Prenatal alcohol exposure and facial morphology in a UK cohort

Laurence J Howe,[1,3] Gemma C Sharp,[1,4] Gibran Hemani,[1] Luisa Zuccolo,[1] Stephen Richmond,[2] Sarah J Lewis,[1,4]

[1] MRC Integrative Epidemiology Unit, Population Health Sciences, Oakfield House, Oakfield Grove, University of Bristol, BS8 2BN, UK

[2] Department of Applied Clinical Research and Public Health, School of Dentistry, Cardiff, UK

[3] Institute of Cardiovascular Science, University College London, UK

[4] Bristol Dental School, University of Bristol, UK

Declarations of interest: none

Supplementary material can be found by accessing the online version of this paper at

Abstract

Background: High levels of prenatal alcohol exposure are known to cause an array of adverse outcomes including foetal alcohol syndrome (FAS); however, the effects of low to moderate exposure are less-well characterised. Previous findings suggest that differences in normal-range facial morphology may be a marker for alcohol exposure and related adverse effects.

Methods: In the Avon Longitudinal Study of Parents and Children, we tested for an association between maternal alcohol consumption and six FAS-related facial phenotypes in their offspring, using both self-report questionnaires and the maternal genotype at rs1229984 in *ADH1B* as measures of maternal alcohol consumption.

Results: In both self-reported alcohol consumption (N=4,233) and rs1229984 genotype (N=3,139) analyses, we found no strong statistical evidence for an association between maternal alcohol consumption and facial phenotypes tested. The directions of effect estimates were compatible with the known effects of heavy alcohol exposure, but confidence intervals were largely centred around zero.

Conclusions: There is no strong evidence, in a sample representative of the general population, for an effect of prenatal alcohol exposure on normal-range variation in facial morphology.

Mendelian randomization; Facial morphology; Alcohol; ALSPAC

1. Introduction

Foetal alcohol spectrum disorders (FASD) refer to a broad range of outcomes relating to prenatal alcohol exposure (Riley et al., 2011). The most extreme phenotype, foetal alcohol syndrome (FAS), was first observed in case-studies of offspring of mothers with clinical alcoholism and is characterised by patterns of extreme foetal malformations such as growth retardation, cognitive issues and craniofacial anomalies (Jones and Smith, 1973).

The potential teratogenic effects of high levels of alcohol exposure are supported by a murine study which demonstrated that alcohol exposure from the human equivalent of 6-months gestation can trigger neurodegeneration of the developing brain (Ikonomidou et al., 2000). However, the impact of lower levels of maternal alcohol consumption is less clear. Individuals exposed to low to moderate levels of alcohol do not typically present with facial, anthropometric or cognitive abnormalities, and there is inconsistent evidence from population studies for an association between maternal alcohol consumption and adverse outcomes in the offspring. Some studies have reported no clear or inconsistent evidence for adverse effects of low to moderate alcohol exposure (Flak et al., 2014) (McCormack et al., 2018) (Kelly et al., 2013) (Mamluk et al., 2017), while other studies have reported evidence that low to moderate alcohol exposure may have modest adverse effects (Lewis et al., 2012; Zuccolo et al., 2013), with a review concluding that lower levels of alcohol exposure may contribute to adverse cognitive outcomes in children (Huizink and Mulder, 2006).

The diagnosis of FASD relates to the presentation of various developmental anomalies, including: stunted anthropometric growth, structural brain anomalies and minor facial anomalies such as a smooth philtrum, a thin upper lip vermillion and shortened palpebral fissures. These dysmorphic patterns can lead to increased risk of adverse social experiences for children with FASD, who may be more likely to present with learning

difficulties and behavioural problems (Hoyme et al., 2005). Traditionally, facial morphology has been phenotyped using facial landmarks, taken either directly from the face or derived from photographs or radiographs. These landmarks are defined by identifiable/describable facial features, e.g. nasion, inner/outer canthi, and can be used to generate Euclidean distances, angles, and ratios (Farkas, et al. 2004; Farkas, et al. 2005; Farkas, et al. 2002). Multiple facial landmarks can be used to generate principal components, geodesic distances, geodesic arrays, facial shells and signatures that categorise facial feature patterns (Abbas, et al. 2018; Hallgrimsson, et al. 2015; Hammond and Suttie 2012; Tsagkrasoulis, et al. 2017). An alternative method involves using anthropometric masks where five landmarks are used to crudely orientate 3D facial shells, which are then non-rigidly mapped on to a template to generate 10,000 quasi landmarks (Claes, et al. 2012). All techniques are valid however simple facial landmarks (for distances and angles) can be used as a proxies to highlight subtle and significant patterns of facial dysmorphia.

FAS-related facial features have been shown to correlate with the severity of structural brain abnormalities and behavioural problems (Riley et al., 2011) (Astley and Clarren, 2001), suggesting that facial morphology may be a marker of alcohol related cognitive impairments. This is supported by a previous study which found that children exposed to alcohol, that have more FAS like facial symptoms, perform worse on psychometric tests relating to verbal IQ and learning (Suttie et al., 2013). It follows that an association between low levels of alcohol exposure and differential facial morphology would support the hypothesis that low levels of alcohol have adverse effects. Indeed, a previous study found evidence that low levels of prenatal alcohol exposure are associated with facial differences in infants aged around 12 months (Muggli et al., 2017).

However, epidemiological studies investigating the effects of self-reported alcohol intake on health outcomes are problematic because alcohol consumption is often correlated with potential confounders such as tobacco smoking, age and socio-economic status. For example, maternal alcohol consumption during pregnancy has been shown to be previously associated with smoking and demographic variables in a UK cohort (Alati et al., 2013). Observational studies have often reported U- or J- shaped curves where moderate alcohol intake can be associated with improved mortality or morbidity (Alati et al., 2005; Marmot and Brunner, 1991; Marmot et al., 1981) but follow-up investigations have suggested that these results may be attributable to residual confounding (Alati et al., 2005; Holmes et al., 2014; Shaper et al., 1988).

Mendelian randomization (MR) is an instrumental variable approach using genetic variants associated with an exposure to explore possible causal relationships between that exposure and an outcome. The underlying premise is that, assuming a random mode of inheritance, genetic variants associated with the exposure are less likely to be associated with confounders than the measured exposure itself, while because genotype is fixed from birth, MR analyses are robust to reverse-causation. MR analyses generating unbiased causal estimates relies on several assumptions; first, that variants used in MR analyses are robustly associated with the exposure, second, that variants do not influence the outcome independently of the exposure, and third, that variants are not associated with confounders of the exposure-outcome relationship (Davey Smith and Ebrahim, 2003; Haycock et al., 2016). The Alcohol Dehydrogenase (*ADH*) genes are a family of genes known to be involved in the production of enzymes that oxidise alcohol (Thomasson et al., 1991). rs1229984 in *ADH1B* is involved in the metabolism of alcohol to acetaldehyde, individuals with one or more risk alleles are more likely to find drinking unpleasant (Luczak et al., 2006). Increased sensitivity to alcohol intake related to this genetic variant has been shown to lead to modified alcohol

intake, including alcohol intake in pregnancy (Zuccolo et al., 2009). Although rs1229984 is common in Asian populations (Eng et al., 2007), it is relatively rare in European populations with a minor allele frequency of 2 to 5% (Zuccolo et al., 2009). Despite this, previous MR studies have successfully utilised this variant as a proxy for alcohol intake in European populations as it has a sizeable effect on alcohol consumption, one or more minor alleles is associated with a 17.2% reduction in weekly alcohol consumption (Holmes et al., 2014; Lawlor et al., 2013; Zuccolo et al., 2013).

In this study, we first investigated whether self-reported low to moderate maternal alcohol intake is associated with facial morphology in the Avon Longitudinal Study of Parents and Children (ALSPAC). Secondly, we used genetic variation in *ADH1B* in an MR framework to estimate the effect of low to moderate maternal alcohol exposure on normal-range facial morphology.

2. Methods

2.1 Study participants

We used data on children from the ALSPAC a longitudinal study that recruited pregnant women living in the former county of Avon (UK) with expected delivery dates between 1 April 1991 and 31 December 1992. The initial number of enrolled pregnancies was 14,541, which resulted in 14,062 live births and 13,988 children alive at the age of 1. When the oldest children were approximately 7 years of age, the initial sample was boosted with eligible cases who had failed to join the study originally. For analyses of children after the age of 7, the total possible sample size is 15,247 pregnancies, resulting in 14,775 live births. Full details of enrolment have been documented elsewhere (Boyd et al., 2012; Golding, 2001). Data was collected from mothers and their partners (during pregnancy and post birth) and from the children (post birth), by self-report questionnaires and clinical

sessions. Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee. The study website contains details of all available data through a searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/dataaccess/datadictionary/).

2.2 Measures

2.2.1 Facial phenotypes of ALSPAC children

A subset of ALSPAC, consisting of 5,253 children, attended a clinic at the age of 15 years, where high-resolution facial images were taken by Konica Minolta Vivid 900 laser scanners. 4,747 individuals had usable images (506 individuals did not complete the assessment, or the scans were of poor quality and consequently excluded). The derivation of the facial phenotypes are described in more detail in a previous publication (Paternoster et al., 2012).

The coordinates of 22 facial landmarks were derived using the scans. In this study, facial phenotypes were defined as 3D Euclidean distances between derived facial landmarks, i.e. the distance between two points in three dimensions. The 3D Euclidean distance between points a and b with coordinates (x, y, z) was calculated as follows: $\sqrt{(a_x - b_x)^2 + (a_y - b_y)^2 + (a_z - b_z)^2}$. To alleviate multiple testing issues, this study tested 6 facial phenotypes known to be related to the FAS spectrum: average eye palpebral length, average eye palpebral width, inter-orbital width, nasal length, lip width and philtrum width (**Figure 1**). The relevance of these distances to FAS have been described previously (Astley and Clarren, 2001).

2.2.2 ALSPAC mothers self-reported alcohol consumption

At around 18 and 32 weeks gestation, ALSPAC mothers completed questionnaires on the average amount and frequency of their alcohol consumption during pregnancy. Mothers were asked questions regarding the number of alcoholic drinks consumed at different stages of their pregnancy and the number of binges (defined as drinking four or more units of alcohol in a day) in the past month. The results of these self-report questionnaires were combined to estimate each mother's weekly alcohol consumption in units (one alcohol unit is equivalent to approximately 8g of ethanol) and classify mothers into three categories (non-drinkers during pregnancy, $\leq$6 units a week and $>$6 units a week). Further details on questionnaire variables and derivation of classifications are contained in **Supplementary Table 1** [1].

2.2.3 Measurement of potential confounders

Maternal and child data pertaining to covariates included in analyses were measured within the ALSPAC study from questionnaires and clinic sessions. Maternal age at delivery was calculated using the difference between the mother and child's dates of birth and gestational age was recorded at birth. Information on maternal smoking was extracted from a questionnaire completed by the mother at 18 weeks gestation; mothers who reported any form of smoking in the first 3 months of pregnancy or in the preceding 2 weeks to the questionnaire were classified as smokers while the non-smokers category included smokers who gave up for pregnancy. Information on maternal education was extracted from a questionnaire completed by the mother at 32 weeks gestation; mothers were asked for their education qualifications and the highest qualification was derived. The height and age of children with available facial scans were recorded at the clinic session.

[1] Supplementary material can be found by accessing the online version of this paper at

2.2.4 ALSPAC mother's ADH1B genotype

The *ADH1B* polymorphism rs1229984 was genotyped by KBioscience using the KASPar chemistry (http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm). Blind duplicates, plate-identifying repeat samples and Hardy–Weinberg equilibrium tests were used as quality control checks (Zuccolo et al., 2009). The SNP had a minor allele frequency of 2.1% in the sample of mothers with complete offspring phenotype data.

2.3 Statistical analysis

2.3.1 Observational analysis

Firstly, to test the association between self-reported maternal alcohol consumption and child facial morphology, we dichotomised mothers into non-drinkers (0 units a week) and mothers reporting alcohol intake (1-6 units a week or >6 units a week). We ran a linear regression of the facial morphology variables on drinking status; adjusting for sex, maternal age, maternal education, maternal smoking and the height and age of the child at the face-shape measurement clinic.

Next, we stratified mothers reporting alcohol intake by alcohol consumption (1-6 units a week and >6 units a week). We re-ran the same analysis, testing differences between the non-drinkers and the two strata separately.

2.3.2 Mendelian randomization analysis

For purposes of this analysis, we used the maternal genotype at the SNP rs1229984 in *ADH1B* as a proxy for self-reported maternal intake. To test assumptions about the association of rs1229984 with other traits, we used the GeneATLAS (http://geneatlas.roslin.ed.ac.uk/) (Canela-Xandri et al., 2017), a data-base of associations between genetic variants and phenotypes in the UK Biobank (Sudlow et al., 2015). For

continuous traits, we presented effect sizes and p-values. For categorical traits, where effect sizes are less interpretable, we presented p-values and direction of effect.

We then ran a linear regression of the child's facial morphology variables on the maternal *ADH1B* SNP, adjusting for child's sex, the first 10 genetic principal components of the mothers, and the height and age of the child at the face-shape measurement clinic. Due to the rarity of the homozygous rare genotype in our modest sample size, we assumed a dominant effect of the rare allele.

3. Results

3.1 Study sample demographics

3.1.1 Observational analysis

Facial phenotype data were available for 4,747 ALSPAC children. We then restricted the sample to maternal-child pairs with complete phenotype data, including; maternal alcohol behaviour during pregnancy, gestational age, maternal age, maternal education, maternal smoking during pregnancy and information on the height and age of the child at the time of the facial scans. The final sample consisted of 4,233 children-mother pairs. More information on the demographics of this sample are contained in **Table 1**.

3.1.2 Mendelian randomization analysis

Again, starting with the 4,747 ALSPAC children with facial phenotype data, we selected maternal-child pairs with the maternal *ADH1B* SNP genotyped and information on the height and age of the child at the time of the facial scans. The final sample consisted of 3,139 child-mother pairs. More information on this sample is contained in **Table 1**.

3.2 Observational analysis

We did not find strong statistical evidence for an association between self-reported maternal alcohol consumption and the 6 facial phenotypes tested, although the directions of effect in our results were compatible with the symptoms of FAS for facial phenotypes tested (**Table 2**). In the stratified analysis, effect sizes of higher magnitude were observed in the >6 units a week for 5 out of 6 phenotypes, suggestive of a possible dose-response relationship although wide confidence intervals prevent stronger conclusions. However, in the stratified analysis we also did not find strong evidence of an association between alcohol exposure and facial morphology (**Supplementary Table 2** [2]).

3.3 Mendelian randomization analysis

3.3.1 Maternal ADH1B SNP, alcohol behaviour and pleiotropy

We confirmed that the *ADH1B* SNP was strongly predictive of alcohol behaviour in our sample; one or more of the rarer A alleles of rs1229984 was associated with reduced odds of reported maternal drinking relative to no drinking OR: 0.54 (95% C.I. 0.38, 0.75; $P < 0.001$). While self-reported maternal drinking was associated with all four potential confounders (maternal education, smoking, gestational age at delivery and maternal age), there was weak evidence for an association between these variables and the *ADH1B* SNP (**Supplementary Table 3** [2]).

[2] Supplementary material can be found by accessing the online version of this paper at

In the GeneATLAS (which used an additive model), the rare A allele was strongly associated with a categorical variable for reduced alcohol intake frequency ($P = 1.59 \times 10^{-148}$). In this much larger sample, there was strong evidence that the SNP is associated with non-alcohol related traits such as variables relating to socio-economic status. Of note, the rare A allele was strongly associated with reduced deprivation on the Townsend deprivation index at recruitment ($P = 6.96 \times 10^{-8}$, each A allele was associated with a 0.097 decrease) and reduced number of vehicles in household ($P=1.9 \times 10^{-10}$). Furthermore, there was evidence of an association with measures of adiposity such as body mass index ($P = 2.65 \times 10^{-11}$, each A allele associated with a 0.17 decrease). Information on the associations between rs1299884 and over 700 phenotypes is publicly available at the following web address: (http://geneatlas.roslin.ed.ac.uk/phewas/?variant=rs1229984&representation=table).

3.3.2 Maternal ADH1B genotype and child's facial morphology

We then tested for association between the maternal *ADH1B* SNP and 6 facial phenotypes. We found no strong statistical evidence that the maternal *ADH1B* SNP was associated with the 6 facial phenotypes, but we did find that the directions of effect were consistent with the symptoms of FAS, as in the observational analysis (**Table 2**).

4. Discussion

In this study, we used self-reported maternal alcohol intake and the maternal genotype for rs1229984 in *ADH1B* to test the hypothesis that alcohol exposure has a detectable effect on normal-range facial variation in the general population. In both observational and MR analyses, we found no clear evidence for an effect. The directions of effect were compatible with the known effects of heavy alcohol exposure, but confidence intervals were wide. The implication of these results is that there is no strong evidence for an effect of alcohol exposure on the FAS-related facial phenotypes tested in our study sample.

Our findings are consistent with the well-characterised difficulties of identifying the effects of prenatal alcohol exposure (Suttie et al., 2013) as well as with the findings of previous studies that found no clear evidence of an association between prenatal alcohol exposure and adverse effects (Flak et al., 2014; Kelly et al., 2013; Mamluk et al., 2017; McCormack et al., 2018). Contrastingly, a previous investigation by Muggli and colleagues found evidence of sub-clinical facial differences at 12 months between offspring of non-drinking mothers and offspring of light to moderate drinking mothers (Muggli et al., 2017). The discordance in results may be explained by differences in the timing and complexity of facial morphology measurements used in both studies; the study by Muggli and colleagues measured facial morphology at age 12 months using a 3-D surface registration algorithm consisting of 69,587 points, whereas our study measured facial morphology using six Euclidean distances at age 15. Poor statistical power is unlikely to explain the lack of replication, because the sample size in our study was more than 10 times that of the study by Muggli and colleagues.

A considerable strength of this study is the use of triangulation of methods (Lawlor et al., 2016); using both self-reported alcohol intake and the *ADH1B* SNP as measures of alcohol exposure and finding concordant results between the two methods. A further advantage is the substantially large sample size used in our analyses, compared to similar previous studies (Astley et al., 1992; Das et al., 2004; Klingenberg et al., 2010; Muggli et al., 2017). However, although our MR analysis included over 3000 mother-child pairs, it may still lack statistical power because of the low frequency of the rs1229984 minor allele. A further limitation of the study is that both the observational and MR analyses may be susceptible to confounding. In theory, MR analyses are less affected by confounding but there is some evidence that the *ADH1B* SNP may be associated with potential confounders such as social class and education (Holmes et al., 2014). It is unclear if these associations relate to downstream effects of alcohol consumption of are related to non-random mating on alcohol consumption. Our use of relatively simple facial phenotypes compared to a previous study (Muggli et al., 2017), that constructed phenotypes from thousands of facial landmarks, may also be a limitation. More detailed facial phenotyping may better capture surface topography and overall facial shape, although it is worth noting that some of the phenotypes tested in this study are included in the latest diagnostic criteria for FAS (Hoyme et al., 2016). A further limitation is that when estimating the effect of maternal genotypes using MR, the child's genotypes may be a confounder if they also affect the phenotype of interest. In this instance, although unlikely, there may be an effect of offspring's alcohol consumption on facial morphology rather than an effect of prenatal exposure (Lawlor et al., 2017). Finally, a previous study has highlighted possible selection and loss to follow-up issues with respect to participation in ALSPAC, which may have affected our analyses (Taylor et al., 2018).

To conclude, in a large sample size of children, we found no strong evidence for an association between maternal alcohol consumption and facial morphology of their offspring. A lack of statistical power in the MR analysis limits the interpretation of the genetic analysis. Currently genetic approaches in the prenatal alcohol exposure area have primarily used ALSPAC, so replication in other cohorts and future meta-analyses of MR studies could allow more definitive conclusions to be made using genetic evidence. The absence of strong evidence for an effect in our well-powered observational analysis suggest that any effect, if one exists, is likely to be small. Further work in ALSPAC could utilise more complex facial phenotyping software to better identify fine-scale facial-structure differences (Claes et al., 2018; Muggli et al., 2017).

REFERENCES

Alati, R., Lawlor, D.A., Najman, J.M., Williams, G.M., Bor, W., O'callaghan, M., 2005. Is there really a 'J-shaped'curve in the association between alcohol consumption and symptoms of depression and anxiety? Findings from the Mater-University Study of Pregnancy and its outcomes. Addiction 100(5), 643-651.

Alati, R., Smith, G.D., Lewis, S.J., Sayal, K., Draper, E.S., Golding, J., Fraser, R., Gray, R., 2013. Effect of prenatal alcohol exposure on childhood academic outcomes: contrasting maternal and paternal associations in the ALSPAC study. PLoS One 8(10), e74844.

Astley, S.J., Clarren, S.K., 2001. Measuring the facial phenotype of individuals with prenatal alcohol exposure: correlations with brain dysfunction. Alcohol and Alcoholism 36(2), 147-159.

Astley, S.J., Clarren, S.K., Little, R.E., Sampson, P.D., Daling, J.R., 1992. Analysis of facial shape in children gestationally exposed to marijuana, alcohol, and/or cocaine. Pediatrics 89(1), 67-77.

Boyd, A., Golding, J., Macleod, J., Lawlor, D.A., Fraser, A., Henderson, J., Molloy, L., Ness, A., Ring, S., Smith, G.D., 2012. Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. International Journal of Epidemiology, 111-127.

Canela-Xandri, O., Rawlik, K., Tenesa, A., 2017. An atlas of genetic associations in UK Biobank. bioRxiv, 176834.

Claes, P., Roosenboom, J., White, J.D., Swigut, T., Sero, D., Li, J., Lee, M.K., Zaidi, A., Mattern, B.C., Liebowitz, C., 2018. Genome-wide mapping of global-to-local genetic effects on human facial shape. Nature Genetics, 1.

Das, U.G., Cronk, C.E., Martier, S.S., Simpson, P.M., McCarver, D.G., 2004. Alcohol dehydrogenase 2* 3 affects alterations in offspring facial morphology associated with maternal ethanol intake in pregnancy. Alcoholism: Clinical and Experimental Research 28(10), 1598-1606.

Davey Smith, G., Ebrahim, S., 2003. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? International Journal of Epidemiology 32(1), 1-22.
Eng, M.Y., Luczak, S.E., Wall, T.L., 2007. ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. Alcohol Research & Health 30(1), 22.
Flak, A.L., Su, S., Bertrand, J., Denny, C.H., Kesmodel, U.S., Cogswell, M.E., 2014. The association of mild, moderate, and binge prenatal alcohol exposure and child neuropsychological outcomes: a meta-analysis. Alcoholism: Clinical and Experimental Research 38(1), 214-226.
Golding, P., Jones and the ALSPAC Study Team, 2001. ALSPAC–the avon longitudinal study of parents and children. Paediatric and Perinatal Epidemiology 15(1), 74-87.
Haycock, P.C., Burgess, S., Wade, K.H., Bowden, J., Relton, C., Smith, G.D., 2016. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. The American Journal of Clinical Nutrition 103(4), 965-978.
Holmes, M.V., Dale, C.E., Zuccolo, L., Silverwood, R.J., Guo, Y., Ye, Z., Prieto-Merino, D., Dehghan, A., Trompet, S., Wong, A., 2014. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 349, g4164.
Hoyme, H.E., Kalberg, W.O., Elliott, A.J., Blankenship, J., Buckley, D., Marais, A.-S., Manning, M.A., Robinson, L.K., Adam, M.P., Abdul-Rahman, O., 2016. Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders. Pediatrics, e20154256.
Hoyme, H.E., May, P.A., Kalberg, W.O., Kodituwakku, P., Gossage, J.P., Trujillo, P.M., Buckley, D.G., Miller, J.H., Aragon, A.S., Khaole, N., 2005. A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria. Pediatrics 115(1), 39-47.
Huizink, A.C., Mulder, E.J., 2006. Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. Neuroscience & Biobehavioral Reviews 30(1), 24-41.
Ikonomidou, C., Bittigau, P., Ishimaru, M.J., Wozniak, D.F., Koch, C., Genz, K., Price, M.T., Stefovska, V., Hörster, F., Tenkova, T., 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 287(5455), 1056-1060.
Jones, K., Smith, D., 1973. Recognition of the fetal alcohol syndrome in early infancy. The Lancet 302(7836), 999-1001.
Kelly, Y., Iacovou, M., Quigley, M.A., Gray, R., Wolke, D., Kelly, J., Sacker, A., 2013. Light drinking versus abstinence in pregnancy–behavioural and cognitive outcomes in 7-year-old children: a longitudinal cohort study. BJOG: An International Journal of Obstetrics & Gynaecology 120(11), 1340-1347.
Klingenberg, C., Wetherill, L., Rogers, J., Moore, E., Ward, R., Autti-Rämö, I., Fagerlund, Å., Jacobson, S., Robinson, L., Hoyme, H., 2010. Prenatal alcohol exposure alters the patterns of facial asymmetry. Alcohol 44(7-8), 649-657.
Lawlor, D., Richmond, R., Warrington, N., McMahon, G., Smith, G.D., Bowden, J., Evans, D.M., 2017. Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them. Wellcome Open Research 2.
Lawlor, D.A., Nordestgaard, B.G., Benn, M., Zuccolo, L., Tybjaerg-Hansen, A., Davey Smith, G., 2013. Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a Mendelian randomization study in the Copenhagen General Population Study. European Heart Journal 34(32), 2519-2528.
Lawlor, D.A., Tilling, K., Davey Smith, G., 2016. Triangulation in aetiological epidemiology. International Journal of Epidemiology 45(6), 1866-1886.
Lewis, S.J., Zuccolo, L., Smith, G.D., Macleod, J., Rodriguez, S., Draper, E.S., Barrow, M., Alati, R., Sayal, K., Ring, S., 2012. Fetal alcohol exposure and IQ at age 8: evidence from a population-based birth-cohort study. PloS One 7(11), e49407.

Luczak, S.E., Glatt, S.J., Wall, T.J., 2006. Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. American Psychological Association.
Mamluk, L., Edwards, H.B., Savović, J., Leach, V., Jones, T., Moore, T.H., Ijaz, S., Lewis, S.J., Donovan, J.L., Lawlor, D., 2017. Low alcohol consumption and pregnancy and childhood outcomes: time to change guidelines indicating apparently 'safe'levels of alcohol during pregnancy? A systematic review and meta-analyses. BMJ Open 7(7), e015410.
Marmot, M., Brunner, E., 1991. Alcohol and cardiovascular disease: the status of the U shaped curve. BMJ 303(6802), 565-568.
Marmot, M., Shipley, M., Rose, G., Thomas, B., 1981. Alcohol and mortality: a U-shaped curve. The lancet 317(8220), 580-583.
McCormack, C., Hutchinson, D., Burns, L., Youssef, G., Wilson, J., Elliott, E., Allsop, S., Najman, J., Jacobs, S., Rossen, L., 2018. Maternal and partner prenatal alcohol use and infant cognitive development. Drug and Alcohol Dependence 185, 330-338.
Muggli, E., Matthews, H., Penington, A., Claes, P., O'Leary, C., Forster, D., Donath, S., Anderson, P.J., Lewis, S., Nagle, C., 2017. Association between prenatal alcohol exposure and craniofacial shape of children at 12 months of age. JAMA Pediatrics.
Paternoster, L., Zhurov, A.I., Toma, A.M., Kemp, J.P., Pourcain, B.S., Timpson, N.J., McMahon, G., McArdle, W., Ring, S.M., Smith, G.D., 2012. Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. The American Journal of Human Genetics 90(3), 478-485.
Riley, E.P., Infante, M.A., Warren, K.R., 2011. Fetal alcohol spectrum disorders: an overview. Neuropsychology Review 21(2), 73.
Roebuck, T.M., Mattson, S.N., Riley, E.P., 1999. Behavioral and Psychosocial Profiles of Alcohol-Exposed Children. Alcoholism: Clinical and Experimental Research 23(6), 1070-1076.
Shaper, A.G., Wannamethee, G., Walker, M., 1988. Alcohol and mortality in British men: explaining the U-shaped curve. The Lancet 332(8623), 1267-1273.
Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., 2015. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Medicine 12(3), e1001779.
Suttie, M., Foroud, T., Wetherill, L., Jacobson, J.L., Molteno, C.D., Meintjes, E.M., Hoyme, H.E., Khaole, N., Robinson, L.K., Riley, E.P., 2013. Facial dysmorphism across the fetal alcohol spectrum. Pediatrics 131(3), e779-e788.
Taylor, A.E., Jones, H.J., Sallis, H., Euesden, J., Stergiakouli, E., Davies, N.M., Zammit, S., Lawlor, D.A., Munafò, M.R., Davey Smith, G., 2018. Exploring the association of genetic factors with participation in the Avon Longitudinal Study of Parents and Children. International Journal of Epidemiology.
Thomasson, H.R., Edenberg, H.J., Crabb, D.W., Mai, X.-L., Jerome, R.E., Li, T.K., Wang, S.P., Lin, Y.T., Lu, R.B., Yin, S.J., 1991. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. American Journal of Human Genetics 48(4), 677.
Zuccolo, L., Fitz-Simon, N., Gray, R., Ring, S.M., Sayal, K., Smith, G.D., Lewis, S.J., 2009. A non-synonymous variant in ADH1B is strongly associated with prenatal alcohol use in a European sample of pregnant women. Human Molecular Genetics 18(22), 4457-4466.
Zuccolo, L., Lewis, S.J., Davey Smith, G., Sayal, K., Draper, E.S., Fraser, R., Barrow, M., Alati, R., Ring, S., Macleod, J., 2013. Prenatal alcohol exposure and offspring cognition and school performance. A 'Mendelian randomization'natural experiment. International Journal of Epidemiology 42(5), 1358-1370.

REFERENCES

Alati, R., Lawlor, D.A., Najman, J.M., Williams, G.M., Bor, W., O'callaghan, M., 2005. Is there really a 'J-shaped'curve in the association between alcohol consumption and symptoms of depression and anxiety? Findings from the Mater-University Study of Pregnancy and its outcomes. Addiction 100, 643-651.
Alati, R., Smith, G.D., Lewis, S.J., Sayal, K., Draper, E.S., Golding, J., Fraser, R., Gray, R., 2013. Effect of prenatal alcohol exposure on childhood academic outcomes: contrasting maternal and paternal associations in the ALSPAC study. PLoS One 8, e74844.
Astley, S.J., Clarren, S.K., 2001. Measuring the facial phenotype of individuals with prenatal alcohol exposure: correlations with brain dysfunction. Alcohol and Alcoholism 36, 147-159.
Astley, S.J., Clarren, S.K., Little, R.E., Sampson, P.D., Daling, J.R., 1992. Analysis of facial shape in children gestationally exposed to marijuana, alcohol, and/or cocaine. Pediatrics 89, 67-77.
Boyd, A., Golding, J., Macleod, J., Lawlor, D.A., Fraser, A., Henderson, J., Molloy, L., Ness, A., Ring, S., Smith, G.D., 2012. Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. International Journal of Epidemiology, 111-127.
Canela-Xandri, O., Rawlik, K., Tenesa, A., 2017. An atlas of genetic associations in UK Biobank. bioRxiv, 176834.
Claes, P., Roosenboom, J., White, J.D., Swigut, T., Sero, D., Li, J., Lee, M.K., Zaidi, A., Mattern, B.C., Liebowitz, C., 2018. Genome-wide mapping of global-to-local genetic effects on human facial shape. Nature Genetics, 1.
Das, U.G., Cronk, C.E., Martier, S.S., Simpson, P.M., McCarver, D.G., 2004. Alcohol dehydrogenase 2* 3 affects alterations in offspring facial morphology associated with maternal ethanol intake in pregnancy. Alcoholism: Clinical and Experimental Research 28, 1598-1606.
Davey Smith, G., Ebrahim, S., 2003. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? International Journal of Epidemiology 32, 1-22.
Eng, M.Y., Luczak, S.E., Wall, T.L., 2007. ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. Alcohol Research & Health 30, 22.
Flak, A.L., Su, S., Bertrand, J., Denny, C.H., Kesmodel, U.S., Cogswell, M.E., 2014. The association of mild, moderate, and binge prenatal alcohol exposure and child neuropsychological outcomes: a meta-analysis. Alcoholism: Clinical and Experimental Research 38, 214-226.
Golding, P., Jones and the ALSPAC Study Team, 2001. ALSPAC–the avon longitudinal study of parents and children. Paediatric and Perinatal Epidemiology 15, 74-87.
Haycock, P.C., Burgess, S., Wade, K.H., Bowden, J., Relton, C., Smith, G.D., 2016. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. The American Journal of Clinical Nutrition 103, 965-978.
Holmes, M.V., Dale, C.E., Zuccolo, L., Silverwood, R.J., Guo, Y., Ye, Z., Prieto-Merino, D., Dehghan, A., Trompet, S., Wong, A., 2014. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 349, g4164.
Hoyme, H.E., Kalberg, W.O., Elliott, A.J., Blankenship, J., Buckley, D., Marais, A.-S., Manning, M.A., Robinson, L.K., Adam, M.P., Abdul-Rahman, O., 2016. Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders. Pediatrics, e20154256.
Hoyme, H.E., May, P.A., Kalberg, W.O., Kodituwakku, P., Gossage, J.P., Trujillo, P.M., Buckley, D.G., Miller, J.H., Aragon, A.S., Khaole, N., 2005. A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria. Pediatrics 115, 39-47.
Huizink, A.C., Mulder, E.J., 2006. Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. Neuroscience & Biobehavioral Reviews 30, 24-41.

Ikonomidou, C., Bittigau, P., Ishimaru, M.J., Wozniak, D.F., Koch, C., Genz, K., Price, M.T., Stefovska, V., Hörster, F., Tenkova, T., 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 287, 1056-1060.
Jones, K., Smith, D., 1973. Recognition of the fetal alcohol syndrome in early infancy. The Lancet 302, 999-1001.
Kelly, Y., Iacovou, M., Quigley, M.A., Gray, R., Wolke, D., Kelly, J., Sacker, A., 2013. Light drinking versus abstinence in pregnancy–behavioural and cognitive outcomes in 7-year-old children: a longitudinal cohort study. BJOG: An International Journal of Obstetrics & Gynaecology 120, 1340-1347.
Klingenberg, C., Wetherill, L., Rogers, J., Moore, E., Ward, R., Autti-Rämö, I., Fagerlund, Å., Jacobson, S., Robinson, L., Hoyme, H., 2010. Prenatal alcohol exposure alters the patterns of facial asymmetry. Alcohol 44, 649-657.
Lawlor, D., Richmond, R., Warrington, N., McMahon, G., Smith, G.D., Bowden, J., Evans, D.M., 2017. Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them. Wellcome Open Research 2.
Lawlor, D.A., Nordestgaard, B.G., Benn, M., Zuccolo, L., Tybjaerg-Hansen, A., Davey Smith, G., 2013. Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a Mendelian randomization study in the Copenhagen General Population Study. European Heart Journal 34, 2519-2528.
Lawlor, D.A., Tilling, K., Davey Smith, G., 2016. Triangulation in aetiological epidemiology. International Journal of Epidemiology 45, 1866-1886.
Lewis, S.J., Zuccolo, L., Smith, G.D., Macleod, J., Rodriguez, S., Draper, E.S., Barrow, M., Alati, R., Sayal, K., Ring, S., 2012. Fetal alcohol exposure and IQ at age 8: evidence from a population-based birth-cohort study. PloS One 7, e49407.
Luczak, S.E., Glatt, S.J., Wall, T.J., 2006. Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. American Psychological Association.
Mamluk, L., Edwards, H.B., Savović, J., Leach, V., Jones, T., Moore, T.H., Ijaz, S., Lewis, S.J., Donovan, J.L., Lawlor, D., 2017. Low alcohol consumption and pregnancy and childhood outcomes: time to change guidelines indicating apparently 'safe'levels of alcohol during pregnancy? A systematic review and meta-analyses. BMJ Open 7, e015410.
Marmot, M., Brunner, E., 1991. Alcohol and cardiovascular disease: the status of the U shaped curve. BMJ 303, 565-568.
Marmot, M., Shipley, M., Rose, G., Thomas, B., 1981. Alcohol and mortality: a U-shaped curve. The lancet 317, 580-583.
McCormack, C., Hutchinson, D., Burns, L., Youssef, G., Wilson, J., Elliott, E., Allsop, S., Najman, J., Jacobs, S., Rossen, L., 2018. Maternal and partner prenatal alcohol use and infant cognitive development. Drug and Alcohol Dependence 185, 330-338.
Muggli, E., Matthews, H., Penington, A., Claes, P., O'Leary, C., Forster, D., Donath, S., Anderson, P.J., Lewis, S., Nagle, C., 2017. Association between prenatal alcohol exposure and craniofacial shape of children at 12 months of age. JAMA Pediatrics.
Paternoster, L., Zhurov, A.I., Toma, A.M., Kemp, J.P., Pourcain, B.S., Timpson, N.J., McMahon, G., McArdle, W., Ring, S.M., Smith, G.D., 2012. Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. The American Journal of Human Genetics 90, 478-485.
Riley, E.P., Infante, M.A., Warren, K.R., 2011. Fetal alcohol spectrum disorders: an overview. Neuropsychology Review 21, 73.
Shaper, A.G., Wannamethee, G., Walker, M., 1988. Alcohol and mortality in British men: explaining the U-shaped curve. The Lancet 332, 1267-1273.
Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., 2015. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Medicine 12, e1001779.

Suttie, M., Foroud, T., Wetherill, L., Jacobson, J.L., Molteno, C.D., Meintjes, E.M., Hoyme, H.E., Khaole, N., Robinson, L.K., Riley, E.P., 2013. Facial dysmorphism across the fetal alcohol spectrum. Pediatrics 131, e779-e788.
Taylor, A.E., Jones, H.J., Sallis, H., Euesden, J., Stergiakouli, E., Davies, N.M., Zammit, S., Lawlor, D.A., Munafò, M.R., Davey Smith, G., 2018. Exploring the association of genetic factors with participation in the Avon Longitudinal Study of Parents and Children. International Journal of Epidemiology.
Thomasson, H.R., Edenberg, H.J., Crabb, D.W., Mai, X.-L., Jerome, R.E., Li, T.K., Wang, S.P., Lin, Y.T., Lu, R.B., Yin, S.J., 1991. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. American Journal of Human Genetics 48, 677.
Zuccolo, L., Fitz-Simon, N., Gray, R., Ring, S.M., Sayal, K., Smith, G.D., Lewis, S.J., 2009. A non-synonymous variant in ADH1B is strongly associated with prenatal alcohol use in a European sample of pregnant women. Human Molecular Genetics 18, 4457-4466.
Zuccolo, L., Lewis, S.J., Davey Smith, G., Sayal, K., Draper, E.S., Fraser, R., Barrow, M., Alati, R., Ring, S., Macleod, J., 2013. Prenatal alcohol exposure and offspring cognition and school performance. A 'Mendelian randomization'natural experiment. International Journal of Epidemiology 42, 1358-1370.