D4.1 Mendelian randomization analyses of lifestyle behaviours

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Definitions and acronyms

ALSPAC	Avon Longitudinal Study of Parents and Children
MR	Mendelian Randomization
PheWAS	Phenome-Wide Association Study

1. Introduction

The aim of this project is to disentangle the association of alcohol, tobacco and caffeine consumption and mental health by using the phenome-wide association study (PheWAS) paradigm. Data from the Avon Longitudinal Study of Parents and Children (ALSPAC), longitudinal pregnancy cohort was used. ALSPAC recruited more than 14 000 pregnant women in 1991 and 1992 and their families have been followed over the years and follow-up is still ongoing. Genotype data in our sample was available for 7977 children and 7921 pregnant women/mothers.

Mendelian randomisation is a causal inference technique that uses genetic variants associated with an exposure as instrumental variables. Genetic variants cannot be influenced by reverse causality and, based on an approximation of Mendel's first and second laws, should not, at a population level, be associated with confounders. They can therefore be used as proxies for an exposure of interest to determine whether that exposure has a causal effect on outcomes of interest. This approach is increasingly widely used in epidemiology, and can be used to explore the effects of intrauterine exposures on offspring outcomes.

Given the large number of outcomes potentially associated with intrauterine exposures, we adopted a PheWAS approach, using polygenic risk scores for tobacco, alcohol and caffeine use in mothers on offspring outcomes at age 7/8 (before they themselves initiate the use of the same substances), and age 13-24 (during which time the offspring would have started using the same substances). We focused our PheWAS on neuropsychiatric offspring outcomes. This approach enables the identification of potential causal pathways between intrauterine exposures and offspring outcomes which can be further interrogated using a range of other causal inference techniques (e.g., additional Mendelian randomisation sensitivity analyses, negative control analyses, etc.).

2. Methods

Our dataset included mental health, substance use, personality and sociodemographic variables for children (age 7-10 years), adolescents (age 13-24 years), pregnant women (measured 18 and 32 weeks of gestation) and mothers (before pregnancy and after children reached age 4). Our dataset included 75 phenotypes for children and adolescents, 58 for pregnant women and 32 for mothers. See the Annex for complete list of variables. Sample sizes varied depending on sub-population and PRS. Maximum sample size available for children was 5800, for adolescents 7800, 7240 for pregnant women and 6800 for mothers.

2.1. Exposures

We created PRS based on recent GWAS results on smoking[1], alcohol[1] and coffee consumption[2]. PRS for alcohol was constructed as a weighted sum of 99 SNPs associated with alcohol use (self-reported average drinks per week). For smoking, PRS was calculated of 378 SNP-s associated with ever smoking regularly. Lifetime smoking is a combined measure of smoking status (current, former, never), age at initiation of smoking in years, smoking heaviness (number of cigarettes smoked per day) and age at smoking cessation in years and in total of 126 independent SNP-s.[3] GWAS of coffee consumption found 8 SNPs associated with cups of coffee drinking per day.

2.2. Outcomes

Phenotypes were selected based on previous findings from the literature and were collected using questionnaires and clinical assessments. If some variables were available at multiple time points, correlations were checked. If variables showed a low or moderate association, both time points were included in the analysis. If variables correlated highly, only the one with the larger sample size was included. A full list of phenotypes selected for inclusion is provided in the Annex (below).

2.3. Statistical Methods

All analyses were performed using STATA v15. Associations between the PRS of the consumption behaviours and phenotypes were calculated using linear and logistic regression analyses. Continuous variables with more than 20% of zero values were considered as zero inflated and transformed to a categorical variable based on the median. Variables that deviated strongly from a normal distribution were rank-transformed to resemble a normal distribution.

Analyses were adjusted for age, gender (in children and adolescents) and the first 10 ancestry-informative principal components from the cohort-specific GWAS. We restricted our sample to singletons. We removed missing values and used complete case analyses. Multiple testing was accounted for by using Bonferroni adjusted p-values (with number of tests defined for each exposure-age combination).

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[1] Liu, M. et al. (2019). Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nature Genetics v51 n2 (201902): 237-244

[2] M C Cornelis, E M Byrne, T Esko, M A Nalls, A Ganna, N Paynter, . . . D I Chasman. (2014). Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption. *Molecular Psychiatry, 20*(5), 647-656

[3] Wootton, R. E. et al. (2018). Causal effects of lifetime smoking on risk for depression and schizophrenia: Evidence from a Mendelian randomisation study. bioRxiv 381301

3.   Results

Results are presented in Tables 1 to 3 (below). In summary, there was no clear evidence of association between genetically-predicted alcohol or caffeine consumption in mothers and offspring neuropsychiatric outcomes at age 7/8. However, there was evidence of an association between maternal genetically-predicted tobacco use and offspring intelligence and conduct disorder at age 7/8 (see Table 1). Although there was weak evidence of association for other offspring phenotypes, these did not survive correction for the large number of statistical tests conducted within the PheWAS.

4.   Conclusions

Our results do not provide strong evidence to support a causal effect of either alcohol or caffeine use in pregnancy on offspring neuropsychiatric outcomes. However, we do find evidence to support a possible causal effect of tobacco use in pregnancy on offspring intelligence and conduct disorder at age 7/8. Previous research using alternative methodologies (e.g., negative control analyses comparing maternal vs paternal smoking during pregnancy on offspring outcomes) provides further support for this possibility. As a next step, it would be valuable to explore this pathway using more sophisticated Mendelian randomisation approaches (e.g., taking into account offspring genotype).

One challenge in studies of intrauterine exposures on offspring outcomes is the possibility of genetic confounding, which Mendelian randomisation approaches are not immune to. There is growing evidence that genetic variants associated with smoking initiation capture, at least in part, a risk taking / impulsivity phenotype. This could in principle account for the results observed here – offspring will inherit 50% of their mother's genotype on average, and if mothers are at high genetic risk of smoking initiation then their offspring also will be. If this risk includes a risk taking / impulsivity, then it may be this latent phenotype, rather than intrauterine tobacco exposure, which is influencing intelligence and conduct disorder. We will conduct sensitivity analyses adjusting for offsprings' own PRS to evaluate where associations we observe are attenuated or disappear entirely.

Another limitation of this study is the relatively small sample size of ALSPAC in the context of a PheWAS. It is possible that small but nevertheless important effects at a population level may have been missed. Moreover, attrition of the sample over time means that the resulting sample for analysis is unlikely to be representative of the underlying population. Therefore the results presented here will require replication in other cohorts, ideally including those which have experienced lower attrition.

This work provides preliminary evidence for a causal effect of tobacco use during pregnancy on offspring neuropsychiatric phenotypes (specifically, intelligence and conduct disorder). However, these results need to be interpreted with particular caution given the possibility of genetic confounding, and the likelihood that genetic variants associated with smoking include an element of variants that are associated with a latent risk taking / impulsivity phenotype.

The triangulation of evidence derived from multiple methodological approaches, each with different (and ideally uncorrelated) sources of bias, will be necessary to resolve this question with certainty.

5. Tables

Outcome	OR	Lower CI	Upper CI	Beta	Lower CI	Upper CI	P-value	N
IQ_8				-0.74118	-1.20155	-0.28081	0.001608	4675
Gr_CD_7				0.027088	0.007663	0.046514	0.006284	5014
bmi84				0.05986	0.00375	0.11597	0.036538	5032
tea_bi8	1.062871	1.000778	1.128816	0.060974	0.000778	0.121169	0.047508	5531
Gr_HYP_7				0.016457	-0.00116	0.034077	0.067148	4918
sleep7_init_bi	0.950747	0.892272	1.013053	-0.05051	-0.11398	0.012968	0.108567	5152
sdqtotdif7				0.0976	-0.0283	0.223499	0.128631	5135
coff_bi8	1.081331	0.966421	1.209903	0.078192	-0.03416	0.19054	0.15659	5495
specphobia10	1.220808	0.915053	1.628728	0.199513	-0.08877	0.487799	0.157453	5102
kq260				-0.01515	-0.0378	0.007497	0.189723	5129
caff_8_MS				0.488792	-0.28857	1.266149	0.217734	4067
anxiety8				-0.02429	-0.07427	0.025682	0.340629	4995
Gr_ODD_7				0.007182	-0.01287	0.027236	0.482662	4945
sleep7_maint_bi	1.017092	0.950978	1.087803	0.016948	-0.05026	0.08416	0.592807	5129
Gr_lifeevents_7				-0.00245	-0.02084	0.015937	0.793975	5169
mfqtotal10				0.011815	-0.07973	0.103361	0.800258	4885
handedness	1.009046	0.914244	1.113677	0.009005	-0.08966	0.107667	0.845698	4849

Table 1 – tobacco

Outcome	OR	Lower CI	Upper CI	Beta	Lower CI	Upper CI	P-value	N
caff_8_MS				0.777552	-0.04129	1.596396	0.06272	4067
mfqtotal10				0.071943	-0.01823	0.162116	0.117859	4885
bmi84				-0.03377	-0.0903	0.022762	0.241626	5032
sdqtotdif7				0.071877	-0.05532	0.199072	0.267988	5135
sleep7_maint_bi	0.970886	0.908987	1.037				0.347762	5129
Gr_HYP_7				0.006829	-0.01132	0.024981	0.460843	4918
sleep7_init_bi	1.013675	0.95206	1.079277				0.645459	5152
Gr_ODD_7				-0.00339	-0.0246	0.017821	0.753922	4492
specphobia10	1.036193	0.80354	1.336206				0.76586	5102
IQ_8				0.068031	-0.39788	0.533947	0.774692	4675
handedness	1.012415	0.922309	1.111323				0.777961	4849
kq260_7				-0.00311	-0.02595	0.019728	0.789342	5129
emotional7				0.004412	-0.04108	0.0499	0.849197	5141
anxiety8				0.00454	-0.0434	0.052482	0.852723	4995
Gr_lifeevents_7				0.000276	-0.0182	0.018749	0.976604	5169

Table 2 – alcohol

Outcome	OR	Lower CI	Upper CI	Beta	Lower CI	Upper CI	P-value	N
specphobia10	0.724259	0.518614	1.011449	-0.32261	-0.6566	0.011384	0.057082	5102
handedness	1.063692	0.968206	1.168596	0.061746	-0.03231	0.155803	0.178137	4849
Gr_HYP_7				0.01213	-0.0058	0.030057	0.184732	4918
Gr_lifeevents_7				0.011342	-0.00719	0.029874	0.230284	5169
Gr_ODD_7				-0.01221	-0.03251	0.008099	0.238667	4945
sleep7_init_bi	0.972335	0.912681	1.035887	-0.02806	-0.09137	0.035258	0.353366	5152
bmi84				0.025724	-0.03255	0.083999	0.386881	5032
sdqtotdif7				0.050777	-0.07726	0.178815	0.436926	5135
mfqtotal10				-0.03379	-0.12705	0.059463	0.47748	4885
kq260				-0.00671	-0.03012	0.016699	0.574152	5129
Gr_CD_7				-0.00486	-0.02428	0.014554	0.623361	5014
anxiety8				-0.0089	-0.05586	0.038052	0.710089	4995
IQ_8				-0.06559	-0.52082	0.38965	0.777614	4675
sleep7_maint_bi	1.000949	0.936118	1.07027	0.000949	-0.06601	0.067911	0.975878	5129

Table 3 – caffeine

6.   Annex

List of phenotypes included in the dataset

Variable	Description	Timepoint
Children		
<u>Mental health variables</u>		
Gr_HYP_7	ADHD total score based on median at age 7	81 months
Gr_CD_7	Conduct disorders total score based on median at age 7	81 months
Gr_ODD_7	ODD total score based on median at age 7	91 months
mfqtotal10	SMFQ total score (complete cases)	9.5 years
emotional7	sdq emotional symptoms score (prorated)	6.8 years
anxiety8	DV: General anxiety symptoms score (complete cases)	7.5 years
sdqtotdif7	dv: total behavioural difficulties score (prorated)	6.7 years
<u>Other variables</u>		
kq260_7	sleep duration at age 7	81 months
Gr_lifeevents_7	Number of life events at age 7 (grouped by median)	81 months
sleep7_init_bi	Problems with sleep initiation in past year (Y/N)	81 months
sleep7_maint_bi	Problems with sleep maintenance in past year (Y/N)	81 months
IQ_8	IQ based on Wechsler-Intelligence scale total score	8 years
<u>Substance use variables</u>		
Caffeine		
caff_8_MS	Total mg/day caffeine from tea, cola, coffee	8 years
tea_bi8	Child drinks caffeinated tea (Y/N)	8 years
coff_bi8	Child drinks caffeinated coffee (Y/N)	8 years
Adolescents		
<u>Mental health variables</u>		
ADHD_16_total	ADHD total core at age 16	16.6 years
CD_16_total	Conduct disorder total score at age 16	16.6 years
Gr_ODD_15	ODD total score based on median at age 15	15.5 years
fh5440_age16	sleep duration at age 16	15.5 years
Gr_lifeevents_16	Number of life events at age 16 (grouped by median)	198 months
cisrdep5sum	Depressive symptoms - Sum of all the 5 depression symptom scores	18 years
mfqtotal18	SMFQ total score	17.5 years
mfqtotal14	total MFQ score @TF2	14 years

ptsd15	15yr PTSD (self-report 6-band computer prediction, ICD-10 and DSM-IV)	15 years
sh_si	Self-harming behaviour	15 years
emotional17	sdq emotional symptoms score (prorated)	16.5 years
cisranx18	Anx: Anxiety score [F17]	17.1 years
cisrphobia18	Phobias: Phobia symptom score [F17]	17.1 years
sdqtotdif17	dv: total behavioural difficulties score (prorated)	16.5 years
eatingdis13	Anorexia	13
psychosis12_pos	Psychosis positive symptoms	12
psychosis16_neg	Psychosis negative symptoms	16
eatingdis16	Anorexia	16
Other variables		
extraversion13	Big-5 personality traits: Extraversion	13
agreeable13	Big-5 personality traits: Agreeableness	13
conscientious13	Big-5 personality traits: Conscientiousness	13
emotion_stable13	Big-5 personality traits: Emotional Stability	13
intellect13	Big-5 personality traits: Intellect	13
sleep15_maint	Maintaining sleep: Number of times YP usually wakes up at night	15
sleep15_init	Initiating sleep: Average time (minutes) YP takes to fall asleep per week	15
exercise14	Frequency respondent did any exercise during the past year	14
Gcse_AC	GCSE grades A*-C	18
Gcse_DG	GCSE grades D-G	18
bmi204	BMI	17
Substance use variables		
Alcohol		
alcfreq18	AUDIT: Freq YP has a drink containing alcohol (Continuous)	17.1 years
auditrisk18	DV: Level of risk identified by alcohol use disorders identification test (AUDIT	17.1 years
alcbinge18	Frequency had 6+ drinks on one occasion	17.1 years
tipsyamt18	No. full drinks needed to feel tipsy/have buzz over last 3 months	17.1 years
dailyalcamt18		18 years
audittotal18		18 years
tipsyamt18		18 years
timesdrank13		
audit24tot	audit score at 24 (totalled differently to previous audit measures due to errors in clinic data collection)	24 years
Tobacco		

age1stsmoked	Age of respondent when first smoked a cigarette	18 years
smoked18	Age when respondent smoked first whole cigarette (years)	18 years
totalcigs18	Number of cigarettes respondent smoked altogether in lifetime	18 years
totalcigs23	Number of cigarettes respondent has smoked altogether in their lifetime	23 years
YPC1371	Number of cigarettes respondent smokes per day, on average	23 years
YPC1381	Number of cigarettes respondent smokes per week, on average	23 years
cannabisfreq	Frequency YP smokes cannabis	16.5 years
smoked14	Respondent has smoked a cigarette (including roll-ups)	14
totalcigs14	Total number of cigarettes that the respondent has smoked	14
eversmoked18	Respondent has ever smoked a whole cigarette (including roll-ups)	18
eversmoked23	Respondent ever smoked a whole cigarette (including roll-ups)	23
cannabis	YP has ever tried cannabis	16.5
Caffeine		
caff_13_MS	Total mg/day caffeine from tea, coffee, cola	13 years
teadaily_caff_13_MS	Tea mg/day caffeine teenager	13 years
coffdaily_caff_13_MS	Coffee mg/day caffeine teenager	13 years
coladaily_caff_13_MS	Cola mg/day caffeine teenager	13 years
Pregnant women		
<u>Mental health variables</u>		
epds_b_bin	Depression	18 weeks gest
epds_c_bin	Depression	32 weeks gest
IPSM18	Eliciting personality: Interpersonal Sensitivity Measure (IPSM)	18 weeks gest
<u>Other variables</u>		
Gr_lifeevents_Preg	Life event scores (pregnancy)	32 weeks gest
imagepercept_18wks	Image perception score during pregnancy	18 weeks gest
imagepercept_change	Image perception change from before to during pregnancy	
feelings_preg	Your Reactions to Becoming a Parent	18 weeks gest
CFactivity_32wks	Activity level compared with other pregnant women	
PA_32wks_bi	Physical activity (Y/N)	32 weeks gest
vompreg	Vomiting	18 weeks gest
<u>Substance use</u>		

Alcohol		
alc_binge_18wk	Alcohol: binging	18 weeks gest
weekly_units_32wk	alcohol per week	32 weeks gest
alc_binge_32wk	Alcohol: binging	32 weeks gest
alcpreg_crave	Craved more alcohol during pregnancy	8 weeks gest
alcpreg_more	Consumed more alcohol during pregnancy	8 weeks gest
alcpreg_nochange	Didn't change alcohol consumption during pregnancy	8 weeks gest
alcpreg_neverdrank	Never has been drinking alcohol	8 weeks gest
alcpreg_stop	Stopped alcohol consumption during pregnancy	8 weeks gest
alcpreg_reduce	Reduced alcohol consumption during pregnancy	8 weeks gest
Tobacco		
smokepreg	Smoking (Y/N)	8 weeks gest
cannabispreg	Cannabis (Y/N)	8 weeks gest
neversmoked	Ever smoked during pregnancy (Y/N)	8 weeks gest
stopsmokepreg	Stopped smoking during pregnancy (Y/N)	8 weeks gest
pregcig_less	Cut down smoking during pregnancy (Y/N)	8 weeks gest
cigchange_crave	Had more or craved more cigarettes (Y/N)	8 weeks gest
Caffeine		
caff_preg_18wks	Total mg/day caffeine pregnancy	18 weeks gest
teadaily_caff_18wks	Tea mg/day caffeine pregnancy	18 weeks gest
coffdaily_caff_18wks	Coffee mg/day caffeine pregnancy	18 weeks gest
coladaily_caff_18wks	Cola mg/day caffeine pregnancy	18 weeks gest
caff_preg_32wks	Total mg/day caffeine pregnancy	32 weeks gest
teadaily_caff_32wks	Tea mg/day caffeine pregnancy	32 weeks gest
coffdaily_caff_32wks	Coffee mg/day caffeine pregnancy	32 weeks gest
coladaily_caff_32wks	Cola mg/day caffeine pregnancy	32 weeks gest
caffpreg_more	Consumed more caffeine during pregnancy	8 weeks gest
caffpreg_neverdrank	Never has been drinking caffeine	8 weeks gest
caffpreg_nochange	Didn't change caffeine consumption during pregnancy	8 weeks gest
caffpreg_less	Reduced caffeine consumption during pregnancy	8 weeks gest
neverdrank_tea	Never drank tea vs. drinking tea	8 weeks gest
stopdrinkpreg_tea	Stopped drinking tea during pregnancy	8 weeks gest
pregtea_less	Reduced tea consumption during pregnancy	8 weeks gest
teachange_cravemore	Craved or had more tea during pregnancy	8 weeks gest
neverdrank_coff	Never drank coffee vs. drinking coffee	8 weeks gest
stopdrinkpreg_coff	Stopped drinking coffee during pregnancy	8 weeks gest
pregcoff_less	Reduced coffee consumption during pregnancy	8 weeks gest
coffchange_crave	Craved or had more coffee during pregnancy	8 weeks gest
neverdrank_cola	Never drank cola vs. drinking cola	8 weeks gest
stopdrinkpreg_cola	Stopped drinking cola during pregnancy	8 weeks gest

pregtea_less	Reduced cola consumption during pregnancy	8 weeks gest
colachange_crave	Craved or had more cola during pregnancy	8 weeks gest
Mothers		
<u>Mental health variables</u>		
CCEI_total_M	Anxiety score mums	134 months (child age)
Bi_EPDS_M	Depression score mums	134 months
Gr_lifeevents_M	Number of life events mums	134 months
d152a	had bulimia Y/N	12 weeks gest
d167a	had drug addiction Y/N	12 weeks gest
d168a	had alcoholism Y/N	12 weeks gest
d169a	had schizophrenia Y/N	12 weeks gest
d170a	had anorexia nervosa Y/N	12 weeks gest
d171a	had severe depression Y/N	12 weeks gest
d172a	had other psychiatric problem Y/N	12 weeks gest
<u>Other variables</u>		
Sleep_dur_M	Sleep duration mums	85 months
KSP_imp	Imuplsivity trait	110 months
KSP_monav	Monotony avoidance trait	110 months
KSP_ang	Anger trait	110 months
KSP_susp	Suspicion trait	110 months
KSP_detach	Detachment trait	110 months
sclass_mums	Social class based on occupation	47 months
Mum_Educ	Mothers highest educational qualifications	61 months
BMI_mums	BMI mothers (measured child age 18)	18 years
PH_act_M	Mother participates in physical activity	18 years
<u>Substance use variables</u>		
Tobacco		
Ever_Smoke_M	Mum has been ever smoker (Yes/No)	18 weeks gest
M_smoking_2W	Number of cigarettes mum has smoked last 2 weeks	97 months
Caffeine		
n_coladaily	cups of cola daily	97 months
Gr_teadaily_M	cups of tea daily	97 months
Gr_coffdaily_M	cups of coffee daily	97 months
Gr_caff_tea_M	caffeine from tea (mg)	97 months
Gr_caff_coff_M	caffeine from coffee (mg)	97 months
Gr_caff_M	Mothers daily caffeine intake through tea, coffee & cola (including persons with missing 1 or 2 drinks)	97 months
Gr_caff_miss_M	Mothers daily caffeine intake through tea, coffee & cola (excluding persons with missing 1 or 2 drinks)	97 months

Alcohol		
Gr_alcoholdaily_M	Mothers total alcohol units daily	97 months
Alc_mums	Mothers pre-pregnancy drinking (never/ever)	18 weeks gest
Binging_mums_5	Number of days in past month that mother had at least 4 units of alcohol	61 months
Gr_totalalc_M4	Mothers total alcohol units weekly	47 months
AUDIT_mums	AUDIT score in mothers (based on risk level)	18 years