposure (max dose/frequency during pregnancy)** | | | |
| None | 269 (3.0) | 1 (0.9) | 268 (3.0) |
| <1 glass per week | 5,480 (60.6) | 69 (63.3) | 5,411 (60.6) |
| 1-6 glasses per week | 2,872 (31.8) | 36 (33.0) | 2,836 (31.7) |
| 7+ glasses per week | 424 (4.7) | 3 (2.8) | 421 (4.7) |
| **Prenatal binge drinking** | | | |
| No | 6,121 (68.3) | 85 (78.0) | 6,036 (68.2) |
| Yes | 2,839 (31.7) | 24 (22.2) | 2,815 (31.8) |
| **Prenatal smoking** | | | |
| No | 6,560 (71.8) | 95 (87.2) | 6,465 (71.6) |
| Yes | 2,573 (28.2) | 14 (12.8) | 2,559 (28.4) |
| **Prenatal illicit drug use** | | | |
| No | 8,674 (96.2) | 107 (98.2) | 8,567 (96.2) |
| Yes | 339 (3.8) | 2 (1.8) | 337 (3.8) |
| **Prenatal calcium supplement use** | | | |
| No | 8,496 (94.3) | 102 (93.6) | 8,394 (94.3) |
| Yes | 517 (5.7) | 7 (6.4) | 510 (5.7) |
| **Prenatal folic acid supplement use** | | | |
| No | 7,256 (80.5) | 85 (78.0) | 7,171 (80.5) |
| Yes | 1,758 (19.5) | 24 (22.0) | 1,734 (19.5) |
| **Prenatal iron supplement use** | | | |
| No | 4,991 (55.4) | 56 (51.4) | 4,935 (55.4) |
| Yes | 4,023 (44.6) | 53 (48.6) | 3,970 (44.6) |
| **Calories (RNI met)** | | | |
| No | 7,450 (90.4) | 101 (92.7) | 7,349 (90.4) |
| Yes | 792 (9.6) | 8 (7.3) | 784 (9.6) |
| **Prenatal stressful life events** | | | |
| Mean (SD) | 7.3 (4.4) | 6.6 (3.8) | 7.3 (4.4) |
| **Weighted prenatal stressful life events** | | | |
| Median (IQR) | 14.0 (15.0) | 13.0 (12.0) | 14.0 (15.0) |
| **Social support score** | | | |
| Mean (SD) | 3.9 (1.0) | 4.2 (0.8) | 3.9 (1.0) |
| **Prenatal anxiety** | | | |
| No | 6,633 (76.1) | 89 (81.7) | 6,544 (76.1) |
| Yes | 2,081 (23.9) | 20 (18.4) | 2,061 (24.0) |
| **Prenatal depression** | | | |
| No | 6,974 (79.5) | 94 (86.2) | 6,880 (79.4) |
| Yes | 1,800 (20.5) | 15 (13.8) | 1,785 (20.6) |
| **Outcome** | | | |
| FASD status | | | |
| Not FASD | 146 (90.1) | 100 (91.7) | 46 (86.8) |
| FASD | 16 (9.9) | 9 (8.3) | 7 (13.2) |

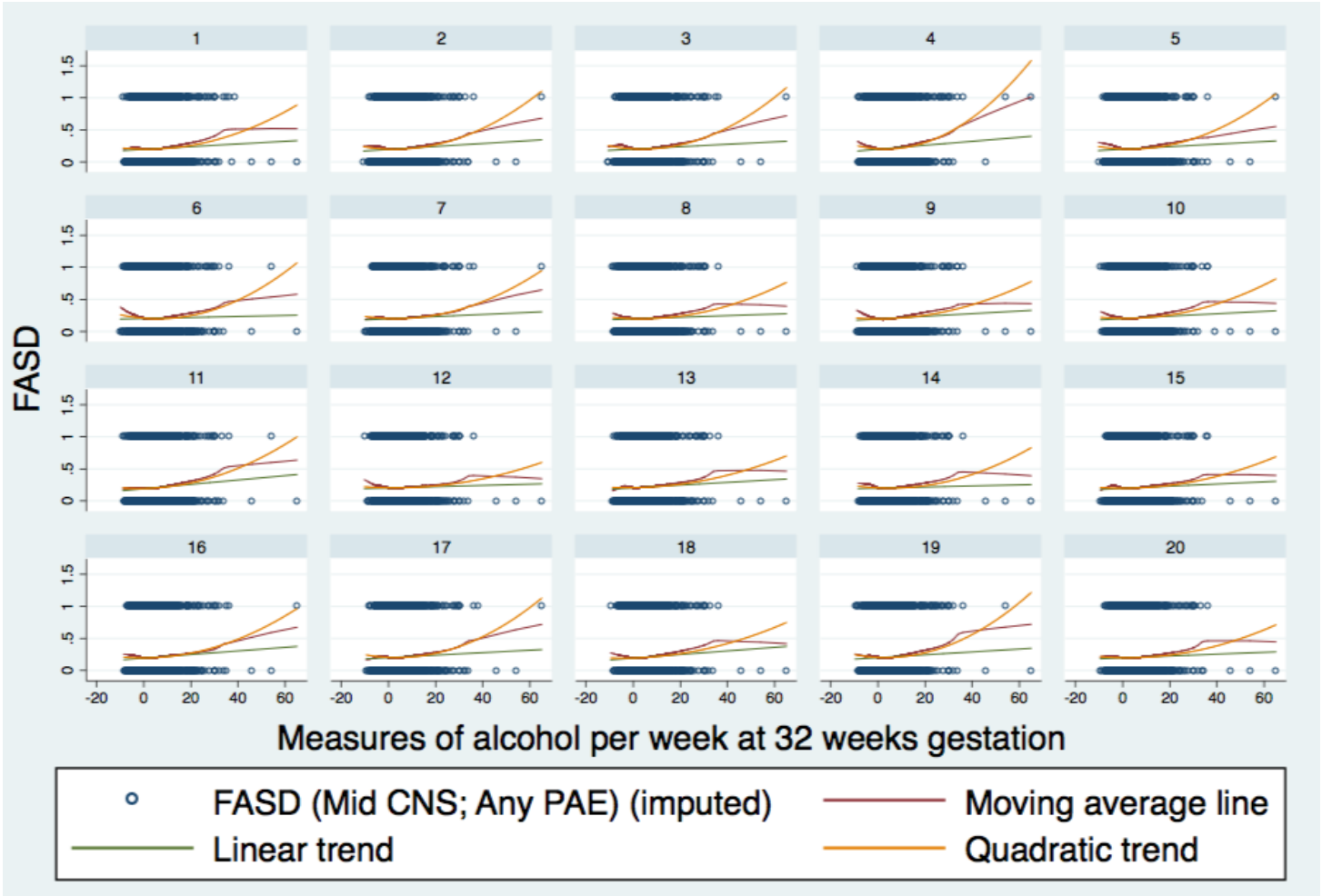
| | Total N = 9,135 N (%)[a] | Included in all risk factor analyses N = 109 (N [%]) | Missing from one or more risk factor analyses[b] N = 9,026 (N [%]) |
|---|---|---|---|
| **Auxiliary variables** | | | |
| **Pregnancy/perinatal complications** | | | |
| No | 6,207 (71.1) | 73 (67.6) | 6,134 (71.2) |
| Yes | 2,520 (28.9) | 35 (32.4) | 2,485 (28.8) |
| **Binge drinking (8 weeks postpartum)** | | | |
| No | 3,102 (53.1) | 71 (65.7) | 3,031 (52.8) |
| Yes | 2,741 (46.9) | 37 (34.3) | 2,704 (47.2) |
| **Postnatal alcohol problems (maternal self-report and AUDIT 5 - 18 years postpartum)** | | | |
| No | 5,405 (77.7) | 74 (67.9) | 5,331 (77.9) |
| Yes | 1,551 (22.3) | 35 (32.1) | 1,516 (22.1) |
| **Parity** | | | |
| 0 | 3,837 (43.7) | 57 (53.3) | 3,780 (43.6) |
| 1 | 3,141 (35.8) | 33 (30.8) | 3,108 (35.8) |
| 2 | 1,305 (14.9) | 11 (10.3) | 1,294 (14.9) |
| >2 | 494 (5.6) | 6 (5.6) | 488 (5.6) |
| **Gestational age at delivery (weeks)** | | | |
| Mean (SD) | 39.5 (1.8) | 39.6 (1.5) | 39.5 (1.8) |
| **Previous miscarriage** | | | |
| 0 | 6,978 (79.2) | 89 (82.4) | 6,889 (79.2) |
| 1 | 1,411 (16.0) | 18 (16.7) | 1,393 (16.0) |
| ≥2 | 418 (4.8) | 1 (0.9) | 417 (4.8) |
| **Ultrasound scan during pregnancy** | | | |
| No | 355 (4.6) | 4 (3.6) | 351 (4.6) |
| Yes | 7,392 (95.4) | 105 (96.3) | 7,287 (95.4) |
| **Maternal BMI (pre-pregnancy)** | | | |
| Underweight | 361 (4.6) | 3 (2.8) | 358 (4.6) |
| Normal | 5,924 (75.4) | 89 (83.2) | 5,835 (75.3) |
| Overweight | 1,180 (15.0) | 14 (13.1) | 1,166 (15.0) |
| Obese | 396 (5.0) | 1 (0.9) | 395 (5.1) |
| **Child sex** | | | |
| Female | 4,433 (48.5) | 65 (40.4) | 4,658 (51.6) |
| Male | 4,702 (51.5) | 44 (59.6) | 4,368 (48.4) |

[a] N varies for each variable due to missing data. Percentages do not always sum to 100 due to rounding.

[b] Missing from one or more of the following risk factor models: prenatal alcohol use pattern, prenatal smoking, prenatal illicit drug use, prenatal nutrition, prenatal stress, prenatal mental health, prenatal social support. Preliminary analyses indicated that missing data patterns were similar for each of the risk factor models.

Appendix 18: Graphs of the association between continuous measures of PAE and the odds of FASD

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16
17
18
19
20
FASD
0
.5
1
1.5
-20
0
20
40
60
Measures of alcohol per week at 32 weeks gestation
FASD (Mid CNS; Any PAE) (imputed)
Moving average line
Linear trend
Quadratic trend

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