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I. Details of search terms used in systematic review

| Database          | embase.com | Medline Ovid | Web of science | Cochrane CENTRAL | Google scholar | Total |
|-------------------|------------|--------------|----------------|-----------------|---------------|-------|
| Count             | 218        | 177          | 250            | 16              | 200           | 861   |
| Unique Count      | 215        | 24           | 129            | 1               | 136           | 505   |

embase.com 218

('instrumental variable analysis'/exp OR ((mendelian* NEAR/3 random*) OR (instrumental* NEAR/3 variab*)):ab,ti) AND ('prenatal exposure'/exp OR 'prenatal drug exposure'/exp OR 'pregnant woman'/exp OR 'pregnancy'/exp OR 'prenatal disorder'/exp OR 'pregnancy disorder'/exp OR 'parameters concerning the fetus, newborn and pregnancy'/exp OR 'prenatal development'/exp OR 'maternal nutrition'/exp OR 'maternal smoking'/exp OR 'Maternal Exposure'/exp OR 'embryonic and placental structures'/exp OR (prenatal* OR perinatal* OR pregnan* OR in*-uter* OR intrauter* OR gestation* OR maternal* OR offspring OR birthweight OR birth-weight OR fetus OR fetal OR foetus OR foetal OR placenta* OR embryo* OR fetomatern* OR PreEclampsia OR Eclampsia):ab,ti)

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(Mendelian Randomization Analysis/ OR ((mendelian* ADJ3 random*) OR (instrumental* ADJ3 variab*)):ab,ti) AND (exp Pregnancy Complications/ OR Maternal Exposure/ OR pregnant women/ OR exp pregnancy/ OR exp Fetal Diseases/ OR exp Pregnancy Complications/ OR exp Birth Weight/ OR exp Infant, Low Birth Weight/ OR Perinatal Mortality/ OR Perinatal Death/ OR Embryology/ OR exp "Embryonic and Fetal Development"/ OR exp Maternal Nutritional Physiological Phenomena/ OR exp Embryonic Structures/ OR (prenatal* OR perinatal* OR pregnan* OR in*-uter* OR intrauter* OR gestation* OR maternal* OR offspring OR birthweight OR birth-weight OR fetus OR fetal OR foetus OR foetal OR placenta* OR embryo* OR fetomatern* OR PreEclampsia OR Eclampsia):ab,ti.)
Cochrane CENTRAL 16

(((mendelian* NEAR/3 random*) OR (instrumental* NEAR/3 variab*)):ab,ti) AND ((prenatal* OR perinatal* OR pregnan* OR in*-uter* OR intrauter* OR gestation* OR maternal* OR offspring OR birthweight OR birth-weight OR fetus OR fetal OR foetus OR foetal OR placenta* OR embryo* OR fetomaternal* OR PreEclampsia OR Eclampsia):ab,ti)

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TS=(((mendelian* NEAR/2 random*) OR (instrumental* NEAR/2 variab*)) AND ((prenatal* OR perinatal* OR pregnan* OR in*-uter* OR intrauter* OR gestation* OR (maternal* NEAR/3 (exposure* OR smoking OR drinking OR alcohol)) OR offspring OR birthweight OR birth-weight OR fetus OR fetal OR foetus OR foetal OR placenta* OR embryo* OR fetomaternal* OR PreEclampsia OR Eclampsia)))

Google scholar

"mendelian randomization|randomisation" |"instrumental variable" prenatal|perinatal|pregnancy|pregnant|"in-uterus"|intrauterine|gestational|maternal|offspring|birthweight |"birth-weight"|fetus|fetal|foetus|foetal|placenta|embryo|fetomaternal

II. Details of extraction procedure

Data points were extracted by the first author (ED); to ensure accuracy in extraction, 5 included studies were randomly chosen for independent extraction by a coauthor (JL). Data points on discussion of MR assumptions were considered in agreement when both authors agreed on the presence/absence of any discussion of violations of the assumption in question.

Sensitivity analyses and limitations were excluded from the comparison checking due to variability in how specific secondary analyses and limitations were categorized. This is because, rather than prespecifying sets of possible limitations and sensitivity analyses of interest, extraction of data points related to both sensitivity analyses and limitations discussed were open-ended to allow for unexpected or unknown analyses and perspectives. This approach meant that each independent extractor could generate an arbitrarily large number of reported limitations and sensitivity analyses based on the same article. Thus, it would be difficult to measure the degree to which independent extractors agreed on datapoints related to sensitivity analyses and limitations discussed, as it is not possible to measure the number of datapoints the two authors agreed were not present in the dataset.
### Supplementary Table 1: Description of Included Studies

| Author  | Year | Title                                                                 | Exposure                          | Outcome                            | Instrument                              | Maternal or Offspring Instrument | Design | Recruited Based on Presence of a Pregnancy |
|---------|------|----------------------------------------------------------------------|-----------------------------------|------------------------------------|-----------------------------------------|----------------------------------|--------|-------------------------------------------|
| Allard  | 2015 | Mendelian randomization supports causality between maternal hyperglycemia and epigenetic regulation of leptin gene in newborns | 2 step: maternal fasting glucose, methylation | 2 step: methylation, cord blood leptin | 2 step: glucose GRS, methylation GRS | 1st step: maternal cohort     | cohort | yes                                       |
| Alwan   | 2012 | Exploring the relationship between maternal iron status and offspring's blood pressure and adiposity: A Mendelian randomization Study | iron                              | blood pressure, waist circumference, BMI | C282Y                                  | maternal cohort                  | cohort | No                                        |
| Bech    | 2006 | Stillbirth and slow metabolizers of caffeine: Comparison by genotypes. | caffeine                          | stillbirth                         | NAT2, CYP1A2, GSTA1                    | maternal nested case-control    | yes (nested in recruited that way) |
| Study | Year | Description | Gene/Marker | Outcome | Cohort Type | Results |
|-------|------|-------------|-------------|---------|-------------|---------|
| Bedard | 2018 | Maternal iron status during pregnancy and respiratory and atopic outcomes in the offspring: A Mendelian randomisation study | hemoglobin | wheezing, asthma, atopy, low lung function | maternal cohort | Yes |
| Bernard | 2018 | Long-chain polyunsaturated fatty acids, gestation duration, and birth size: A Mendelian randomization study using fatty acid desaturase variants | omega 3 and omega 6 PUFAs | gestational duration, birthweight, birth length | maternal and offspring cohort | yes |
| Binder | 2013 | The causal effect of red blood cell folate on genome-wide methylation in cord blood: a Mendelian randomization approach | folate | genome-wide methylation | maternal cross-sectional | Yes |
| Bonilla | 2012 | Maternal and offspring fasting glucose and type 2 diabetes-associated genetic variants and cognitive function at age 8: A Mendelian randomization study in the Avon Longitudinal Study of Parents and Children | fasting glucose, type 2 diabetes | IQ at age 8 genetic risk score | maternal and offspring cohort | Yes |
| First Name | Year | Title                                                                 | Outcome | SNP | Study Type | Cohort | Causal Inference |
|------------|------|----------------------------------------------------------------------|---------|-----|------------|--------|------------------|
| Bonilla    | 2012 | Vitamin B-12 Status during Pregnancy and Child's IQ at Age 8: A Mendelian Randomization Study in the Avon Longitudinal Study of Parents and Children | vitamin B12, IQ at age 8 | rs492602, rs1047781, rs96-6756 | maternal cohort | Yes |
| Caramaschi | 2017 | Exploring a causal role of DNA methylation in the relationship between maternal vitamin B12 during pregnancy and child's IQ at age 8, cognitive performance and educational attainment: a two-step Mendelian randomization study. | 2 step: vitamin B12, methylation | 2 step: rs492602 + rs1047781 for vitamin b12, rs5750236, rs1890131 for methylation | maternal, offspring in 2nd stpe | cohort | Yes |
| Caramaschi | 2018 | Maternal smoking during pregnancy and autism: using causal inference methods in a birth cohort study | smoking heaviness, autism spectrum disorder | rs1051730 | maternal cohort | Yes |
| Evans      | 2018 | Elucidating the role of maternal environmental exposures on offspring health and disease using two-sample Mendelian randomization | maternal type 2 diabetes, birthweight | 403 SNP GRS | maternal and offspring | cross-sectional, UK biobank | No |
| Author   | Year | Title                                                                 | Outcome Measures                                                                 | Methodology                                                                 | Population | Study Design | Result |
|----------|------|----------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------|--------------|--------|
| Geng     | 2018 | Maternal central obesity and birth size: A Mendelian randomization analysis | waist-to-hip ratio adjusted for BMI, hip circumference adjusted for BMI, waist circumference adjusted for BMI | birth weight, birth length, head circumference | maternal   | 2 sample cross-sectional, seems to be same studies that developed GRS in? | No     |
| Granell  | 2008 | The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort | folate | atopy, asthma | MTHFR C677T | maternal | cohort | yes |
| Howe     | 2019 | Prenatal alcohol exposure and facial morphology in a UK cohort | alcohol | facial morphology | rs1229984 | maternal | cohort | Yes |
| Humphriss| 2013 | Prenatal alcohol exposure and childhood balance ability: Findings from a UK birth cohort study | alcohol | 3 composite balance scores (dynamic balance, static balance eyes open, static balance eyes closed) | ADH1B rs1229984 | maternal | cohort | Yes |
| Author | Year | Title                                                                 | Outcomes/Variables | Methodology | Study Design | Results | Data Sources |
|--------|------|----------------------------------------------------------------------|--------------------|-------------|-------------|---------|--------------|
| Hwang  | 2019 | Using a two-sample Mendelian randomization design to investigate a possible causal effect of maternal lipid concentrations on offspring birth weight | HDL cholesterol, LDL cholesterol, triglycerides | birthweight 96, 82, and 60 SNP GRS | maternal, controlled for offspring using SEM | 2 sample summary results, UK biobank and EGG | uK biobank no, EGG mostly yes |
| Korevaar | 2014 | Soluble Flt1 and Placental Growth Factor are novel determinants of newborn thyroid dysfunction: the generation r study. | TSH, FT4, sFlt1, PIGF | GRS | offspring | cohort | yes |
| Lawlor | 2008 | Exploring the developmental overnutrition hypothesis using parental-offspring associations and FTO as an instrumental variable | BMI, fat mass at age 9-11 | FTO | maternal | cohort | Yes |
| Lawlor | 2017 | Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them | BMI, BMI, fat mass index | GRS | maternal | cohort | Yes |
| Last Name | Year | Title                                                                 | Index | Outcome 1                        | Outcome 2                        | Index 2 | Cohort | Status |
|-----------|------|----------------------------------------------------------------------|-------|----------------------------------|----------------------------------|---------|--------|--------|
| Lee       | 2013 | Mendelian randomization analysis of the effect of maternal homocysteine during pregnancy, as represented by maternal MTHFR C677T genotype, on birth weight | homocysteine | birthweight | MTHFR C677T | maternal | cohort | Yes    |
| Lewis     | 2009 | Body composition at age 9 years, maternal folate intake during pregnancy and methyltetrahydrofolate reductase (MTHFR) C677T genotype | folate intake | total weight, total body fat mass, total lean mass | MTHFR C677T | maternal and offspring | cohort | yes    |
| Lewis     | 2012 | Fetal Alcohol Exposure and IQ at Age 8: Evidence from a Population-Based Birth-Cohort Study | alcohol | cognitive score (IQ at age 8) | 10 SNP in ADH4, ADH1A, ADH1B, ADH7 (rs4699714, rs3763894, rs4148884, rs2866151, rs975833, rs1229966, rs2066701, rs4147536, rs1229984, rs284779) | both | cohort | Yes    |
| Lewis     | 2014 | Maternal iron levels early in pregnancy are                          | iron  | IQ at age 8                      | GRS based on maternal            | maternal | cohort | Yes    |
| Study                        | Year | Description                                                                 | Indexes/References                                                                 |
|------------------------------|------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Mamasoulia                   | 2013 | Association between C677T Polymorphism of Methylene Tetrahydrofolate reductase and Congenital Heart Disease: Meta-Analysis of 7697 Cases and 13,125 Controls. Circ Cardiovasc Genet | rs1799945, rs1800562, rs4820268 |
| Morales                      | 2011 | Maternal C-reactive protein levels in pregnancy are associated with wheezing and lower respiratory tract infections in the offspring | CRP, wheezing, LRTI, rs1205, rs1983204, rs344008, rs6795327, rs7637701, rs11929637 |
| Morales                      | 2016 | Genome-wide DNA methylation study in human placenta identifies novel loci associated with maternal smoking during pregnancy | methylation at top-ranked cpg site for placental methylation in smokers, rs1799945, rs1800562, rs4820268 |
| Murray                       | 2016 | Moderate alcohol drinking in pregnancy increases risk for children's persistent alcohol conduct problem trajectories (6 measures of GRS ADH1A, AHD1B) | alcohol, conduct problem trajectories, GRS ADH1A rs2866151, rs975833, AHD1B |
| Study                        | Year | Title                                                                 | Substance | Outcomes                                                                 | Genotype  | Study Design | Findings       |
|------------------------------|------|------------------------------------------------------------------------|-----------|--------------------------------------------------------------------------|-----------|--------------|----------------|
| Richmond                     | 2016 | DNA methylation and BMI: Investigating identified methylation sites at HIF3A in a causal framework | BMI       | HIF3A methylation                                                        | GRS       | maternal     | cohort          | Yes            |
| Richmond                     | 2017 | Using Genetic Variation to Explore the Causal Effect of Maternal Pregnancy Adiposity on Future Offspring Adiposity: A Mendelian Randomisation Study | BMI       | BMI, fat mass index                                                       | GRS       | maternal     | cohort          | yes            |
| von Hinke Kessler Scholder   | 2014 | Alcohol Exposure In Utero and Child Academic Achievement               | alcohol   | academic achievement (KS1, KS2, KS3, GCSE)                               | ADH1B rs1229984 | maternal, controlling for offspring | cohort        | Yes            |
| Shaheen                      | 2014 | Prenatal alcohol exposure and childhood atopic disease: A Mendelian randomization approach | alcohol   | childhood atopic disease                                                 | ADH1B rs1229984 | maternal     | cohort          | Yes            |
| Steenweg-de Graaff           | 2012 | Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study | folate    | CBCL emotional and behavioral score                                       | MTHFR C677T | maternal     | cohort          | yes            |
| Author    | Year | Study Title                                                                 | Exposure | Outcome                  | SNP            | Analysis Method                                         | COHORTS                                   |
|-----------|------|------------------------------------------------------------------------------|----------|--------------------------|----------------|---------------------------------------------------------|--------------------------------------------|
| Steer     | 2011 | Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC) | folate   | BMC, BMD, BA             | MTHFR C677T    | maternal cohort                                         | yes                                        |
| Taylor    | 2014 | Maternal smoking during pregnancy and offspring smoking initiation: assessing the role of intrauterine exposure | smoking  | latent class of offspring smoking initiation | rs1051730      | maternal cohort                                         | yes                                        |
| Thompson  | 2019 | Association of maternal circulating 25(OH)D and calcium with birth weight: A mendelian randomisation analysis | vitamin D, calcium | birth weight | separate 7 SNP GRS | maternal | 2 sample: various GWAS+ UKB cross-sectional, sensitivity analyses: ALSPAC/EFSOC H cohort | ALSPAC/EFSOC H yes, UKB no |
| Tyrell    | 2016 | Genetic evidence for causal relationships between maternal obesity related traits and birth weight. | BMI, fasting glucose, diabetes, triglycerids, HDL, blood pressure, vitamin D, adiponectin | birthweight | GRS          | maternal | meta-analysis of multiple cohorts                       | yes for all |
| Wehby     | 2011 | A genetic instrumental variables analysis of the effects of prenatal smoking on birth weight: Evidence from two samples | smoking  | birthweight              | 14 SNPs        | maternal | 1 cohort, 1 cross-sectional                            | yes for Norway, no for AddHealth         |
| Author   | Year | Title                                                                 | Outcome(s)                        | SNPs                                      | Study Design   | Study Design Type | All   |
|----------|------|----------------------------------------------------------------------|-----------------------------------|-------------------------------------------|----------------|-------------------|-------|
| Wehby    | 2011 | Genes as instruments for studying risk behavior effects: An application to maternal smoking and orofacial clefts | smoking, orofacial cleft          | 4 SNPs (rs1435252, rs1930139, rs1547272, rs2743467) | maternal       | case-control      | yes   |
| Wehby    | 2013 | Genetic instrumental variable studies of effects of prenatal risk factors | smoking, Alcohol use, obesity, birthweight | smoking: rs12914385, rs1051730, alcohol: ADH1B rs1229984, BMI: rs8050136 | maternal       | cohort (multiple) | yes for both |
| Yajnik   | 2014 | Maternal homocysteine in pregnancy and offspring birthweight: Epidemiological associations and Mendelian randomization analysis | homocysteine, birthweight         | MTHFR rs1801133                           | maternal       | 2 cohorts         | yes   |
| Zerbo    | 2016 | Maternal mid-pregnancy C-reactive protein and risk of autism spectrum disorders: the early markers for autism study | CRP, autism spectrum disorder     | rs3116656, rs2794520                     | maternal       | nested case-control | yes   |
| Zhang    | 2015 | Assessing the Causal Relationship of Maternal Height on Birth Size and Gestational Age at | maternal height, birth weight, GRS | GRS                                       | maternal       | 3 case-control nested w/in cohorts | yes for all |
| Authors   | Year | Title                                                                 | Exposure  | Outcome                          | SNP        | Analysis Type                          | Cohort  | Sensitivity |
|----------|------|----------------------------------------------------------------------|-----------|----------------------------------|------------|---------------------------------------|---------|-------------|
| Zuccolo  | 2013 | Prenatal alcohol exposure and offspring cognition and school performance. A 'Mendelian randomization' natural experiment | alcohol (1st trimester) | IQ at age 8, educational attainment | rs1229984  | maternal, sensitivity analysis with offspring | yes     |             |
### Table 1 Continued

| 1: Author | 2: Year | 3: Title                                                                 | 10: Point Estimation? | 11: F-statistic | 12: Assumption 1 Violations Discussed | 13: Assumption 2 Violations Discussed | 14: Assumption 3 Violations Discussed |
|-----------|---------|-------------------------------------------------------------------------|------------------------|-----------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Allard    | 2015    | Mendelian randomization supports causality between maternal hyperglycemia and epigenetic regulation of leptin gene in newborns | point estimate         |                 |                                      |                                      | Population Stratification            |
| Alwan     | 2012    | Exploring the relationship between maternal iron status and offspring's blood pressure and adiposity: A Mendelian randomization Study. | point estimate         | 10 Weak Instrument Bias | Pleiotropy, Exposure Measurement Error, Offspring Genotype Path |
| Bech      | 2006    | Stillbirth and slow metabolizers of caffeine: Comparison by genotypes. | instrument-outcome association | Weak Instrument Bias |                                      |                                      |
| Bedard    | 2018    | Maternal iron status during pregnancy and respiratory and atopic outcomes in the offspring: A Mendelian randomisation study | point estimate         |                 | Pleiotropy, Exposure Measurement Error, Offspring Genotype Path |
| Bernard   | 2018    | Long-chain polyunsaturated fatty acids, gestation duration, and birth size: A Mendelian randomization study using fatty acid desaturase variants | instrument-outcome association | Weak Instrument Bias | Pleiotropy, Exposure Measurement Error |
| Binder    | 2013    | The causal effect of red blood cell folate on genome-wide methylation in cord blood: a Mendelian randomization approach | point estimate         | 4.876 Weak Instrument Bias | 2nd or 3rd Assumption - General |
| Bonilla   | 2012    | Maternal and offspring fasting glucose and type 2 diabetes-associated genetic variants and | instrument-outcome association |               | Offspring Genotype Path               | Population Stratification            |
| Study | Year | Description | Methodology | Confounding Factors |
|-------|------|-------------|-------------|---------------------|
| Bonilla | 2012 | Vitamin B-12 Status during Pregnancy and Child's IQ at Age 8: A Mendelian Randomization Study in the Avon Longitudinal Study of Parents and Children | instrument-outcome association | Exposure Measurement Error, Offspring Genotype Path, Population Stratification |
| Caramaschi | 2017 | Exploring a causal role of DNA methylation in the relationship between maternal vitamin B12 during pregnancy and child's IQ at age 8, cognitive performance and educational attainment: a two-step Mendelian randomization study. | point estimate | Pleiotropy, Exposure Measurement Error, Population Stratification |
| Caramaschi | 2018 | Maternal smoking during pregnancy and autism: using causal inference methods in a birth cohort study | instrument-outcome association | Pleiotropy, Exposure Measurement Error |
| Evans | 2018 | Elucidating the role of maternal environmental exposures on offspring health and disease using two-sample Mendelian randomization | point estimate | Weak Instrument Bias, Pleiotropy, Offspring Genotype Path, Population Stratification |
| Geng | 2018 | Maternal central obesity and birth size: A Mendelian randomization analysis | point estimate | Weak Instrument Bias, Pleiotropy, Postnatal Effects of Genotype, Offspring Genotype Path, Population Stratification |
| Granell | 2008 | The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort | instrument-outcome association | Weak Instrument Bias |
| Author   | Year | Title                                                                 | Methodology                                                                 | Instrument-Outcome Association | Bias Assumptions                                                                                   | Assortative Mating          |
|----------|------|------------------------------------------------------------------------|----------------------------------------------------------------------------|--------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------|
| Howe     | 2019 | Prenatal alcohol exposure and facial morphology in a UK cohort         | instrument-outcome association                                            |                                 | Pleiotropy, Offspring Genotype Path                                                            | Assortative Mating          |
| Humphriss| 2013 | Prenatal alcohol exposure and childhood balance ability: Findings from a UK birth cohort study | instrument-outcome association                                            | Weak Instrument Bias, Cannot Prove Assumption 1 (Reduced Form) | Pleiotropy                                                                       | Population Stratification  |
| Hwang    | 2019 | Using a two-sample Mendelian randomization design to investigate a possible causal effect of maternal lipid concentrations on offspring birth weight | point estimate                                                            |                                 | Pleiotropy, Postnatal Effects of Genotype, Exposure Assumed Constant Over Pregnancy, Offspring Genotype Path | Population Stratification  |
| Korevaar | 2014 | Soluble Flt1 and Placental Growth Factor are novel determinants of newborn thyroid dysfunction: the generation r study. J clin Endocrinol Metab | instrument-outcome association                                            |                                 | Exposure Assumed Constant Over Pregnancy                                                        |                             |
| Lawlor   | 2008 | Exploring the developmental overnutrition hypothesis using parental-offspring associations and FTO as an instrumental variable | point estimate                                                            | 12.9, 10.1 after adjustment for offspring genotype | Pleiotropy, Postnatal Effects of Genotype, Offspring Genotype Path | Population Stratification  |
| Lawlor   | 2017 | Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them | point estimate                                                            | >45                             | Weak Instrument Bias                                                                           | Pleiotropy, Postnatal Effects of Genotype, Offspring Genotype Path | Population Stratification, Assortative Mating |
| Author  | Year | Description                                                                                                                                                                                                 | Type                  | Assumption                     |
|---------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------------------------|
| Lee     | 2013 | Mendelian randomization analysis of the effect of maternal homocysteine during pregnancy, as represented by maternal MTHFR C677T genotype, on birth weight                                                                 | point estimate        | Pleiotropy, 2nd or 3rd Assumption - General |
| Lewis   | 2009 | Body composition at age 9 years, maternal folate intake during pregnancy and methylenetetrahydrofolate reductase (MTHFR) C677T genotype                                                                                                                                  | instrument-outcome association | Exposure Measurement Error |
| Lewis   | 2012 | Fetal Alcohol Exposure and IQ at Age 8: Evidence from a Population-Based Birth-Cohort Study                                                                                                             | instrument-outcome association | Weak Instrument Bias           |
| Lewis   | 2014 | Maternal iron levels early in pregnancy are not associated with offspring IQ score at age 8, findings from a Mendelian randomization study                                                                       | instrument-outcome association | Pleiotropy, Offspring Genotype Path |
| Mamasoula | 2013 | Association between C677T Polymorphism of Methylene Tetrahydrofolate reductase and Congenital Heart Disease: Meta-Analysis of 7697 Cases and 13,125 Controls. Circ Cardiovasc Genet | instrument-outcome association | 2nd or 3rd Assumption - General |
| Morales | 2011 | Maternal C-reactive protein levels in pregnancy are associated with wheezing and lower respiratory tract infections in the offspring                                                                              | instrument-outcome association | Weak Instrument Bias           |
| Morales | 2016 | Genome-wide DNA methylation study in human placenta identifies novel loci associated with maternal smoking during pregnancy                                                                                | point estimate         | Pleiotropy, Exposure Assumed Constant Over Pregnancy, 2nd or 3rd Assumption - General |
|         |      |                                                                                                                                                                                                            | Winner's Curse         | Pleiotropy, Exposure Measurement Error, |


| Name          | Year | Description                                                                 | Associated Variables | Weak Instrument Bias | Bias Assumptions                                                                 | Population Stratification |
|---------------|------|------------------------------------------------------------------------------|-----------------------|----------------------|----------------------------------------------------------------------------------|----------------------------|
| Murray        | 2016 | Moderate alcohol drinking in pregnancy increases risk for children's persistent conduct problems: causal effects in a Mendelian randomisation study | instrument-outcome association | Cannot Prove Assumption 1 (Reduced Form) | Pleiotropy, Exposure Measurement Error, Offspring Genotype Path | Population Stratification |
| Richmond      | 2016 | DNA methylation and BMI: Investigating identified methylation sites at HIF3A in a causal framework | point estimate | 45.7 in offspring | Pleiotropy, Exposure Measurement Error, Offspring Genotype Path | Population Stratification |
| Richmond      | 2017 | Using Genetic Variation to Explore the Causal Effect of Maternal Pregnancy Adiposity on Future Offspring Adiposity: A Mendelian Randomisation Study | point estimate | minimum 45 | Weak Instrument Bias | Population Stratification |
| Scholder      | 2014 | Alcohol Exposure In Utero and Child Academic Achievement | point estimate | 1.38-24.76 | Weak Instrument Bias | Population Stratification |
| Shaheen       | 2014 | Prenatal alcohol exposure and childhood atopic disease: A Mendelian randomization approach | instrument-outcome association | | Offspring Genotype Path | Population Stratification |
| Steenweg-de Graaff | 2012 | Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study | instrument-outcome association | | Exposure Assumed Constant Over Pregnancy, Offspring Genotype Path, 2nd or 3rd Assumption - General | Population Stratification |
| Steer         | 2011 | Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC) | instrument-outcome association | | Offspring Genotype Path | Population Stratification |
| Authors   | Year | Title                                                                 | Type                        | Point Estimate | Bias Issues                                                                 | Stratification Issues                                      |
|----------|------|-----------------------------------------------------------------------|-----------------------------|----------------|-----------------------------------------------------------------------------|-------------------------------------------------------------|
| Taylor   | 2014 | Maternal smoking during pregnancy and offspring smoking initiation: assessing the role of intrauterine exposure | instrument-outcome association |                | Pleiotropy, Exposure Measurement Error, Postnatal Effects of Genotype       | Population Stratification                                   |
| Thompson | 2019 | Association of maternal circulating 25(OH)D and calcium with birth weight: A mendelian randomisation analysis | point estimate              |                | Weak Instrument Bias                                                        | Pleiotropy, Exposure Measurement Error, Exposure Assumed Constant Over Pregnancy, Offspring Genotype Path |
| Tyrell   | 2016 | Genetic evidence for causal relationships between maternal obesity related traits and birth weight. JAMA | point estimate              |                | Weak Instrument Bias                                                        | Pleiotropy, Postnatal Effects of Genotype, Offspring Genotype Path |
| Wehby    | 2011 | A genetic instrumental variables analysis of the effects of prenatal smoking on birth weight: Evidence from two samples | point estimate              | 3.3-4.4        | Weak Instrument Bias                                                        | Pleiotropy                                                  |
| Wehby    | 2011 | Genes as instruments for studying risk behavior effects: An application to maternal smoking and orofacial clefts | point estimate              | 3.33           | Weak Instrument Bias                                                        | Pleiotropy, Exposure Measurement Error                      |
| Wehby    | 2013 | Genetic instrumental variable studies of effects of prenatal risk factors | point estimate              | 0.66-35.486    | Weak Instrument Bias                                                        | Pleiotropy, Postnatal Effects of Genotype                   |
| Yajnik   | 2014 | Maternal homocysteine in pregnancy and offspring birthweight: Epidemiological associations and Mendelian randomization analysis | point estimate              |                | Pleiotropy, Offspring Genotype Path                                          | Population Stratification                                   |
| Zerbo    | 2016 | Maternal mid-pregnancy C-reactive protein and risk of autism spectrum disorders: the early markers for autism study | instrument-outcome association |                |                                                                             | Population Stratification                                   |
| Author  | Year | Title                                                                                                                                       | Approach | Pleiotropy, Offspring Genotype Path | Population Stratification |
|---------|------|----------------------------------------------------------------------------------------------------------------------------------------------|----------|------------------------------------|---------------------------|
| Zhang   | 2015 | Assessing the Causal Relationship of Maternal Height on Birth Size and Gestational Age at Birth: A Mendelian Randomization Analysis | point estimate | Pleiotropy, Offspring Genotype Path | Assortative Mating        |
| Zuccolo | 2013 | Prenatal alcohol exposure and offspring cognition and school performance. A 'Mendelian randomization' natural experiment | instrument-outcome association | Cannot Prove Assumption 1 (Reduced Form) | Pleiotropy, Exposure Measurement Error, Postnatal Effects of Genotype, 2nd or 3rd Assumption - General | Population Stratification |
Table 1 Continued

| 1: Author | 2: Year | 3: Title                                                                 | 15: Other Limitations Discussed                      | 16: Falsification Techniques | 17: Exposure Stratification/Testing |
|-----------|---------|--------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------|-----------------------------------|
| Allard    | 2015    | Mendelian randomization supports causality between maternal hyperglycemia and epigenetic regulation of leptin gene in newborns | Outcome Measurement Error, Low Power               | Alternative MR Methods       | No                                |
| Alwan     | 2012    | Exploring the relationship between maternal iron status and offspring’s blood pressure and adiposity: A Mendelian randomization Study. | Low Power                                              | Covariate Balance            | No                                |
| Bech      | 2006    | Stillbirth and slow metabolizers of caffeine: Comparison by genotypes. | Low Power                                              | Covariate Balance            | Yes                               |
| Bedard    | 2018    | Maternal iron status during pregnancy and respiratory and atopic outcomes in the offspring: A Mendelian randomisation study | Selection Bias, Low Power                             | Covariate Balance, Alternative MR Methods | No                                |
| Bernard   | 2018    | Long-chain polyunsaturated fatty acids, gestation duration, and birth size: A Mendelian randomization study using fatty acid desaturase variants | Outcome Measurement Error, Low Power               |                              | No                                |
| Binder    | 2013    | The causal effect of red blood cell folate on genome-wide methylation in cord blood: a Mendelian randomization approach | Modeling Assumptions, Outcome Measurement Error, Low Power |                              | No                                |
| Bonilla   | 2012    | Maternal and offspring fasting glucose and type 2 diabetes-associated genetic variants and cognitive function at age 8: A Mendelian randomization study in the Avon Longitudinal Study of Parents and Children | Modeling Assumptions, Low Power                      | Covariate Balance            | No                                |
### Supplementary Material: Prenatal Mendelian Randomization Systematic Review

| Author      | Year | Title                                                                 | Modeling Assumptions | Alternative MR Methods | Covariate Balance | Notes          |
|-------------|------|----------------------------------------------------------------------|-----------------------|------------------------|-------------------|----------------|
| Bonilla     | 2012 | Vitamin B-12 Status during Pregnancy and Child's IQ at Age 8: A Mendelian Randomization Study in the Avon Longitudinal Study of Parents and Children | Selection Bias, Low Power, Limited Generalizability |                       | Yes               |                |
| Caramaschi  | 2017 | Exploring a causal role of DNA methylation in the relationship between maternal vitamin B12 during pregnancy and child's IQ at age 8, cognitive performance and educational attainment: a two-step Mendelian randomization study. | Low Power, Limited Generalizability |                       | No                |                |
| Caramaschi  | 2018 | Maternal smoking during pregnancy and autism: using causal inference methods in a birth cohort study | Low Power |                       | Yes               |                |
| Evans       | 2018 | Elucidating the role of maternal environmental exposures on offspring health and disease using two-sample Mendelian randomization | Modeling Assumptions | Alternative MR Methods | No                |                |
| Geng        | 2018 | Maternal central obesity and birth size: A Mendelian randomization analysis | Modeling Assumptions, Limited Generalizability | Alternative MR Methods | No                |                |
| Granell     | 2008 | The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort | Modeling Assumptions, Selection Bias, Outcome Measurement Error |                       | Yes               |                |
| Howe        | 2019 | Prenatal alcohol exposure and facial morphology in a UK cohort | Outcome Measurement Error |                       | No                |                |
| Humphriss   | 2013 | Prenatal alcohol exposure and childhood balance ability: Findings from a UK birth cohort study | Outcome Measurement Error, Low Power, Limited Generalizability |                       | No                |                |
| Author     | Year | Title                                                                 | Limitations and Methods                                                                 | Covariate Balance | Alternative MR Methods | Low Power | Selection Bias | No |
|------------|------|----------------------------------------------------------------------|----------------------------------------------------------------------------------------|-------------------|------------------------|-----------|----------------|----|
| Hwang      | 2019 | Using a two-sample Mendelian randomization design to investigate a possible causal effect of maternal lipid concentrations on offspring birth weight | Limited Generalizability, Alternative MR Methods                                         | No                |                        |           |                |     |
| Korevaar   | 2014 | Soluble Flt1 and Placental Growth Factor are novel determinants of newborn thyroid dysfunction: the generation r study. J clin Endocrinol Metab | Low Power                                                                             | No                |                        |           |                |     |
| Lawlor     | 2008 | Exploring the developmental overnutrition hypothesis using parental-offspring associations and FTO as an instrumental variable | Selection Bias, Low Power, Covariate Balance                                            | No                |                        |           |                |     |
| Lawlor     | 2017 | Using Mendelian randomization to determine causal effects of maternal pregnancy (intrauterine) exposures on offspring outcomes: Sources of bias and methods for assessing them | Low Power, Covariate Balance, Alternative MR Methods                                    | No                |                        |           |                |     |
| Lee        | 2013 | Mendelian randomization analysis of the effect of maternal homocysteine during pregnancy, as represented by maternal MTHFR C677T genotype, on birth weight | Selection on Pregnancy, Low Power, Limited Generalizability                               | Yes               |                        |           |                |     |
| Lewis      | 2009 | Body composition at age 9 years, maternal folate intake during pregnancy and methyltetrahydrofolate reductase (MTHFR) C677T genotype |                                                                                         | No                |                        |           |                |     |
| Lewis      | 2012 | Fetal Alcohol Exposure and IQ at Age 8: Evidence from a Population-Based Birth-Cohort Study | Selection Bias                                                                         | Yes               |                        |           |                |     |
| Lewis      | 2014 | Maternal iron levels early in pregnancy are not associated with offspring IQ score at age 8, findings from a Mendelian randomization study | Modeling Assumptions, Low Power, Covariate Balance                                       | No                |                        |           |                |     |
| Mamasoula  | 2013 | Association between C677T Polymorphism of Methylene Tetrahydrofolate reductase and Congenital Heart Disease: Meta-Analysis of 7697 Cases and 13,125 Controls. Circ Cardiovasc Genet | Modeling Assumptions                                                                    | No                |                        |           |                |     |
| Morales    | 2011 | Maternal C-reactive protein levels in pregnancy are associated with wheezing and lower respiratory tract infections in the offspring | Low Power                                                                              | No                |                        |           |                |     |
| Name               | Year | Title                                                                 | Issues                                      | Alternative MR Methods | Covariate Balance |
|--------------------|------|-----------------------------------------------------------------------|---------------------------------------------|-------------------------|-------------------|
| Morales            | 2016 | Genome-wide DNA methylation study in human placenta identifies novel loci associated with maternal smoking during pregnancy | Low Power                                  |                         | No                |
| Murray             | 2016 | Moderate alcohol drinking in pregnancy increases risk for children's persistent conduct problems: causal effects in a Mendelian randomisation study | Selection Bias, Low Power                  | Covariate Balance       | Yes               |
| Richmond           | 2016 | DNA methylation and BMI: Investigating identified methylation sites at HIF3A in a causal framework | Modeling Assumptions, Selection Bias, Low Power | Covariate Balance       | No                |
| Richmond           | 2017 | Using Genetic Variation to Explore the Causal Effect of Maternal Pregnancy Adiposity on Future Offspring Adiposity: A Mendelian Randomisation Study | Modeling Assumptions, Selection Bias, Low Power | Covariate Balance, Alternative MR Methods | No                |
| Scholder           | 2014 | Alcohol Exposure In Utero and Child Academic Achievement               | Modeling Assumptions                        | Weight Function, Covariate Balance | No                |
| Shaheen            | 2014 | Prenatal alcohol exposure and childhood atopic disease: A Mendelian randomization approach | Selection Bias, Low Power                  | Covariate Balance       | Yes               |
| Steenweg-de Graaff | 2012 | Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study | Modeling Assumptions, Selection Bias        |                         | Yes               |
| Steer              | 2011 | Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC) | NA                                          |                         | No                |
| Taylor             | 2014 | Maternal smoking during pregnancy and offspring smoking initiation: assessing the role of intrauterine exposure | Low Power, Limited Generalizability         |                         | Yes               |
| Thompson           | 2019 | Association of maternal circulating 25(OH)D and calcium with birth weight: A mendelian randomisation analysis | Outcome Measurement Error                  | Alternative MR Methods   | No                |
| Tyrell             | 2016 | Genetic evidence for causal relationships between maternal obesity related traits and birth weight. JAMA | Modeling Assumptions, Low Power             | Covariate Balance       | No                |
| Author      | Year | Title                                                                 | Modeling Assumptions                                                                 | Overidentification Tests | Covariate Balance |
|-------------|------|----------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------|-------------------|
| Wehby       | 2011 | A genetic instrumental variables analysis of the effects of prenatal smoking on birth weight: Evidence from two samples | Model Assumptions                                                                  | No                       |                   |
| Wehby       | 2011 | Genes as instruments for studying risk behavior effects: An application to maternal smoking and orofacial clefts | Model Assumptions                                                                  | No                       |                   |
| Wehby       | 2013 | Genetic instrumental variable studies of effects of prenatal risk factors | Model Assumptions, Low Power, Limited Generalizability                              | No                       |                   |
| Yajnik      | 2014 | Maternal homocysteine in pregnancy and offspring birthweight: Epidemiological associations and Mendelian randomization analysis | Low Power                                                                         | No                       |                   |
| Zerbo       | 2016 | Maternal mid-pregnancy C-reactive protein and risk of autism spectrum disorders: the early markers for autism study | Outcome Measurement Error, Low Power                                               | No                       |                   |
| Zhang       | 2015 | Assessing the Causal Relationship of Maternal Height on Birth Size and Gestational Age at Birth: A Mendelian Randomization Analysis | Selection Bias                                                                     | Alternative MR Methods   | No                |
| Zuccolo     | 2013 | Prenatal alcohol exposure and offspring cognition and school performance. A 'Mendelian randomization' natural experiment | Low Power                                                                         | Covariate Balance        | Yes               |
IV. Assumptions required for point estimation

Investigators can test whether there is a non-null effect of the exposure on the outcome for at least one individual in the study population, and can estimate bounds for the average causal effect using only the 3 instrumental variable assumptions discussed in the main text [1, 2].

To estimate the average causal effect of an exposure X on an outcome Y, using an instrument Z in the total study population, investigators must assume one of the following conditions hold [2].

4a. The effect of X on Y is identical (constant) for all individuals in the population:
\[ E(Y_{x=x} - Y_{x=0} | X=x) = E(Y_{x=x} - Y_{x=0} | X=0) \]

4b. No effect modification by the instrument Z in all levels of the exposure X:
\[ E(Y_{x=x} - Y_{x=0} | X=x, Z=z) = E(Y_{x=x} - Y_{x=0} | X=x, Z=z̅) \]
Or equivalently
\[ E(Y_{x=x} - Y_{x=0} | X=x, Z=z) = E(Y_{x=x̅} - Y_{x=0} | X=x, Z=z) \]

Recent research has found that the average causal effect can also be identified, even in the presence of violations of the second and third assumption, under one of two alternative assumptions by an additional variable J, as can be seen in Supplemental Figure 1 [3]. In this case, the usual 3 IV assumptions are replaced by the following:

1'. \( Z \perp X | J \)

2'. \( Z \perp U | X \)

3'. \( Z \perp Y | (J, U, X) \)

4'. \( Y^X \perp (Z, X) | (J, U) \)

Under these conditions, point estimation of the average causal effect is possible if one of the two following conditions hold:

4c. No additive U-Z interaction on \( E(X | Z, J, U) \):
\[ E(X | Z = z, J, U) - E(X | Z = 0, J, U) = E(X | Z = z, J) - E(X | Z = 0, J) \]

4d. No additive U-X interaction on the average causal effect of \( X \) on \( Y \):
\[ E(Y^{X=x} - Y^{X=0} | J, U) = E(Y^{X=x} - Y^{X=0} | J) \]
If the above assumptions are not plausible for a particular analysis, researchers can estimate the average causal effect within the compliers, those individuals for whom $X^{Z=a} > X^{Z=b}$ for all $a > b$ [2]. This value is also known as the local average treatment effect, or LATE. In order to estimate this quantity, researchers must assume:

4e. The causal effect of Z on X is monotonic, that is, it only works in one direction for every individual in the study population. Formally, $X^Z$ is a nondecreasing function of z on the support of Z.

V. Interpretation of certain additional point-estimating assumptions in pregnancy Mendelian randomization

Four studies in this review reported additional point-estimating assumptions and their targeted estimand. Of these, 3 assumed monotonicity (assumption 4e) in order to estimate the average causal effect among the compliers, and 1 assumed no effect modification by the instrument in all levels of the exposure (assumption 4b) to estimate the average causal effect in the total study population.

In the context of certain pregnancy exposures, there is evidence that conditions 4a, 4b, 4c, and 4d are unreasonable. When genes related to alcohol metabolism are used as instruments for maternal drinking during pregnancy, fetal exposure to alcohol and alcohol metabolites will depend on maternal intake and the speed at which the mother can metabolize alcohol, as well as other environmental factors. For the same level of maternal alcohol intake, offspring of slow metabolizers will have a longer exposure to alcohol, and would be at greater risk of negative health outcomes [5]. This means that the average causal effect of alcohol exposure on offspring outcomes will be modified by the level of the maternal genetic variant proposed as an instrument, violating conditions 4a, 4b, 4c, and 4d. For this reason, most studies of alcohol use during pregnancy in this review focused on a testing approach, rather than point estimation. The same logic applies to other metabolism-related genetic variants proposed as instruments for substance use behaviors, like smoking and caffeine use. In these cases, studies may choose to focus on approaches with weaker assumptions, such as the complier average treatment effect, testing approaches, or bounds.

It is important to note that, in prenatal MR proposing maternal genetic factors as instruments, the interpretation of “compliers” and the complier average causal effect (described in Appendix III above) are different than the usual interpretation in MR studies or most studies using instrumental variable analyses[4]. This is because a mother-child pair’s compliance status is determined by the relationship between a mother’s genetics and exposure, while the average causal effect of interest occurs in the offspring of those mothers. In typical MR and instrumental variable studies, the proposed instrument, exposure, and outcome are all measured within the same individual. In those cases, under condition 4e, researchers can estimate the average causal effect among the compliers. In contrast, in pregnancy MR designs, under condition 4e, researchers can estimate the average...
causal effect among the offspring of mothers who are compliers, although the offspring themselves would not necessarily be compliers.

Supplemental Figure 1: DAG representing an instrumental variable model with violation of assumption 3 by J. Under this model, valid estimation of $E(Y_{X=x} - Y_{X=0})$ is possible using the alternative assumptions presented by Wang and Tchetgen Tchetgen [3].
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