Prenatal alcohol exposure and childhood atopic disease: A Mendelian randomization approach

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Background: Alcohol consumption in western pregnant women is not uncommon and could be a risk factor for childhood atopic disease. However, reported alcohol intake may be unreliable, and associations are likely to be confounded.

Objective: We aimed to study the relation between prenatal alcohol exposure and atopic phenotypes in a large population-based birth cohort with the use of a Mendelian randomization approach to minimize bias and confounding.

Methods: In white mothers and children in the Avon Longitudinal Study of Parents and Children (ALSPAC) we first analyzed associations between reported maternal alcohol consumption during pregnancy and atopic outcomes in the offspring measured at 7 years of age (asthma, wheezing, hay fever, eczema, atopy, and total IgE). We then analyzed the relation of maternal alcohol dehydrogenase (ADH1B) genotype (rs1229984) with these outcomes (the A allele is associated with faster metabolism and reduced alcohol consumption and, among drinkers, would be expected to reduce fetal exposure to ethanol).

Results: After controlling for confounders, reported maternal drinking in late pregnancy was negatively associated with childhood asthma and hay fever (adjusted odds ratio [OR] per category increase in intake: 0.91 [95% CI, 0.82-1.01] and 0.87 [95% CI, 0.78-0.98], respectively). However, maternal ADH1B genotype was not associated with asthma comparing carriers of the A allele with persons homozygous for G allele (OR, 0.98 [95% CI, 0.78-0.98], respectively). In the case of alcohol, a nonsynonymous variant in the alcohol dehydrogenase (ADH1B) gene is thought to associate with alcohol consumption in pregnancy which have evaluated childhood atopic outcomes. However, causal inference can be strengthened in observational studies with the use of Mendelian randomization to reduce measurement error and to minimize bias and confounding.
Abbreviations used
ADH: Alcohol dehydrogenase
ALSPAC: Avon Longitudinal Study of Parents and Children (ALSPAC)
GWAS: Genome Wide Association Study
PCA: Principal Components Analysis

Longitudinal Study of Parents and Children (ALSPAC), a United Kingdom population-based birth cohort, the maternal ADH1B gene variant was strongly associated with alcohol use before and during pregnancy, suggesting that it could be used as a reliable instrument to study the consequences of prenatal (and periconceptional) alcohol exposure. Indeed, to study the relation between fetal alcohol exposure and cognitive development, we recently used a Mendelian randomization approach in ALSPAC to determine whether genetic variants in alcohol metabolizing genes were associated with childhood IQ.

In ALSPAC, we have first analyzed whether reported alcohol intake by mothers in pregnancy is associated with risk of atopic disease in the offspring and then examined the relation between the maternal ADH1B gene variant and these outcomes.

METHODS
Subjects
ALSPAC is a population-based birth cohort that recruited 14,541 predominantly white pregnant women resident in the Bristol area of the United Kingdom during 1990 to 1992. There were 14,062 live born children, and 13,988 of these children were alive at 1 year of age and subsequently were followed up. The cohort has been followed since birth with annual questionnaires and, since 7 years of age, with objective measures in annual research clinics. The study protocol has been described previously, and further information can be found at http://www.alspac.bris.ac.uk. Ethics approval for all aspects of data collection was obtained from the ALSPAC Ethics and Law Committee (IRB 00003312) and the Local Research Ethics Committees.

Exposures
Mothers were asked 8 weeks into their pregnancy to report their current alcohol consumption (how many measures of beer, wine, spirit, or other alcoholic drinks per day on weekdays and weekends), from which the total units/week were derived and then grouped to avoid categories with small numbers (never, 1-2 units/week, 3-4 units/week, ≥5 units/week). At 18 weeks of gestation mothers were asked to recall their alcohol consumption just before the current pregnancy (derived categories: never, <1 unit/week, 1-6 units/week, >7 units/week) and in the first 3 months of pregnancy (never, <1 unit/week, >1 unit/week). (Asking about drinking habits just before pregnancy is likely to capture intake in week 12 months. Atopy at 7 years was defined as a positive reaction (any detectable well) to Dermatophagoides pteronyssinus, cat, or grass (after subtracting positive saline reactions from histamine and allergen wheals, and excluding children unreactive to 1% histamine). (Atopy defined in this way identified 96% of children sensitized to 26 other allergens in this cohort.) Serum total IgE (in kU/L) was measured by fluoroluminun assay with the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Confounders
In multivariate analyses we controlled for the following potential confounders: maternal factors during pregnancy (smoking, infections, anxiety score, antibiotic use, and paracetamol use); other maternal factors (educational level; housing tenure; financial difficulties; body mass index; age; parity; history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length; postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, antibiotic and paracetamol use in infancy, number of younger siblings, body mass index at age 7).

Genotyping
The majority of maternal DNA samples were extracted from whole blood and white cells taken during pregnancy, and a minority were buccal DNA extracted from mouthwash samples. A single nucleotide polymorphism (rs1229984 in ADH1B) was typed by KBiosciences Ltd (Hoddesdon, Herts, United Kingdom; www.kbioscience.co.uk) with a competitive allele-specific PCR system (KASPar). The genotype distribution was compatible with no departure from Hardy-Weinberg equilibrium, and the genotyping success rate was >93.3% and error rate <0.25%.

Statistical analyses
Although the ALSPAC population is largely white, we adopted 2 strategies to reduce the possibility of confounding by population substructure, because the rs1229984 variant is highly ethnically stratified. First, mother-child pairs were excluded from all analyses if the mother’s reported ethnicity was non-white or unknown. Second, to address possible residual confounding by population substructure, we controlled for 10 variables derived by principal components analysis (PCA) from ALSPAC genome-wide association study (GWAS) data.

We used logistic regression to analyze relations of maternal ADH1B genotype with binary outcomes and linear regression to analyze associations with log-transformed total IgE. Analyses of reported alcohol intake in pregnancy were performed before and after controlling for confounders; tests for trend were performed by analyzing reported alcohol exposure associations as linear per category effects. In view of the rarity of the homozygous mutant genotype (A:A), persons with this genotype were grouped together with heterozygotes (G:A), and we assumed a dominant effect for analysis. Previous analyses of this single nucleotide polymorphism in ALSPAC mothers in relation to alcohol intake suggested that this was appropriate. We also performed a priori stratified analyses to explore potential interactions between maternal ADH1B genotype and reported alcohol intake in pregnancy. All analyses were performed with Stata version 10.1 (Stata Corporation, College Station, Tex).

RESULTS
Table I shows the association between reported alcohol intake in the last 2 months of pregnancy and other maternal characteristics among mothers for whom data were complete for at least 1 childhood atopic outcome. Mothers who reported drinking alcohol were more likely than mothers who reported never drinking to be older, better educated, financially better off, and to have taken paracetamol in pregnancy. In contrast, Table II shows that the distribution of maternal ADH1B genotype broadly does not vary according to maternal or offspring characteristics.
| Alcohol consumption (units/wk) | Never, no. (%) (N = 3780) | <1, no. (%) (N = 2947) | ≥1, no. (%) (N = 1475) |
|-------------------------------|-----------------------------|------------------------|------------------------|
| **Mother’s age**              |                             |                        |                        |
| <20 y                         | 108 (3)                     | 40 (1)                 | 9 (1)                  |
| 20-24 y                       | 684 (18)                    | 338 (11)               | 81 (5)                 |
| 25-29 y                       | 1564 (41)                   | 1258 (43)              | 466 (32)               |
| 30-34 y                       | 1077 (28)                   | 983 (33)               | 630 (43)               |
| ≥35 y                         | 347 (9)                     | 328 (11)               | 289 (20)               |
| **Parity**                    |                             |                        |                        |
| 0                             | 1727 (46)                   | 1284 (44)              | 619 (42)               |
| 1                             | 1255 (33)                   | 1095 (37)              | 527 (36)               |
| ≥2                            | 716 (19)                    | 510 (17)               | 299 (20)               |
| Unknown                       | 82 (2)                      | 58 (2)                 | 30 (2)                 |
| **Maternal body mass index (kg/m²)** |                       |                        |                        |
| <18.5                         | 171 (5)                     | 109 (4)                | 54 (4)                 |
| 18.5-24.99                    | 2527 (67)                   | 2108 (72)              | 1083 (73)              |
| 25-29.99                      | 549 (15)                    | 403 (14)               | 188 (13)               |
| ≥30                           | 217 (6)                     | 116 (4)                | 53 (4)                 |
| Unknown                       | 316 (8)                     | 211 (7)                | 97 (7)                 |
| **Mother’s education level**  |                             |                        |                        |
| <Ordinary level               | 1098 (29)                   | 558 (19)               | 225 (15)               |
| Ordinary level                | 1468 (39)                   | 1089 (37)              | 387 (26)               |
| ≥Advanced level               | 1198 (32)                   | 1292 (44)              | 861 (58)               |
| Unknown                       | 16 (<1)                     | 8 (<1)                 | 2 (<1)                 |
| **Housing tenure**            |                             |                        |                        |
| Owned/mortgaged               | 2915 (77)                   | 2477 (84)              | 1261 (85)              |
| Rented (public housing)       | 436 (12)                    | 194 (7)                | 64 (4)                 |
| Rented (nonpublic housing)    | 257 (7)                     | 152 (5)                | 99 (7)                 |
| Unknown                       | 172 (5)                     | 124 (4)                | 51 (3)                 |
| **Financial difficulties**    |                             |                        |                        |
| None                          | 1425 (38)                   | 1111 (38)              | 657 (45)               |
| Some                          | 1337 (35)                   | 1144 (39)              | 499 (34)               |
| Many                          | 904 (24)                    | 627 (21)               | 279 (19)               |
| Unknown                       | 114 (3)                     | 65 (2)                 | 40 (3)                 |
| **Maternal asthma**           |                             |                        |                        |
| 246 (7)                       | 175 (6)                     | 81 (5)                 |
| **Maternal eczema**           |                             |                        |                        |
| 426 (11)                      | 358 (12)                    | 179 (12)               |
| **Maternal rhinoconjunctivitis** |                       |                        |                        |
| 665 (18)                      | 585 (20)                    | 292 (20)               |
| **Maternal migraine**         |                             |                        |                        |
| 577 (15)                      | 398 (14)                    | 188 (13)               |
| **Maternal anxiety score in pregnancy** |                       |                        |                        |
| 0-4                           | 1920 (51)                   | 1514 (51)              | 757 (51)               |
| 5-9                           | 1218 (32)                   | 951 (32)               | 468 (32)               |
| ≥10                           | 335 (9)                     | 269 (9)                | 145 (10)               |
| Unknown                       | 307 (8)                     | 213 (7)                | 105 (7)                |
| **Prenatal tobacco exposure (maximum in pregnancy)** |                       |                        |                        |
| Not exposed                   | 1659 (44)                   | 1436 (49)              | 695 (47)               |
| Passive only                  | 1267 (34)                   | 967 (33)               | 472 (32)               |
| Mother 1-9/d                  | 283 (7)                     | 225 (8)                | 145 (10)               |
| Mother 10-19/d                | 395 (10)                    | 211 (7)                | 101 (7)                |
| Mother ≥20/d                  | 158 (4)                     | 97 (3)                 | 54 (4)                 |
| Unknown                       | 18 (<1)                     | 11 (<1)                | 8 (1)                  |
| **Maternal paracetamol use in early pregnancy** |                       |                        |                        |
| Nonuser                       | 1873 (50)                   | 1260 (43)              | 600 (41)               |
| User                          | 1850 (49)                   | 1654 (56)              | 857 (58)               |
| Unknown                       | 57 (2)                      | 33 (1)                 | 18 (1)                 |
| **Maternal paracetamol use in late pregnancy** |                       |                        |                        |
| Nonuser                       | 2219 (59)                   | 1580 (54)              | 774 (52)               |
| User                          | 1447 (38)                   | 1296 (44)              | 662 (45)               |
| Unknown                       | 114 (3)                     | 71 (2)                 | 39 (3)                 |
| **Antibiotic use in late pregnancy** |                       |                        |                        |
| Not exposed                   | 203 (5)                     | 209 (7)                | 93 (6)                 |
| Maternal cold/flu in pregnancy | 1478 (39)                   | 1230 (42)              | 659 (45)               |
| Maternal urinary infection in pregnancy | 198 (5)                     | 157 (5)                | 79 (5)                 |
| Maternal other infections in pregnancy | 185 (5)                     | 191 (6)                | 97 (7)                 |

Includes all women with complete data for at least 1 childhood outcome (wheeze, eczema, hay fever, asthma, atopy, IgE) and self-reported alcohol consumption in the last 2 months of pregnancy.
TABLE II. Maternal characteristics by maternal ADH1B genotype

| ADH1B genotype | GG, no. (%) | AA/GA, no. (%) |
|----------------|-------------|---------------|
| (N = 5033)     | (N = 268)   |

- **Mother’s age**
  - <20 y: 86 (2) 3 (1)
  - 20-24 y: 671 (13) 37 (14)
  - 25-29 y: 2056 (41) 105 (39)
  - 30-34 y: 1657 (33) 93 (35)
  - ≥35 y: 563 (11) 30 (11)

- **Parity**
  - 0: 2275 (45) 118 (44)
  - 1: 1750 (35) 92 (34)
  - ≥2: 909 (18) 52 (19)
  - Unknown: 99 (2) 6 (2)

- **Maternal body mass index (kg/m²)**
  - <18.5: 208 (4) 14 (5)
  - 18.5-24.99: 3460 (69) 192 (72)
  - 25-29.99: 726 (14) 35 (13)
  - ≥30: 248 (5) 9 (3)
  - Unknown: 391 (8) 18 (7)

- **Mother’s education level**
  - <Ordinary level: 1186 (24) 47 (18)
  - Ordinary level: 1811 (36) 108 (40)
  - ≥Advanced level: 2023 (40) 113 (42)
  - Unknown: 13 (<1) 0 (0)

- **Housing tenure**
  - Owned/mortgaged: 4093 (81) 222 (83)
  - Rented (public housing): 439 (9) 23 (9)
  - Rented (nonpublic housing): 297 (6) 16 (6)
  - Unknown/other: 204 (4) 7 (3)

- **Financial difficulties**
  - None: 1957 (39) 104 (39)
  - Some: 1816 (36) 93 (35)
  - Many: 1127 (22) 66 (25)
  - Unknown: 133 (3) 5 (2)

- **Maternal other infections in pregnancy**
  - None: 314 (6) 16 (6)
  - Maternal cold/flu in pregnancy: 2060 (41) 105 (39)
  - Maternal urinary infection in pregnancy: 274 (5) 10 (4)
  - Maternal other infections in pregnancy: 292 (6) 12 (4)

- **Antibiotic use in early pregnancy**
  - Non user: 2275 (45) 118 (44)
  - User: 2691 (53) 133 (50)
  - Unknown: 75 (1) 3 (1)

(Continued)
TABLE III. Associations between self-reported alcohol consumption before and during pregnancy and childhood asthma

| Mother’s alcohol intake before pregnancy (units/wk) | Univariate analysis, OR (95% CI) | Multivariate analysis, OR (95% CI) |
|--------------------------------------------------|---------------------------------|----------------------------------|
| Never (n = 446)                                  | 1.0                             | 1.0                              |
| <1 (n = 2860)                                    | 0.88 (0.66-1.18)                | 0.93 (0.69-1.26)                 |
| 1-6 (n = 3410)                                   | 0.85 (0.64-1.14)                | 0.92 (0.68-1.25)                 |
| ≥7 (n = 890)                                     | 0.81 (0.57-1.14)                | 0.84 (0.59-1.21)                 |
| Test for trend (N = 7606)                        | 0.95 (0.86-1.04)*               | 0.96 (0.87-1.05)*                |
| P value                                          | .229                            | .383                             |

Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)

| Never (n = 4728)                                  | 1.0                             | 1.0                              |
| 1-2 (n = 973)                                     | 0.97 (0.78-1.20)                | 0.96 (0.77-1.20)                 |
| 3-4 (n = 524)                                     | 1.06 (0.81-1.39)                | 1.03 (0.78-1.37)                 |
| ≥5 (n = 659)                                     | 1.02 (0.79-1.30)                | 0.93 (0.71-1.20)                 |
| Test for trend (N = 6884)                        | 1.00 (0.94-1.09)*               | 0.98 (0.91-1.06)*                |
| P value                                          | .808                            | .667                             |

Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)

| Never (n = 3356)                                  | 1.0                             | 1.0                              |
| <1 (n = 3088)                                     | 0.96 (0.82-1.11)                | 0.91 (0.78-1.06)                 |
| ≥1 (n = 1148)                                     | 0.89 (0.72-1.09)                | 0.81 (0.65-1.00)                 |
| Test for trend (N = 7592)                        | 0.95 (0.86-1.04)*               | 0.90 (0.81-0.99)*                |
| P value                                          | .262                            | .047                             |

Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)

| Never (n = 3410)                                  | 1.0                             | 1.0                              |
| <1 (n = 2668)                                     | 0.92 (0.79-1.08)                | 0.92 (0.78-1.08)                 |
| ≥1 (n = 1327)                                     | 0.83 (0.68-1.02)                | 0.83 (0.67-1.02)                 |
| Test for trend (N = 7405)                        | 0.91 (0.83-1.00)*               | 0.91 (0.82-1.01)*                |
| P value                                          | .062                            | .074                             |

Mother’s binge drinking (days in previous month at 18 wk of pregnancy)

| Never (n = 6358)                                  | 1.0                             | 1.0                              |
| 1-2 (n = 630)                                     | 1.11 (0.87-1.42)                | 0.95 (0.74-1.23)                 |
| ≥3 (n = 516)                                     | 0.98 (0.75-1.30)                | 0.84 (0.63-1.12)                 |
| Test for trend (N = 7504)                        | 1.02 (0.90-1.15)*               | 0.92 (0.81-1.05)*                |
| P value                                          | .783                            | .235                             |

OR, Odds ratio.
*OR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.

maternal ADH1B and reported intake of alcohol in pregnancy; such an interaction, with a weaker or absent effect of the gene in mothers reporting no alcohol intake, would have been expected if there was a causal effect of prenatal alcohol exposure.33 The lack of evidence of association between maternal ADH1B genotype and atopic outcomes in the offspring also suggests that maternal consumption of alcohol before, or periconception, is unlikely to influence childhood asthma and allergies; in ALSPAC the same nonsynonymous variant of ADH1B is strongly associated with alcohol use just before, as well as during, pregnancy.28

Given the principles underpinning Mendelian randomization,34,35 our genetic findings are much less likely to be confounded by lifestyle or environmental factors than the weak negative associations we observed between reported maternal alcohol intake in late pregnancy and risk of asthma and hay fever in the offspring. Mothers who drank alcohol in pregnancy tended to be older, better educated, and financially better off than mothers who abstained. Although we controlled for a large number of socioeconomic and other background characteristics in the analyses of reported maternal drinking, the possibility of residual or uncontrolled confounding remains. Furthermore, the negative associations between reported maternal intake and childhood asthma and hay fever conflict with the few previous epidemiologic studies that have suggested a positive relation between prenatal

TABLE IV. Associations between self-reported alcohol consumption before and during pregnancy and childhood hay fever

| Mother’s alcohol intake before pregnancy (units/wk) | Univariate analysis, OR (95% CI) | Multivariate analysis, OR (95% CI) |
|--------------------------------------------------|---------------------------------|----------------------------------|
| Never (n = 449)                                  | 1.0                             | 1.0                              |
| <1 (n = 2875)                                    | 1.28 (0.87-1.87)                | 1.27 (0.86-1.89)                 |
| 1-6 (n = 3428)                                   | 1.26 (0.87-1.84)                | 1.17 (0.79-1.73)                 |
| ≥7 (n = 903)                                     | 1.15 (0.74-1.77)                | 1.03 (0.66-1.62)                 |
| Test for trend (N = 7655)                        | 1.0 (0.90-1.11)*               | 0.96 (0.86-1.07)*                |
| P value                                          | .944                            | .437                             |

Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)

| Never (n = 4753)                                  | 1.0                             | 1.0                              |
| 1-2 (n = 977)                                     | 1.02 (0.81-1.30)                | 0.95 (0.74-1.22)                 |
| 3-4 (n = 530)                                     | 1.02 (0.74-1.39)                | 1.04 (0.75-1.44)                 |
| ≥5 (n = 670)                                     | 0.70 (0.51-0.97)                | 0.71 (0.50-0.99)                 |
| Test for trend (N = 6930)                        | 0.93 (0.85-1.01)*               | 0.93 (0.84-1.02)*                |
| P value                                          | .095                            | .109                             |

Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)

| Never (n = 3370)                                  | 1.0                             | 1.0                              |
| <1 (n = 3100)                                     | 0.83 (0.70-0.99)                | 0.84 (0.70-1.01)                 |
| ≥1 (n = 1172)                                     | 0.88 (0.70-1.11)                | 0.91 (0.71-1.17)                 |
| Test for trend (N = 7642)                        | 0.91 (0.81-1.02)*               | 0.93 (0.82-1.04)*                |
| P value                                          | .103                            | .203                             |

Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)

| Never (n = 3434)                                  | 1.0                             | 1.0                              |
| <1 (n = 2678)                                     | 0.92 (0.77-1.09)                | 0.85 (0.71-1.03)                 |
| ≥1 (n = 1346)                                     | 0.83 (0.66-1.05)                | 0.77 (0.60-0.99)                 |
| Test for trend (N = 7458)                        | 0.91 (0.82-1.02)*               | 0.87 (0.78-0.98)*                |
| P value                                          | .101                            | .024                             |

Mother’s binge drinking (days in previous month at 18 wk of pregnancy)

| None (n = 6396)                                  | 1.0                             | 1.0                              |
| 1-2 (n = 633)                                     | 0.91 (0.68-1.23)                | 0.96 (0.71-1.31)                 |
| ≥3 (n = 523)                                     | 0.73 (0.51-1.04)                | 0.78 (0.54-1.33)                 |
| Test for trend (N = 7552)                        | 0.87 (0.74-1.02)*               | 0.90 (0.77-1.06)*                |
| P value                                          | .079                            | .219                             |

OR, Odds ratio.
*OR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.
alcohol exposure and atopic outcomes. Although there is some evidence for a protective effect of alcohol on allergic airway inflammation and airway hyperresponsiveness in an adult mouse model of allergic asthma, the mechanisms underlying such an effect and the relevance of these observations to the inception of asthma after prenatal exposure in humans are unclear. In addition, other animal data indicate detrimental effects of prenatal exposure on fetal lung growth and development. The findings for hay fever, if real, would be expected to be mediated through an effect on atopy, yet we did not find similar negative associations between reported intake and atopy or IgE. We therefore think it is unlikely that prenatal alcohol exposure in pregnancy protects against asthma and other atopic disease.

In ALSPAC, mothers carrying the minor A allele consumed less alcohol during pregnancy. Previous in vitro data suggested that carriers of this allele metabolize alcohol up to 100 times faster than persons homozygous for the G allele and consequently consume less alcohol to avoid the negative effects of high acetaldehyde concentrations; this in turn would reduce exposure of the fetus to alcohol, which readily crosses the placenta. Although a more recent study has suggested that this variant makes a minor contribution to the variation in alcohol metabolism in vivo, lack of power because of the variant’s low frequency in European populations is the most likely explanation for this.

The utility of ADH1B genotype as a Mendelian randomization instrument has been clearly demonstrated in relation to alcohol-induced cancer.

**Strengths and limitations**

Our study has a number of strengths, including ALSPAC’s population-based prospective design and rich data on maternal reported alcohol use in pregnancy, potential confounders and detailed phenotypic outcome measurements. In addition, few other birth cohorts have maternal DNA available. Measurement of maternal ADH1B genotype enabled us to use a Mendelian randomization approach to investigate the relation between prenatal alcohol exposure and atopic outcomes. In contrast, most previous epidemiologic studies of the effects of maternal alcohol use in pregnancy on offspring health have relied on reported consumption, an approach which is highly susceptible to bias and confounding. Although there was potential for associations with ADH1B genotype to be confounded by population substructure, we reduced this possibility by excluding those with reported non-white ethnicity and by performing sensitivity analyses in which we controlled for PCA variables derived from GWAS data.

**TABLE V. Associations between maternal ADH1B genotype and childhood atopic outcomes**

| Outcome by ADH1B genotype | No. | Univariate maternal effect estimate* (95% CI) | No. of reduced data set† | Univariate maternal effect estimate* (95% CI) | Multivariate maternal effect estimate* (95% CI) |
|---------------------------|-----|---------------------------------------------|--------------------------|---------------------------------------------|---------------------------------------------|
| Asthma                    |     |                                             |                          |                                             |                                             |
| G:G                       | 4524| 1.0                                         | 3266                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 231 | 0.98 (0.66-1.47)                            | 171                      | 0.86 (0.53-1.41)                            | 0.90 (0.54-1.51)                            |
| Total                     | 4755|                                            | 3437                     |                                             |                                             |
| P value                   |     |                                             |                          | .937                                        | .571                                        | .699                                        |
| Wheezing                  |     |                                             |                          |                                             |                                             |
| G:G                       | 4575| 1.0                                         | 3280                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 237 | 1.12 (0.75-1.67)                            | 171                      | 1.04 (0.64-1.68)                            | 1.08 (0.65-1.79)                            |
| Total                     | 4812|                                            | 3451                     |                                             |                                             |
| P value                   |     |                                             |                          | .582                                        | .867                                        | .754                                        |
| Eczema                    |     |                                             |                          |                                             |                                             |
| G:G                       | 4567| 1.0                                         | 3295                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 234 | 1.01 (0.71-1.43)                            | 170                      | 1.07 (0.72-1.60)                            | 1.14 (0.75-1.73)                            |
| Total                     | 4801|                                            | 3465                     |                                             |                                             |
| P value                   |     |                                             |                          | .961                                        | .730                                        | .539                                        |
| Hay fever                 |     |                                             |                          |                                             |                                             |
| G:G                       | 4556| 1.0                                         | 3248                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 236 | 1.11 (0.71-1.72)                            | 171                      | 1.12 (0.67-1.87)                            | 1.15 (0.67-1.97)                            |
| Total                     | 4792|                                            | 3419                     |                                             |                                             |
| P value                   |     |                                             |                          | .653                                        | .673                                        | .616                                        |
| Atopy                     |     |                                             |                          |                                             |                                             |
| G:G                       | 3571| 1.0                                         | 2811                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 193 | 1.09 (0.77-1.55)                            | 152                      | 1.12 (0.76-1.65)                            | 1.23 (0.82-1.84)                            |
| Total                     | 3764|                                            | 2963                     |                                             |                                             |
| P value                   |     |                                             |                          | .610                                        | .571                                        | .327                                        |
| IgE                       |     |                                             |                          |                                             |                                             |
| G:G                       | 2852| 1.0                                         | 2404                     | 1.0                                         | 1.0                                         |
| A:A/G:A                  | 158 | 1.07 (0.82-1.40)                            | 133                      | 0.97 (0.72-1.30)                            | 0.96 (0.72-1.30)                            |
| Total                     | 3010|                                            | 2537                     |                                             |                                             |
| P value                   |     |                                             |                          | .606                                        | .815                                        | .806                                        |

OR, Odds ratio.

*OR (geometric mean ratio for IgE analysis).
†Data set reduced because of incomplete PCA variables derived from GWAS data.
interactions with reported alcohol intake. We also accept that we have not taken into account the ability of the fetus to metabolize alcohol and hence the potential effect of gene variants in the child on atopic outcomes. Nevertheless, we would argue that the maternal ADH1B gene is likely to have a greater influence on fetal alcohol exposure because it is so strongly associated with maternal drinking behavior. As with any longitudinal study, data were not complete on exposures, outcomes, and confounders for the whole cohort. Therefore, we cannot rule out the possibility that exclusion of children without complete information might have biased our findings for reported alcohol intake; however, importantly, it is unlikely that missing genotype data would bias the main genetic results.

Public health implications

In conclusion, with the use of a Mendelian randomization approach, we have not found evidence to suggest that alcohol consumption in pregnancy increases the risk of childhood atopic outcomes. Nor did associations with reported intake suggest increased risks. Nevertheless, because of other potential risks to the developing fetus of low-level alcohol consumption, pregnant women should still heed current advice to abstain from consuming alcohol.38,39

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

Clinical implications: Although pregnant women should be advised to abstain from alcohol, this study has reassuringly found no evidence that alcohol exposure in utero increases the risk of atopic disease in the offspring.

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| Outcome by ADH1B genotype | Mother’s alcohol intake (units/wk) never | Mother’s alcohol intake (units/wk) ≥1 |
|---------------------------|----------------------------------------|-------------------------------------|
|                           | No. | Adjusted estimate* (95% CI) | No. | Adjusted estimate* (95% CI) |
| Asthma†                   |     |                          |     |                          |
| G:G                       | 2001| 1.0                      | 2372| 1.0                      |
| G:A/A:A                  | 121 | 0.94 (0.53-1.68)         | 103 | 1.14 (0.62-2.10)         |
| Total                     | 4597|                          |      |                          |
| Wheezing‡                 |     |                          |     |                          |
| G:G                       | 2024| 1.0                      | 2400| 1.0                      |
| G:A/A:A                  | 124 | 1.26 (0.72-2.19)         | 105 | 0.87 (0.43-1.73)         |
| Total                     | 4653|                          |      |                          |
| Eczema§                   |     |                          |     |                          |
| G:G                       | 2018| 1.0                      | 2397| 1.0                      |
| G:A/A:A                  | 121 | 0.89 (0.52-1.52)         | 105 | 1.23 (0.75-2.02)         |
| Total                     | 4641|                          |      |                          |
| Hay fever||                 |     |                          |     |                          |
| G:G                       | 2017| 1.0                      | 2388| 1.0                      |
| G:A/A:A                  | 124 | 0.98 (0.52-1.87)         | 104 | 1.23 (0.62-2.46)         |
| Total                     | 4633|                          |      |                          |
| Hay fever||                 |     |                          |     |                          |
| G:G                       | 1538| 1.0                      | 1894| 1.0                      |
| G:A/A:A                  | 102 | 1.29 (0.80-2.09)         | 83  | 1.12 (0.65-1.95)         |
| Total                     | 3617|                          |      |                          |
| Atopy¶                    |     |                          |     |                          |
| G:G                       | 1191| 1.0                      | 1557| 1.0                      |
| G:A/A:A                  | 85  | 1.23 (0.85-1.78)         | 70  | 0.91 (0.61-1.36)         |
| Total                     | 2903|                          |      |                          |

GMR, Geometric mean ratio.
*Estimates (OR [GMR for IgE analysis]) are adjusted for all confounders listed in Methods section.
†P for interaction = .664.
‡P for interaction = .384.
§P for interaction = .636.
∥P for interaction = .710.
¶P for interaction = .288.
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**TABLE E1.** Association between maternal \(\text{ADH1B}\) genotype and reported maternal alcohol intake before and during pregnancy

| ADH1B genotype | GG, No. (%) (N = 5033) | GA/AA, No. (%) (N = 268) |
|----------------|-------------------------|--------------------------|
| **Mother’s alcohol intake before pregnancy (units/wk)** | | |
| Never | 275 (5) | 18 (7) |
| <1 | 1820 (36) | 126 (47) |
| 1-6 | 2273 (45) | 102 (38) |
| ≥7 | 599 (12) | 20 (7) |
| Unknown | 66 (1) | 2 (1) |
| **Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)** | | |
| Never | 3074 (61) | 197 (74) |
| 1-2 | 686 (14) | 30 (11) |
| 3-4 | 342 (7) | 17 (6) |
| ≥5 | 454 (9) | 9 (3) |
| Unknown | 477 (9) | 15 (6) |
| **Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)** | | |
| Never | 2130 (42) | 138 (51) |
| <1 | 2035 (40) | 99 (37) |
| ≥1 | 795 (16) | 27 (10) |
| Unknown | 73 (1) | 4 (1) |
| **Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)** | | |
| Never | 2211 (44) | 143 (53) |
| <1 | 1722 (34) | 81 (30) |
| ≥1 | 898 (18) | 34 (13) |
| Unknown | 202 (4) | 10 (4) |
| **Mother’s binge drinking (days in previous month at 18 wk of pregnancy)** | | |
| None | 4164 (83) | 237 (88) |
| 1-2 | 441 (9) | 14 (5) |
| ≥3 | 351 (7) | 13 (5) |
| Unknown | 77 (2) | 4 (1) |

Includes all women with complete data for at least 1 childhood outcome and ADH1B genotype.
| Table E2. Associations between self-reported alcohol consumption before and during pregnancy and childhood wheezing |
|--------------------------------------------------|
| **Mother’s alcohol intake before pregnancy (units/wk)** |
| **Never (n = 452)** | 1.0 | 1.0 |
| <1 (n = 2888) | 0.91 (0.66-1.24) | 0.96 (0.69-1.32) |
| ≥1-6 (n = 3443) | 0.88 (0.65-1.20) | 0.92 (0.67-1.28) |
| ≥7 (n = 904) | 0.96 (0.67-1.37) | 0.95 (0.65-1.38) |
| Test for trend (N = 7687) | 0.99 (0.90-1.09) | 0.98 (0.89-1.08) |
| **P value** | .820 | .703 |
| **Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)** |
| **Never (n = 4779)** | 1.0 | 1.0 |
| 1-2 (n = 981) | 1.08 (0.87-1.34) | 1.03 (0.82-1.30) |
| 3-4 (n = 530) | 1.12 (0.85-1.49) | 1.08 (0.80-1.44) |
| ≥5 (n = 671) | 1.05 (0.81-1.37) | 0.95 (0.73-1.25) |
| Test for trend (N = 6961) | 1.03 (0.96-1.11)* | 0.99 (0.92-1.08)* |
| **P value** | .439 | .957 |
| **Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)** |
| **Never (n = 3386)** | 1.0 | 1.0 |
| <1 (n = 3113) | 1.04 (0.89-1.22) | 0.98 (0.83-1.16) |
| ≥1 (n = 1174) | 1.05 (0.85-1.30) | 0.95 (0.76-1.19) |
| Test for trend (N = 7673) | 1.03 (0.93-1.14)* | 0.98 (0.88-1.09)* |
| **P value** | .575 | .664 |
| **Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)** |
| **Never (n = 3445)** | 1.0 | 1.0 |
| <1 (n = 2696) | 0.94 (0.80-1.11) | 0.91 (0.76-1.08) |
| ≥1 (n = 1346) | 0.98 (0.80-1.20) | 0.92 (0.74-1.14) |
| Test for trend (N = 7487) | 0.98 (0.89-1.08)* | 0.95 (0.85-1.06)* |
| **P value** | .706 | .335 |
| **Mother’s binge drinking (days in previous month at 18 wk of pregnancy)** |
| **None (n = 6423)** | 1.0 | 1.0 |
| 1-2 (n = 637) | 0.97 (0.74-1.27) | 0.85 (0.64-1.13) |
| ≥3 (n = 523) | 1.12 (0.85-1.49) | 0.99 (0.74-1.33) |
| Test for trend (N = 7583) | 1.04 (0.92-1.18)* | 0.97 (0.84-1.11)* |
| **P value** | .536 | .619 |

**OR:** Odds ratio.

*OR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.
### TABLE E3. Associations between self-reported alcohol consumption before and during pregnancy and childhood eczema

|                           | Univariate analysis, OR (95% CI) | Multivariate analysis, OR (95% CI) |
|---------------------------|----------------------------------|-----------------------------------|
| **Mother’s alcohol intake before pregnancy (units/wk)** |                                   |                                   |
| Never (n = 451)           | 1.0                              | 1.0                               |
| <1 (n = 2880)             | 1.08 (0.82-1.43)                 | 1.03 (0.77-1.36)                  |
| 1-6 (n = 3438)            | 1.11 (0.85-1.46)                 | 1.03 (0.78-1.37)                  |
| ≥7 (n = 904)              | 1.13 (0.83-1.54)                 | 0.99 (0.72-1.38)                  |
| Test for trend (N = 7673) | 1.03 (0.95-1.12)                 | 0.99 (0.92-1.09)                  |
| P value                   | .438                             | .970                              |
| **Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)** |                                   |                                   |
| Never (n = 4766)          | 1.0                              | 1.0                               |
| 1-2 (n = 980)             | 1.07 (0.89-1.29)                 | 1.04 (0.86-1.26)                  |
| 3-4 (n = 530)             | 1.00 (0.79-1.28)                 | 1.02 (0.79-1.32)                  |
| ≥5 (n = 670)              | 1.13 (0.91-1.40)                 | 1.12 (0.90-1.40)                  |
| Test for trend (N = 6946) | 1.03 (0.97-1.10)*                | 1.03 (0.97-1.10)*                 |
| P value                   | .303                             | .343                              |
| **Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)** |                                   |                                   |
| Never (n = 3379)          | 1.0                              | 1.0                               |
| <1 (n = 3107)             | 0.99 (0.87-1.13)                 | 0.98 (0.86-1.13)                  |
| ≥1 (n = 1174)             | 1.06 (0.89-1.26)                 | 1.08 (0.90-1.31)                  |
| Test for trend (N = 7660) | 1.02 (0.94-1.11)*                | 1.03 (0.94-1.12)*                 |
| P value                   | .649                             | .554                              |
| **Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)** |                                   |                                   |
| Never (n = 3436)          | 1.0                              | 1.0                               |
| <1 (n = 2692)             | 1.03 (0.90-1.18)                 | 0.98 (0.85-1.13)                  |
| ≥1 (n = 1346)             | 1.18 (0.99-1.39)                 | 1.11 (0.93-1.33)                  |
| Test for trend (N = 7474) | 1.08 (0.99-1.17)*                | 1.04 (0.95-1.14)*                 |
| P value                   | .077                             | .356                              |
| **Mother’s binge drinking (days in previous month at 18 wk of pregnancy)** |                                   |                                   |
| None (n = 6411)           | 1.0                              | 1.0                               |
| 1-2 (n = 637)             | 0.84 (0.66-1.05)                 | 0.84 (0.66-1.07)                  |
| ≥3 (n = 522)              | 0.92 (0.72-1.18)                 | 0.93 (0.72-1.20)                  |
| Test for trend (N = 7570) | 0.93 (0.83-1.04)*                | 0.94 (0.83-1.06)*                 |
| P value                   | .227                             | .297                              |

OR, Odds ratio.

*OR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.
### TABLE E4. Associations between self-reported alcohol consumption before and during pregnancy and childhood atopy

|                                      | Univariate analysis, OR (95% CI) | Multivariate analysis, OR (95% CI) |
|--------------------------------------|----------------------------------|-----------------------------------|
| **Mothers alcohol intake before pregnancy (units/wk)** |                                  |                                   |
| Never (n = 330)                      | 1.0                              | 1.0                               |
| <1 (n = 2287)                       | 1.12 (0.83-1.51)                 | 1.09 (0.80-1.48)                 |
| 1-6 (n = 2717)                      | 1.21 (0.90-1.62)                 | 1.08 (0.80-1.47)                 |
| ≥7 (n = 725)                        | 1.40 (1.01-1.94)                 | 1.22 (0.87-1.73)                 |
| Test of trend (N = 6059)            | 1.11 (1.02-1.20)*                | 1.05 (0.96-1.14)*                |
| P value                             | .014                             | .304                              |
| **Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)** |                                  |                                   |
| Never (n = 3713)                    | 1.0                              | 1.0                               |
| 1-2 (n = 780)                       | 1.07 (0.89-1.29)                 | 1.05 (0.86-1.27)                 |
| 3-4 (n = 427)                       | 0.99 (0.78-1.27)                 | 1.01 (0.78-1.30)                 |
| ≥5 (n = 525)                        | 0.80 (0.63-1.01)                 | 0.80 (0.63-1.03)                 |
| Test for trend (N = 5445)           | 0.95 (0.89-1.02)*                | 0.95 (0.89-1.02)*                |
| P value                             | .167                             | .198                              |
| **Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)** |                                  |                                   |
| Never (n = 2614)                    | 1.0                              | 1.0                               |
| <1 (n = 2502)                       | 0.98 (0.86-1.12)                 | 1.00 (0.87-1.15)                 |
| ≥1 (n = 939)                        | 0.96 (0.80-1.15)                 | 0.96 (0.79-1.17)                 |
| Test for trend (N = 6055)           | 0.98 (0.90-1.07)*                | 0.99 (0.90-1.08)*                |
| P value                             | .639                             | .776                              |
| **Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)** |                                  |                                   |
| Never (n = 2615)                    | 1.0                              | 1.0                               |
| <1 (n = 2133)                       | 1.04 (0.90-1.19)                 | 1.01 (0.87-1.16)                 |
| ≥1 (n = 1098)                       | 0.99 (0.83-1.18)                 | 0.92 (0.76-1.10)                 |
| Test for trend (N = 5846)           | 1.0 (0.92-1.09)*                 | 0.96 (0.88-1.15)*                |
| P value                             | .965                             | .429                              |
| **Mother’s binge drinking (days in previous month at 18 wk of pregnancy)** |                                  |                                   |
| None (n = 5021)                     | 1.0                              | 1.0                               |
| 1-2 (n = 527)                       | 0.96 (0.77-1.20)                 | 0.99 (0.78-1.24)                 |
| ≥3 (n = 419)                        | 0.80 (0.61-1.03)                 | 0.86 (0.66-1.13)                 |
| Test for trend (N = 5967)           | 0.91 (0.81-1.02)*                | 0.94 (0.83-1.06)*                |
| P value                             | .101                             | .319                              |

OR, Odds ratio.

*OR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.
TABLE E5. Associations between self-reported alcohol consumption before and during pregnancy and childhood IgE

|                          | Univariate analysis, GMR (95% CI) | Multivariate analysis, GMR (95% CI) |
|--------------------------|-----------------------------------|------------------------------------|
| **Mother’s alcohol intake before pregnancy (units/wk)** |                                   |                                    |
| Never (n = 254)          | 1.0                               | 1.0                                |
| <1 (n = 1816)            | 1.07 (0.86-1.34)                  | 1.11 (0.89-1.38)                   |
| 1-6 (n = 2179)           | 1.09 (0.88-1.35)                  | 1.16 (0.93-1.44)                   |
| ≥7 (n = 554)             | 0.96 (0.75-1.23)                  | 1.04 (0.81-1.34)                   |
| Test for trend (N = 4803) | 0.98 (0.92-1.05)                  | 1.01 (0.95-1.08)                   |
| P value                  | .602                              | .715                               |
| **Mother’s current alcohol intake at 8 wk of pregnancy (units/wk)** |                                   |                                    |
| Never (n = 2920)         | 1.0                               | 1.0                                |
| 1-2 (n = 649)            | 1.0 (0.87-1.16)                   | 1.01 (0.87-1.16)                   |
| 3-4 (n = 345)            | 1.10 (0.91-1.32)                  | 1.08 (0.90-1.30)                   |
| ≥5 (n = 436)             | 1.08 (0.91-1.27)                  | 1.06 (0.89-1.25)                   |
| Test for trend (N = 4350) | 1.03 (0.98-1.08)*                 | 1.02 (0.97-1.08)*                  |
| P value                  | .268                              | .390                               |
| **Mother’s alcohol intake in first 3 mo of pregnancy (units/wk)** |                                   |                                    |
| Never (n = 2066)         | 1.0                               | 1.0                                |
| <1 (n = 2002)            | 1.10 (0.99-1.22)                  | 1.06 (0.96-1.18)                   |
| ≥1 (n = 731)             | 1.09 (0.95-1.26)                  | 1.06 (0.92-1.22)                   |
| Test for trend (N = 4799) | 1.06 (0.99-1.13)*                 | 1.04 (0.97-1.11)*                  |
| P value                  | .095                              | .306                               |
| **Mother’s alcohol intake during last 2 mo of pregnancy (units/wk)** |                                   |                                    |
| Never (n = 2033)         | 1.0                               | 1.0                                |
| <1 (n = 1730)            | 1.00 (0.90-1.12)                  | 0.98 (0.88-1.10)                   |
| ≥1 (n = 897)             | 1.08 (0.95-1.23)                  | 1.05 (0.92-1.20)                   |
| Test for trend (N = 4660) | 1.04 (0.97-1.10)*                 | 1.02 (0.95-1.09)*                  |
| P value                  | .283                              | .584                               |
| **Mother’s binge drinking (days in previous month at 18 wk of pregnancy)** |                                   |                                    |
| None (n = 3980)          | 1.0                               | 1.0                                |
| 1-2 (n = 426)            | 0.97 (0.82-1.15)                  | 0.93 (0.79-1.10)                   |
| ≥3 (n = 323)             | 0.93 (0.77-1.12)                  | 0.89 (0.73-1.07)                   |
| Test for trend (N = 4729) | 0.96 (0.89-1.05)*                 | 0.94 (0.86-1.02)*                  |
| P value                  | .403                              | .158                               |

GMR, Geometric mean ratio.
*GMR per category increase in exposure. Multivariate analysis of maternal alcohol intake adjusts for all confounders listed in Methods section.