The association of alcohol polygenic risk scores with mental health outcomes: A multi-generational analysis in the Avon Longitudinal Study of Parents and Children

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Abstract

*Background:* Increased alcohol consumption often co-occurs with mental health problems; however, we do not currently fully understand whether this is due to confounding, shared biological mechanisms, or causal effects. *Design:* We analysed a polygenic risk score (PRS) composed of single nucleotide polymorphisms (SNPs) reliably associated with patterns of adult alcohol consumption to test: 1) if this PRS is associated with consumption during pregnancy and adolescence, 2) if child alcohol PRS is associated with mental health phenotypes, and 3) if maternal alcohol PRS is associated with offspring alcohol phenotypes and mental health. We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC). Additional substance abuse behaviours and mental health/behavioural outcomes were also investigated at different life stages across both generations (alcohol phenotypes $n=22$; health phenotypes $n=91$). The availability of data from early life on the same participants (pre-alcohol use around ages 7-10 years) provided a negative control, in contrast to that in ages of alcohol use (13-24 years). *Findings:* The adult alcohol PRS was associated with consumption phenotypes during pregnancy (strongest signal for alcohol frequency at 18 weeks’ gestation: $p=1.01\times10^{-5}$) but offspring alcohol PRS did not predict offspring alcohol consumption at age 13-24 years. We found evidence for an association of maternal PRS with own perinatal depression ($p=0.02$) and decreased offspring intellectual ability ($p=0.016$). *Conclusions:* An alcohol PRS derived from GWAS of alcohol use in the general population was shown to be associated with frequency and amount of alcohol consumed during pregnancy, and maternal depression at 32 weeks gestation. The associations between alcohol PRS with mother’s depression and offspring intellectual ability are consistent with previous studies, adding to the validity of using this alcohol PRS in future aetiological studies.

*Keywords:* alcohol, mental health, polygenic risk score, PheWAS, ALSPAC
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Introduction

Alcohol use frequently co-occurs with mental health problems (1-3), but we do not yet fully understand the nature and extent of these associations. Previous research has suggested worse mental and physical health outcomes in offspring prenatally exposed to alcohol (4-6), but there is also evidence of a J-shaped relationship between pre- and post-natal maternal alcohol use and offspring mental health (7, 8). This might imply potential health benefits for offspring and mothers who consume moderate amounts of alcohol, such as reduced offspring behavioural problems (8-10). However, these apparent benefits may be due to confounding, rather than a causal effect of alcohol use (6). One way to address problems of residual confounding is to use genetic markers for alcohol use as proxies for the exposure (11, 12).

Genetic variants have been identified that are associated with increased alcohol consumption in adults (13), but to date there have been no genome-wide association studies (GWAS) of alcohol consumption in pregnant women. Maternal metabolism is altered during pregnancy to meet the physiological demands on the mother, as well as to promote healthy development of the fetus (14-16). In order to explore intergenerational effects of maternal alcohol use during pregnancy using GWAS results, SNPs identified in the general population require validation in pregnant women. For example, the \( ADH1B \) gene is involved in metabolizing ethanol and has been linked with alcohol dependence in adults (17). Zuccolo and colleagues confirmed that a functional variant in the \( ADH1B \) gene is also associated with alcohol consumption before and during pregnancy.

A recent study of 941,280 participants, discovered 99 genetic variants robustly associated with increased alcohol use (13). We combined these SNPs to create separate polygenic risk scores (PRS) from maternal and offspring genetic data to use as instrumental variables. It is currently unknown whether these PRS are suitable instruments for alcohol consumption during pregnancy and adolescence. Therefore, our first aim was to confirm that these SNPs (identified in a general population sample) show a similar pattern of association in
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pregnant women, and adolescence. Our second aim was to investigate if these alcohol PRS were associated with maternal and offspring mental health using a phenome wide association study (PheWAS) design. Our third aim was to examine the association between maternal alcohol PRS and offspring alcohol and mental health phenotypes. If associations between maternal alcohol PRS and offspring phenotypes are due to shared biological (in this case genetic) contributions to both exposure and outcome variables, we might expect weaker associations for maternal alcohol PRS and offspring phenotypes, compared to own alcohol PRS and mental health phenotypes (in either mothers or offspring). This is because offspring share 50% of their variegated genotype with their mother, and we would therefore expect the observed effects to be roughly half the size of those in the mother’s analyses in the presence of a causal intergeneration effect. This study used longitudinal data on multiple generations (mother and offspring) and at different timepoints available from the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

Study population

Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study. The initial number of pregnancies enrolled is 14,541 (for these at least one questionnaire has been returned or a “Children in Focus” clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above. The number of new pregnancies not in the initial sample (known as Phase I
enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 24 is 913 (456, 262 and 195 recruited during Phases II, III and IV respectively), resulting in an additional 913 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper and its update (18-20). The total sample size for analyses using any data collected after the age of seven is therefore 15,454 pregnancies, resulting in 15,589 fetuses. Of these 14,901 were alive at 1 year of age. A 10% sample of the ALSPAC cohort, known as the Children in Focus (CiF) group, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: [http://www.bristol.ac.uk/alspac/researchers/our-data/](http://www.bristol.ac.uk/alspac/researchers/our-data/).

**Genotyping and quality control**

Children from the ALSPAC cohort were genotyped using the Illumina HumanHap550 quad chip genotyping platforms. Mothers from ALSPAC were genotyped using the Illumina human 660W-quad array at Centre National de Génotypage (CNG). Further detail of the quality control process is described elsewhere (21).

**Alcohol polygenic risk score**

A recent large scale GWAS (13) used 941,280 individuals and identified 99 SNPs related to the number of alcoholic drinks consumed per week using a threshold of 5x10⁻⁸, explaining 2.5% of the variance in drinks per week. A total of 34 cohorts were meta-analysed and drinks per week was broadly defined as the average number of alcoholic drinks (aggregated across all alcohol types) consumed per week (see Supplementary Methods). We
calculated a PRS for alcohol use based on these 99 conditionally independent genome-wide significant SNPs (13) (see Table S1), weights were based on the effect estimates reported in the original publication. The PRS for alcohol use were calculated both for the mothers and offspring using PLINK V1.

**Phenotyping**

Targeted phenotypes were selected for available alcohol use phenotypes \((n = 22)\) and mental health/behavioural phenotypes \((n = 91)\) within ALSPAC. For maternal phenotypes \((n = 30;\) see Fig. 1), variables were recorded during pregnancy (8 to 32 weeks gestation). For the child’s phenotypes \((n = 61;\) see Fig. 2), primary outcome variables were selected as close to age 18 years as possible and negative control variables were selected as close to age 7 years as possible, before alcohol initiation was likely to have occurred. Further details of variable selection are given in Supplementary Methods.

**Mother phenotypes**

All maternal phenotypes were self-reported. Measures of maternal alcohol use during pregnancy were continuous measures of, alcohol consumption in months 1-3 of pregnancy, number of days mothers drank 4+ units of alcohol in the past month at 18 and 32 weeks gestation, total weekly units of alcohol consumed at 32 weeks gestation. Binary measures were, the most consumed alcoholic beverage (compared to other alcoholic drinks during pregnancy) at 18 weeks gestation, being wine, beer or lager, spirits, sherry or port, other types of alcohol. As well as measures indicating change in alcohol consumption during pregnancy or not (increased use/stopped use/no change/increased craving/never used), for mothers who normally consumed alcohol and those who normally did not consume alcoholic drinks. An overview of included phenotypes can be seen in Fig. 1.
Measures of maternal mental health and other substance use collected at 8 weeks gestation were: changes (increased use/stopped use/no change/increased craving/never used) to caffeine and tobacco consumption. Collected at 18 weeks gestation were: neurotic symptomatology (Crown Crisp Experiential Index), hypersensitivity to personal rejection, image perception within the last four weeks, image perception change from 3 months pre-pregnancy to 18 weeks gestation, reactions towards becoming a parent, illicit drug use, smoked during pregnancy, taken cannabis during pregnancy, vomited during pregnancy and if mothers had ever smoked in their lifetime. Collected at 32 weeks gestation were: total caffeine consumption, highest maternal education qualification, mother’s perception of their own physical activity compared to other pregnant women of a similar age. Depression (Edinburgh Postnatal Depression Scale (EPDS) (22)) was measured at both 18 and 32 weeks gestation, with scores ≥13 indicating depression diagnosis.

Demographic measures included were: maternal socioeconomic status, adverse life events (asked during pregnancy at 32 weeks gestation), and a combined measure of average income (measured at 33 and 47 months).

Child phenotypes

Offspring phenotypes were included as close to age 18 as possible; further analyses using the same phenotypes (where available) as close to age 7 as possible were included as negative controls. Measures of adolescent alcohol use self-reported at age 18 were: alcohol frequency, amount of alcohol consumed daily, number of days they drank 4+ units of alcohol in the past month, number of drinks it took to feel tipsy, total score for hazardous drinking behaviours from the Alcohol Use Disorders Identification Test (AUDIT) and number of times...
they had consumed alcohol. Total AUDIT score was also included at age 24. An overview of child and adolescent phenotypes included can be seen in Fig. 2.

Adolescent substance use measures were: caffeine intake, ever tried cannabis, frequency of cannabis use, ever smoked tobacco, age first smoked tobacco, total number of cigarettes smoked in lifetime and number of cigarettes smoked per day and per week.

Adolescent mental health measures included were: personality (agreeableness, extraversion, emotional stability, intellectual ability and conscientiousness), phobia symptoms, total anxiety score, depression (Clinical Interview Schedule-Revised (CIS-R) (23) and Short Mood and Feelings Questionnaire (SFMQ) (24)), psychotic like symptoms (PLIKS), negative psychotic symptom scores (Community Assessment of Psychotic Experiences (CAPE) (25)), total emotional symptoms, total problem score, total attention deficit hyperactive disorder (ADHD) score, conduct disorder (Strengths and Difficulties Questionnaire (SDQ) (26, 27)), oppositional defiant disorder (ODD), post-traumatic stress disorder (Development and Wellbeing Assessments (DAWBA)), initiating and difficulty maintaining sleep, average sleep duration on a school night, diagnosis of autism, presence of an eating disorder, and ever self-harmed with suicidal intent.

Adolescent demographic measures included were: body mass index (BMI), frequency of exercise, negative life events, IQ and if obtained a GCSE grade at levels A*-C, or D-G.

Negative control measures collected around age 7 were: number of hours the child normally sleeps during term-time, difficulty initiating and maintaining sleep, presence of ODD, conduct disorder symptoms, hyperactivity symptoms, emotional symptoms score, and total behavioural difficulties, general anxiety symptoms scale, child’s IQ and daily caffeine intake, total depression score and clinical diagnosis of specific phobias.

Child demographic measures included BMI and number of negative life events experienced at age 7. A measure of handedness was included as a further negative control which we did not expect to be associated with the PRS regardless of own alcohol exposure.
Statistical analysis

Using Stata version 15.1, linear and logistic regression analyses were used to investigate whether own alcohol PRS were associated with 1) alcohol consumption for mothers during pregnancy and in adolescence for offspring, and 2) mental health phenotypes (for mothers during pregnancy, and for children both pre-alcohol use around ages 7-10 years, and post-alcohol use around ages 13-24 years). In addition, similar analyses were used to investigate whether maternal alcohol PRS was associated with 3) offspring alcohol and mental health phenotypes (to test for possible intergenerational effects). All analyses were performed separately in children and mothers. Analyses were adjusted for sex (in the children only), age (at questionnaire completion or clinic attendance), and the first 10 ancestry-informative principal components.

Sensitivity analyses were conducted using Bonferroni-corrected p-values (see Supplementary Methods). Due to the conservative nature of Bonferroni correction where tests are not independent, permutation tests were also conducted accounting for any degree of correlation between the variables in each test.

Results

Aim 1) Alcohol use PRS and alcohol phenotypes. Maternal alcohol PRS previously shown in the general population were validated for maternal alcohol use during pregnancy (see Fig. 3). Each SD increase in genetic score for alcohol consumption was associated with a 0.04 unit increase in the number of drinks consumed each week at 18 weeks gestation (95% CI 0.02 to 0.06, \( p = 1.01 \times 10^{-3} \)), 0.03 unit increase in the number of days mothers binge drank at 18 weeks gestation (95% CI 0.01 to 0.05, \( p = 9.19 \times 10^{-4} \)), 0.04 unit increase in the number of days mothers binge drank at 32 weeks gestation (95% CI 0.02 to 0.06, \( p = 2.37 \times 10^{-4} \)) and 0.25 unit
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increase in the in the number of drinks consumed each week at 32 weeks gestation (95% CI 0.04 to 0.36, $p = 1.70 \times 10^{-5}$) (Fig. 3). There was little evidence that offspring PRS for alcohol use was associated with any of the offspring alcohol measures at ages 13 or 18 years (Fig. 4).

[Insert Fig. 3 about here]

Aim 2) Alcohol use PRS and mental health phenotypes (during pregnancy, age ~7 and age ~18). Maternal alcohol PRS was associated with increased maternal depression at 32 weeks gestation (OR = 1.09, 95% CI = 1.02 to 1.18, $p = 0.022$), see Table S4. There was no clear evidence of association between maternal alcohol PRS and any of the other mental health phenotypes (Fig. 3). There was little evidence that offspring alcohol PRS were associated with any of the child mental health phenotypes (see Table S5).

[Insert Fig. 4 about here]

Aim 3) Intergenerational. Maternal alcohol PRS and offspring alcohol and mental health phenotypes (age ~7 and age ~18). Maternal alcohol PRS were associated with increased offspring AUDIT total score ($\beta = 0.184$, 95% CI = 0.02, to 0.35, $p = 0.028$) (Table S3, and Fig. 5). Of the measured offspring phenotypes, maternal alcohol PRS were associated with decreased scores in intellectual ability at age 13 ($\beta = -0.209$, 95% CI = -0.38 to -0.04, $p = 0.016$, see Tables S3 and S6). Maternal alcohol PRS showed little evidence of association with any of the other offspring outcomes after adjustment for multiple testing (see Table S6).

[Insert Fig. 5 about here]
Sensitivity analyses. After permutation analyses, the strength of evidence persisted for maternal alcohol PRS associating with maternal depression at 32 weeks gestation: $p = 0.016$. Whereas the intergenerational association between maternal PRS and offspring phenotypes was attenuated (see Tables S7 to S9).

Discussion

We have shown that PRS for alcohol consumption reliably associates with consumption in pregnancy, but not in adolescence/young adulthood. This suggests that alcohol PRS derived in the general population is associated with consumption patterns during pregnancy and can be applied in future epidemiological analysis focused on this timing for exposure. Offspring alcohol PRS was not a valid predictor of consumption in adolescence, however, this was not entirely unexpected – the PRS created within the current study was based on cohorts with a much older average age (13). In adolescents and younger adults, drinking patterns are not yet fully established and may be more subject to social influences (29). Previous studies have shown that alcohol use increases over time from adolescence to adulthood (30).

We subsequently investigated associations between both maternal and offspring PRS for increased alcohol consumption, and multiple mental health outcomes. We found evidence of association between maternal PRS and increased maternal depression at 32 weeks gestation, but no strong evidence for offspring outcomes. Alcohol abuse is shown to have high comorbidity with mental health problems (31, 32), and these results may suggest a potential causal effect of alcohol consumption on maternal depression, or a shared biological contribution to both alcohol consumption and mental health. Mendelian randomization studies have previously identified a causal role of genetic liability for major depression and alcohol dependence (33, 34). Our finding is in line with this literature, and it extends it to lower non-pathological alcohol use levels in a general population of pregnant women.
The offspring alcohol PRS was also not shown to be associated with any of the mental health outcomes in the offspring subpopulation, at ages ~7 and ~18 years. The outcomes at age 7 provided a negative control, because the offspring are unlikely to have started consuming alcohol themselves. Therefore, any associations are likely to be due to horizontal pleiotropy or maternal drinking, rather than the offspring’s own alcohol use. However, as the offspring alcohol PRS did not predict offspring alcohol consumption, we cannot make inferences about the association between alcohol use and mental health. The sample sizes for offspring phenotypes were also relatively small as they were mostly collected around age 18, when the cohort has experienced significant attrition. Larger sample sizes would increase statistical power and potentially identify weaker associations.

We also investigated the effect of maternal alcohol PRS on offspring phenotypes to elucidate potential intergenerational associations. Within the intergenerational analyses, maternal alcohol PRS were associated with offspring increased risk of hazardous alcohol use (AUDIT), but no other alcohol measures. The association with hazardous use could be due to genetic nurture, with offspring being more exposed to a parent engaging in high levels of alcohol use. The lack of association with other outcomes could again be due to the younger age of the adolescents having not yet fully established their drinking behaviour, or the smaller sample size and consequently reduced statistical power. Maternal alcohol PRS was also associated with decreased scores for intellectual ability. This could be either due to a causal intrauterine effect, offspring’s own alcohol use (which is unlikely as we did not find an association with offspring alcohol use), or (as previously discussed) pleiotropic effects. As we had already shown maternal PRS to be validated as a measure of increased maternal alcohol use, this would suggest that offspring of mothers with increased levels of alcohol PRS would have likely been exposed to greater amounts of prenatal alcohol exposure (PAE). Permutation testing attenuated the strength of evidence in support of the intergenerational associations for offspring cognitive
outcomes. However, these observed associations in the main analyses were already weak, as PRS typically only explain a small proportion of phenotypic variance.

A key strength of this study is the validation of the alcohol PRS in pregnant women, which has to date not yet been previously investigated. However, several limitations should also be considered when interpreting these results. First, the small sample sizes reduced our power to detect true associations. Future follow-up studies should use cohorts with larger sample sizes to address how alcohol PRS may influence mental health trajectories within the two generations of pregnant mothers and their offspring. Our results therefore warrant replication. Second, we did not conduct longitudinal analyses. Third, there was modest sample overlap between ALSPAC and the GWAS in which the SNPs for alcohol consumption were identified (13); the GWAS included 8913 participants from ALSPAC out of a total sample size of 941,280. However, this is unlikely to introduce substantial bias as the size of this overlap compared to the total included in the GWAS is minimal.

The current methods and data do not allow us to draw firm conclusions regarding mechanism, as the associations we observe could arise in a variety of ways. The intergenerational analyses suggest a causal pathway from mothers’ alcohol PRS to offspring outcomes. This is consistent with a body of literature demonstrating adverse effects of PAE on offspring cognition (35-37). Increased post-natal maternal alcohol use may also influence offspring outcomes, for example via how the mother interacts with or parents her child or environmental pathways (38). However, these findings could also be due to horizontal pleiotropy, or shared genetic liability between increased alcohol use and mental health. However, since we did not observe any associations between child’s own alcohol PRS and child mental health outcomes this is perhaps unlikely, particularly as the offspring outcomes where there was evidence of an association with maternal PRS occurred before the offspring were likely to have begun consuming alcohol.
Our findings validate the alcohol PRS as a reliable predictor for maternal alcohol consumption during pregnancy, but caution that the same PRS might not be a suitable instrument to use in adolescent populations. The additional analyses we carried out as part of our PheWAS approach (aim 2) replicated well-known epidemiological associations (e.g., of maternal alcohol use in pregnancy and lower offspring cognition), extending the known association of alcohol abuse and depression (39) to lower levels of drinking, and within pregnancy. This application of the targeted PheWAS analyses in this richly characterised multigenerational cohort should therefore be viewed as more than simply a proof-of-principle approach. Nevertheless, our results should be interpreted in the context of the study’s limitations, and in particular that this approach alone cannot fully disentangle if associations are evidencing causal pathways or are due to pleiotropy. Follow-up analyses focused on causal inference (such as Mendelian randomisation) should be carried out to formally test these and future PheWAS findings.
Declarations of interest

None.

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Fig. 1: Phenotypes included in PheWAS for mothers during pregnancy
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Fig. 2: Phenotypes included in PheWAS for children and adolescents
Fig. 3: Associations between maternal alcohol PRS and maternal alcohol exposures and mental health phenotypes. Each datapoint represents an individual phenotype, colour coded by phenotypic category.
Fig. 4: Associations between offspring alcohol PRS and offspring alcohol exposures, mental health phenotypes at pre-drinking age and drinking initiation age. Each datapoint represents an individual phenotype, colour coded by phenotypic category.
Fig. 5: Associations between maternal alcohol PRS and offspring alcohol exposures and mental health phenotypes at pre-drinking age and drinking initiation age (intergenerational). Each datapoint represents an individual phenotype, colour coded by phenotypic category.
