DNA methylation analysis of amplicons from individuals exposed to maternal tobacco use during pregnancy, and offspring conduct problems in childhood and adolescence

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Abstract

Maternal tobacco smoking during pregnancy is a large driver of health inequalities and a higher prevalence of conduct problem has been observed in exposed offspring. Further, maternal tobacco use during pregnancy can also alter offspring DNA methylation. However, currently, limited molecular evidence have been found to support this observation. Thus we aim to examine the association between maternal tobacco use in pregnancy and whether offspring Conduct problems is mediated by tobacco exposure-induced via DNA methylation differences. Understanding the etiology of the causal link will be crucial in the early identification and treatment of CP in children and adolescents. DNA was sourced from the Christchurch Health and Development Study, a longitudinal birth cohort studied for over 40 years in New Zealand. Bisulfite-based amplicon sequencing of 10 loci known to play a role in neurodevelopment, or with associations with CP phenotypes, was undertaken. We identified nominally significant differential DNA methylation at specific CpG sites in *CYP1A1*, *ASH2L* and *MEF2C* in individuals with Conduct problems who were exposed to tobacco *in utero*. We conclude that environmentally-induced DNA methylation differences could play a role in the observed link between maternal tobacco use during pregnancy and childhood/adolescent Conduct problems However, larger sample sizes are needed to produce an adequate amount of power to investigate this interaction further.

Introduction

Mothers who smoked tobacco during pregnancy have a higher prevalence of offspring developing a conduct disorder phenotype compared to mothers who did not smoke (Wakschlag et al. 1997). This association has been proven in several different cohort studies and the observation have remained following adjustment for various other confounding factors, for example, socio economic status, maternal age, substance abuse, parental anti-social personality, and maladaptive parenting (Wakschlag et al. 1997; Joelsson et al. 2016). However, there is limited molecular evidence to suggest a link between *in utero* tobacco exposure and offspring conduct disorder, thus a direct link between *in utero* tobacco exposure and Conduct problems (CP) remains elusive. Previously we conducted a pilot study assessing differential DNA methylation in a small cohort of individuals who were exposed to tobacco *in utero*, with sub-groups of individuals defined as having high conduct disorder scores (Noble et al. 2021). We found nominally significant DNA methylation changes with this interaction in several genes associated with neurodevelopment (cite epic in utero paper). Due to the limitations of using a small sample size combined with an array containing a large number of loci, results were underpowered, therefore observations were unable to reach genome wide significance. However, the biological relevance of these nominally significant CpG loci to the CP phenotype, combined with further research which has suggested an epigenetic link between *in utero* tobacco exposure and ADHD (Sengupta et al. 2017), implies that the link between DNA methylation and CP development in tobacco-exposed offspring should be investigated more fully.

Here, we will further pursue this hypothesis, by exploring differential methylation in genes that have known roles during *in utero* neurodevelopment and CP phenotypes, to understand whether DNA methylation may help explain the relationship between *in utero* tobacco exposure and development of CP in offspring. We applied a targeted approach via bisulfite-based amplicon sequencing (BSAS) of regions of genes involved in neurodevelopment. Amplicon sequencing has the ability to interrogate a region of the genome, therefore specifically targeting consecutive CpG sites in a row. We then assessed differential methylation in the DNA of participants from the Christchurch Health and Development Study (CHDS) whose mothers consumed tobacco during pregnancy, with high and low CP scores, and compared

this to controls who were not exposed. This approach allows us to specifically ask whether DNA methylation at genes involved in neurodevelopment and CP phenotypes are specifically differentially methylated in the DNA of offspring with CP, who were exposed to tobacco *in utero*. A significant interaction here would provide further support for a role for DNA methylation in the link between *in utero* exposure and CP development, something which has so far proved elusive.

Methods

Sample

A sub-group of individuals from the CHDS were selected for this study (Table 1). This longitudinal study originally included 97% of all the children (N= 1265) born in the Christchurch, New Zealand urban region during a period in mid-1977 and has been studied at 24 time points from birth to age 40 (N= 987 at age 30). All participants were aged between 28-30 when blood samples and DNA was extracted.

For the subsets studied in this report, CHDS participants were chosen based on their *in utero* tobacco exposure status, their adult smoking status, and their CP scores. Group 1 consisted of individuals who were exposed *in utero* to tobacco smoke, and never smokers at the time blood samples were taken (N= 32). Group 2 consisted of individuals who were exposed *in utero* to tobacco smoke and were themselves regular smokers at the time the blood was taken (N= 32). Group 3 consisted of individuals who were not exposed to tobacco *in utero*, and never smokers at the time blood was taken (N= 32). *In utero* tobacco exposure was defined as 10+ cigarettes per day throughout pregnancy. Within each group of 32, 16 individuals were selected with a 'high' score on a measure of childhood CP at age 7-9 years and 16 with a 'low' score. Severity of childhood CP was assessed using an instrument that combined selected items from the Rutter and Conners child behaviour checklists (Rutter M 1970; Conners 1970, 1969; Fergusson, Horwood, and Lloyd 1991) as completed by parents and teachers at annual intervals from 7-9 years. Parental and teacher reports were summed and averaged over the three years (Fergusson, Horwood, and Ridder 2005) to derive a robust scale measure of the extent to which the child exhibited conduct disordered/oppositional behaviours (mean (SD)=

50.1(7.9) ; range 41-97). For the purposes of this report a 'high' score was defined as falling into the top quartile of the score distribution (scores >53) and a 'low' score was defined as scores < 46.

Bisulfite-based amplicon sequencing

Bisulfite-based amplicon sequencing (BSAS) was carried out as described (Noble et al. 2020). DNA was extracted from whole blood samples using the Kingfisher Flex System (Thermo Scientific, Waltham, MA USA). DNA was quantified via nanodrop (Thermo Scientific, Waltham, MA USA). Bisulfite treatment was carried out using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA, USA) as per the manufacturer's instructions. DNA samples were then diluted to a final concentration of 100 ng/µl.

Amplicons for sequencing (Table 2 and Supplementary Table 1) were picked based upon several criteria: i) previously published differential DNA methylation in response to *in utero* tobacco smoking; ii) known associations with *in utero* brain development, and; iii) known associations with CP phenotypes. Primers were then designed to flank the CpG sites of interest, ~350 base pairs (bp) in total, or to amplify the promoter region of the gene if a specific CpG site was not known. Multiple pairs of primers were designed to amplify larger regions.

Bisulfite-converted DNA was amplified via PCR, using KAPA Taq HotStart DNA Polymerase (Sigma, Aldrich) under the following conditions: 95 °C for 10 min, 95 °C for 30 sec, 59 °C for 20 sec, 72 °C for 7 min, and held at 4 C° using the Mastercycler Nexus (Eppendorf, Australia). This was then cycled a total of 40 times. PCR products were purified with the Zymo DNA Clean & Concentrator Kit™ (Zymo Research, Irvine, CA, USA).

Following PCR, DNA was cleaned up with Agencourt® AMPure® XP beads (Beckman Coulter) and washed with 80% ethanol and allowed to air-dry. DNA was then eluted with 52.5 µl of 10 mM Tris pH 8.5 before being placed back into the magnetic stand. Once the supernatant had cleared, 50 µl was aliquoted for the experiment. DNA samples were quantified using the Quant-iT™ PicoGreen™ dsDNA Assay kit (Thermo Fisher) using the FLUROstar® Omega (BMG Labtech).

147 Samples were processed using the Illumina MiSeq™ 500 cycle Kit V2 and
148 sequenced on the Illumina MiSeq™ system by Massey Genome Service
149 (Palmerston North). Illumina MiSeq™ sequences were trimmed using SolexaQA++
150 software (Cox, Peterson, and Biggs 2010) and aligned to FASTA bisulfite converted
151 reference sequences using the package Bowtie2 (version 2.3.4.3) Each individual
152 read was then aligned to all reference sequences using the methylation-specific
153 package Bismark (Krueger and Andrews 2011).

154

155 **Statistics**

156 Differential DNA methylation was assessed using the package edgeR (Chen et al.).
157 Coverage level was set to greater or equal to ‘‘8’’ across unmethylated and
158 methylated counts, as recommended by (Chen et al.). Two models were used – the
159 first was a bivariate model, to assess differences between the *in utero* exposed to
160 tobacco compared to the non-exposed control group (model 1).

$$Y \sim U + AS + e$$

161 The second was a multiple regression to assess the interaction term *in utero*
162 maternal smoke exposure and offspring conduct problem score (high or low, model
163 2).

$$Y \sim U + C + AS + U{:}C + e$$

164 Where, *Y* is defined as the methylation M ratio, *U* is the exposed/unexposed *in utero*
165 to maternal smoking, *C* is conduct problem score with high conduct problem score <
166 53 and low conduct problem core < 46, *AS* adult smoker/non-smoker and *e* is the
167 unexplained variation or error tem.

168 This model was fitted with both ANOVA parameters and with contrasts between *in*
169 *utero* exposure groups (exposed – non-exposed) within CP score levels. Top tables
170 were constructed using the topTags function in edgeR, Log fold change, average log
171 counts per million, and in some cases F statistic and were calculated and nominal
172 significance was given for $P < 0.05$, these were then corrected using FDR. Scatter
173 plots with the inclusion of confidence intervals were constructed from log
174 transformed normalised methylated and unmethylated counts.

Results

Here we assessed DNA methylation within 10 separate genes (Table 2). DNA sequence data for 15 amplicons from these 10 genes (Supplementary Table 1) was generated, comprising a total of 280 CpG sites. These CpG sites included a combination of sites previously identified as differentially methylated, as well as amplification of all CpGs within the promoter region of genes associated with *in utero* neurodevelopment and CP phenotypes (Table 2). Differential methylation across these CpG sites was calculated to address whether any were specifically differentially methylated in individuals with CP, in response to *in utero* tobacco exposure.

Quantification of DNA methylation at previously reported CpG sites in response to *in utero* exposure to tobacco

Initially, we attempted to validate in our cohort (age ~28-30 years) five CpG sites which have been previously reported to be differentially methylated in the DNA of cord blood from newborns, and whole blood from children and adolescents (ages newborn to 17) in response to *in utero* tobacco exposure (Table 1). Data were partitioned into those individuals exposed *in utero*, and those who were not (model 1), to assess whether or not BSAS could detect previously reported CpG sites (Table 3).

AHRR (cg05575921) displayed a 3.1% decrease in DNA methylation between exposed and non-exposed individuals, at a nominal P value of 0.02. This site has been previously identified as hypomethylated in adult tobacco smokers, as well as in postnatal cord blood samples between *in utero* tobacco-exposed and non-exposed individuals. The probe cg05549655 in the gene *CYP1A1* displayed a 5.19% increase in DNA methylation in the *in utero* exposed group, however, this site did not reach nominal statistical significance in our cohort. Cg09935388 and cg09662411 in *GFI1* were unable to be replicated as differentially methylated between the exposed and the non-exposed groups (no significant change in β values). Both CpG sites show

hypomethylation, supporting previous observations of differential methylation within this gene. *CNTNAP2* (cg2594950) was similarly unable to be validated in our cohort using the method BSAS.

Differentially methylated CpGs under the interaction of *in utero* tobacco exposure and CP

Differential methylation dependent on both *in utero* exposure and CP score was found at 10 loci in six genes at nominal significance level, none were significant after correcting for false discovery rate (Table 4).

Of these CpG sites, five of the 10 CpG sites were found in the following genes: *CYP1A1*, *GFI1*, ASH2L *and GRIN2b*. Differential methylation between *in utero* exposed and non-exposed associated with for high conduct scores. No nominal significance from the interaction observed in association with low conduct scores. The top three CpG sites with nominal significance under the interaction are displayed in Figure 1. Here, differential methylation is found in response to high CP score and no differences are seen between the exposed and non-exposed low CP groups (Figure 1).

Discussion

In utero tobacco exposure is known to alter DNA methylation at the genome-wide level in offspring (Joubert, Haberg, et al. 2012; Joubert, Felix, Yousefi, Bakulski, Just, Breton, Reese, Markunas, Richmond, Xu, et al. 2016) (Joubert Bonnie et al. 2012; Richmond et al. 2014). The later-life implications of these tobacco-induced DNA methylation changes are unclear, however, an association between *in utero* tobacco exposure and CP has previously been observed (Sengupta et al. 2017). Given the complex etiology of CP phenotypes (Acosta, Arcos-Burgos, and Muenke 2004; Beaver et al. 2007; Salvatore and Dick 2018) and the vast array of socioeconomic variables associated with tobacco use (Lantz et al. 1998), proving a

causal link between maternal smoking and offspring CP is inherently challenging. Previously we quantified tobacco-induced DNA methylation changes that associate with CP phenotypes in offspring exposed to tobacco *in utero* (via maternal smoking) using the Illumina EPIC array, with results indicating that methylation was altered at genes that may have roles in neurodevelopment and CP phenotypes. However, due to a combination of a comparatively small sample size relative to the number of loci on the array, only nominal significance was observed. The data suggested a role for DNA methylation in the link between exposure and CP, so here we chose a panel of genes with known roles in these things, to see if methylation is changed at these loci too. Due to our small sample size we need to try an alternative approach. We've shown that BSAS previously is very good for targeting differential methylated so here we use BSAS at some targeted genes to see if we can detect differential methylation specific to the interaction between high CP score and exposure?

Validation of previously identified differentially methylated CpG from *in utero* tobacco exposure

First, we asked whether differentially methylated CpGs that have been previously associated with *in utero* tobacco exposure were supported by this cohort. Here, we present validation of differential methylation of a CpG site within the gene *AHRR* (cg05575921). *AHRR* is a well-defined tobacco smoking gene, which is consistently represented in tobacco methylation data. *AHRR* has previously been found to be differentially methylated in response to *in utero* tobacco exposure (Richmond, Simpkin, Woodward, Gaunt, Lyttleton, McArdle, Ring, Smith, Timpson, Tilling, Davey Smith, et al. 2015; Joubert, Håberg, Nilsen, Wang, Vollset, Murphy, Huang, Hoyo, Midttun, Cupul-Uicab, et al. 2012; de Vocht et al. 2015). Four other CpG sites investigated here due to previous association with *in utero* tobacco exposure were not differentially methylated in our data. However, the direction of methylation change was supported at all five sites investigated (Tehranifar et al. 2018; Rauschert et al. 2019; Rotroff et al. 2016). We suggest that further investigation in a larger cohort may lead to nominal significance at the sites in *CYP1A1*, *CNTNAP2,* and *GFI1*.

Identification of *in utero* exposure-related differentially methylated CpG sites that are specific to individuals with high CP scores

Epidemiological data suggests that there is an increased association between *in utero* tobacco exposure and behavioural disorder in children and adolescents (Carter et al. 2008; Mick et al. 2002). Thus, here, we investigated DNA methylation changes induced by *in utero* tobacco exposure as a potential molecular mechanism of dysfunction that could link the phenotypic trait of CP to maternal tobacco use during pregnancy. We therefore analysed DNA methylation patterns within our gene panel in response to *in utero* tobacco exposure and its interaction with CP status. A total of 10 CpG sites in seven genes were found to display nominal significance in DNA methylation in response to *in utero* tobacco exposure and CP in this cohort (Table 4).

In the 10 CpG sites we identified under the interaction, *CYP1A1* showed greater magnitude differential methylation in high CP scores (exposed *in utero* vs. non-exposed with high CPS), with reduced reversed or no evidence of differential methylation at the same sites with low CP score. This indicates that within the observed nominal methylation changes the interaction was being driven in the high CP score group. One gene (*ASH2L*), contained three nominally significantly differentially methylated CpG sites, and *CYP1A1* and *MEF2C* both had two.

CYP1A1 (Cytochrome P450 family 1 subfamily A member 1) is a well-established marker for *in utero* tobacco smoke exposure (Richmond et al. 2018; Lee Ken et al. 2015; Richmond et al. 2014; Tehranifar et al. 2018). Neither of the two sites we observed under the interaction have probes at these locations on the Illumina array system, thus emphasising a benefit of amplicon sequencing compared to an array-based method. Variant differences in *CYP1A1* have previously been associated with child behavioural problems at age 2, from prenatal maternal environmental tobacco smoke (Hsieh et al. 2010). This highlights the need for this gene to be further investigated for its role in the development of conduct problems following *in utero* tobacco exposure.

Three CpG sites from the gene *ASH2L* (ASH2 like histone lysine methyltransferase complex subunit) showed in consistent levels of differential methylation in response to *in utero* tobacco exposure and CP, with two displaying hyper- and one hypomethylation. *ASH2L* has been found to interact with *MEF2C* (Myocyte enhancer

299 factor 2C) to mediate changes in histone 3 lysine 4 trimethylation (H3K4me3) (Jung
300 et al.). Here, we detected nominal significance at two CpG sites within *MEF2C* (chr5,
301 88179596 and 88179541). Both of these sites were associated with a greater level of
302 hypomethylation in participants who were exposed to tobacco *in utero* with high CP
303 scores in this cohort, although not at the FDR significance level. *MEF2C* plays a role
304 in neural crest formation during development, where tissue-specific inactivation of
305 the gene results in embryonic lethality (Verzi et al. 2007). Further, *MEF2* interacts
306 with oxytocin, which is affiliated with prosocial behaviours (Kosfeld et al. 2005; Zak,
307 Stanton, and Ahmadi 2007). Alterations to oxytocin have been shown to change the
308 morphology of neurons via *MEF2A* (Meyer et al. 2018; Meyer et al. 2020). Functional
309 roles of the gene in relation to early neuronal development still remain unclear,
310 however it is thought to play a crucial role (Harrington et al. 2016). Recent research
311 in animal models suggests that nicotine-dependent induction of the *ASH2L* and
312 *MEF2C* complex during development induces alterations that could lead to
313 fundamental changes in the brain (Jung et al.).

314 While we cannot assert causality, our targeted approach shows that *in utero* tobacco
315 exposure may be altering methylation at CpG sites associated with neural
316 phenotypes which persist into adulthood and are then associated with increased risk
317 of high CP.

318

319 **Conclusion**

320 Here we have presented preliminary data to suggest that the association between
321 maternal tobacco use during pregnancy and the development of CP in children and
322 adolescents may in part be mediated by altered DNA methylation, induced by *in*
323 *utero* tobacco exposure during development, at genes that have roles in *in utero*
324 brain development and CP phenotypes. We acknowledge the limitations of this
325 study described above, however, the data presented here are suggestive of a role
326 for DNA methylation in the link between *in utero* tobacco exposure and offspring CP.
327 Our findings should stimulate further study using larger sample sizes.

328 **Abbreviations**

329 CP Conduct problems

CHDS Christchurch health and development study

BSAS Bisulfite based amplicon sequencing

SIDS Sudden infant death syndrome

ADHD Attention-deficit hyperactivity disorder

DOHaD Developmental origins of human health and disease

GFI1 Growth Factor Independent one transcriptional repressor

CPS Conduct disorder score

AHRR Aryl hydrocarbon receptor repressor

ASH2L ASH2 like histone lysine methyltransferase complex subunit

BDNF Brain-derived neurotrophic factor,

CNTNAP2 Contactin associated protein 2

CYP1A1 Cytochrome P450 Family 1 Subfamily A Member 1

DUSP6 Dual specificity phosphatase 6

GRIN2b Glutamate Ionotropic Receptor NMDA Type Subunit 2B

MEF2C Myocyte enhancer factor 2C

PRDM8 PR/SET Domain 8

FC Fold change

CPM Counts per million

FDR False discovery rate

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Availability of data

Upon request.

Contributions

AJN-molecular lab work, data analysis, and major contributor to manuscript. JFP-study design, data analysis, and major contributor to manuscript. ADN- data

analysis. JMB and LJH study design, provided DNA samples via CHDS. MAK- study design and over view. AJO- study design, molecular lab work, major contributor to manuscript and source of funding. All authors read and approved the final manuscript.

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Ethics declarations

All aspects of the study were approved by the Southern Health and Disability Ethics Committee, under application number CTB/04/11/234/AM10 “Collection of DNA in the Christchurch Health and Development Study”.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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572 Table 1 - CHDS subsets selected for analysis. The range of conduct problem scores
573 in each category is indicated in brackets. A score of 53 or more is the top quartile for
574 CP, a score of 60 or more the top decile for CP.

	Group 1 Exposed *in utero* and never smokers	Group 2 Exposed *in utero* and a regular smoker	Group 3 Not *exposed in utero* and never smokers
	n= 32	n= 32	n= 32
Sex			
Male	69%	72%	60%
Female	31%	28%	40%
Tobacco smoking status at the time of blood collection			
Never	100%	0%	100%
Regular	0%	100%	0%
Conduct problem score (CPS)			
Low CPS (<46)	n= 16 (42-46)	n= 16 (42-46)	n= 16 (41-43)
High CPS (>53)	n= 16 (53-75)	n= 16 (60-85)	n= 16 (53-68)

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591 *Table 2* - Genes selected to investigate the link between in utero tobacco exposure
592 and CP.

Gene	Function	Significance
AHRR (Joubert, Håberg, Nilsen, Wang, Vollset, Murphy, Huang, Hoyo, Midttun, and Cupul-Uicab 2012; Richmond, Simpkin, Woodward, Gaunt, Lyttleton, McArdle, Ring, Smith, Timpson, Tilling, Smith, et al. 2015; de Vocht et al. 2015; van Otterdijk, Binder, and Michels 2017; Rotroff et al. 2016)	Mediates toxicity of dioxin (found in cigarette smoke)	Hypomethylated in tobacco smokers and their offspring
ASH2L (Li et al.)	Histone lysine methyltransferase	Associated with schizophrenia
BDNF (Skogstrand et al. ; Jiao et al.)	Nerve growth factor	Promotes neuronal survival. Implicated in neurodegenerative disease
CNTNAP2 (Joubert, Felix, Yousefi, Bakulski, Just, Breton, Reese, Markunas, Richmond, and Xu 2016; Richmond, Simpkin, Woodward, Gaunt, Lyttleton, McArdle, Ring, Smith, Timpson, Tilling, Smith, et al. 2015; Rzehak et al. 2016)	Neurexin family – functions in vertebrate nervous system	Implicated in schizophrenia, autism, ADHD, intellectual disability. Hypomethylated in offspring of maternal smoking
CYP1A1 (Suter et al. 2010; Rotroff et al. 2016; Joubert, Håberg, Nilsen, Wang, Vollset, Murphy, Huang, Hoyo, Midttun, and Cupul-Uicab 2012; Richmond, Simpkin, Woodward, Gaunt, Lyttleton, McArdle, Ring, Smith, Timpson, Tilling, Smith, et al. 2015;	Monooxygenase – expression is induced by hydrocarbons found in cigarette smoke	Hypomethylated in offspring of maternal smoking

de Vocht et al. 2015; van Otterdijk, Binder, and Michels 2017)		
DUSP6 (Demontis et al. 2017)	Protein phosphatase, cellular proliferation and differentiation	Regulates neurotransmitter homeostasis
GFI1 (Rotroff et al. 2016; Joubert, Håberg, Nilsen, Wang, Vollset, Murphy, Huang, Hoyo, Midttun, and Cupul-Uicab 2012; van Otterdijk, Binder, and Michels 2017)	Zinc finger protein - transcriptional repressor	Part of a complex that controls histone modifications and gene silencing. Hypermethylated in offspring of maternal smoking
GRIN2B (Riva et al. 2015)	Glutamate receptor – expressed early in the brain and is required for normal brain development	Mutations associated with autism, ADHD, schizophrenia
MEF2C (Demontis et al. 2017)	MEF2C is associated with hippocampal-dependent learning and memory	MEF2C is crucial for normal neuronal development. Associated with ADHD
PRDM8 (Joubert, Felix, Yousefi, Bakulski, Just, Breton, Reese, Markunas, Richmond, and Xu 2016)	Histone methyltransferase - Controls expression of genes involved in neural development and neuronal differentiation	Hypomethylated in offspring of maternal smoking

Table 3 - Previously reported CpG sites showing differential DNA methylation in response to *in utero* tobacco exposure, and their average methylation values in individuals from this cohort.

Gene	Illumina ID	Exposed *in*	Non-	β difference	P value

		utero β value	exposed *in utero* β value		
AHRR	cg05575921	72.287	75.448	-3.161	0.022
CNTNAP2	cg2594950	3.845	3.860	-0.014	0.991
CYP1A1	cg05549655	26.894	21.699	5.195	0.425
GFI1	cg09935388	75.151	75.330	-0.582	0.055
GFI1	cg09662411	95.837	97.400	-1.583	0.274

Table 4 - CpG sites where differential methylation between conduct problem scores differs with *in utero* exposure at P< 0.05. Log Fold Change (FC) and P values (unadjusted) from log ratio tests for the effect on normalized methylation ratios of: (1) interaction between *in utero* exposure and Conduct Problem Score, (2) *In utero* exposed - non-exposed contrast within Low CPS and (3) within High CPS

631 participants. Loci with nominally significant (P< 0.05) interaction shown, all FDR P
632 values > 0.05.

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Gene	CpG location	Interaction[1]		Low CPS[2]		High CPS[3]	
		Log FC	*P value*	*Log FC*	*P value*	*Log FC*	*P value*
CYP1A1	Chr15, 75019290	-2.013	0.010	0.344	0.493	-1.669	0.005
GFI1	Chr1, 92947705	-0.957	0.011	0.002	0.992	-0.955	0.001
ASH2L	Chr8, 37962878	1.257	0.024	-0.447	0.253	0.811	0.042
MEF2C	Chr5, 88179596	-1.679	0.040	0.678	0.174	-1.000	0.122
DUSP6	Chr12, 89746588	-1.444	0.041	0.864	0.107	-0.580	0.204
ASH2L	Chr8, 37962657	-0.199	0.042	0.052	0.455	-0.147	0.033
CYP1A1	Chr15, 75019127	-1.221	0.045	0.403	0.319	-0.819	0.072
ASH2L	Chr8, 37962901	1.250	0.046	-0.561	0.205	0.688	0.121
GRIN2b	Chr12, 14133359	2.711	0.048	0.121	0.903	2.832	0.004
MEF2C	Chr5, 88179541	-1.336	0.050	0.615	0.139	-0.720	0.190

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