

[10.1071/RD21108](https://doi.org/10.1071/RD21108)

Reproduction, Fertility and Development

Supplementary Material

DNA methylation analysis using bisulphite-based amplicon sequencing of individuals exposed to maternal tobacco use during pregnancy, and offspring conduct problems in childhood and adolescence[†]

Alexandra J. Noble^{A,}, John F. Pearson^B, Alasdair D. Noble^C, Joseph M. Boden^D, L. John Horwood^D, Martin A. Kennedy^B, and Amy J. Osborne^A*

^ASchool of Biological Sciences, University of Canterbury, Christchurch, New Zealand.

^BDepartment of Pathology and Biomedical Sciences, University of Otago, Christchurch, New Zealand.

^CAgResearch, Lincoln Research Centre, Christchurch, New Zealand.

^DDepartment of Psychological Medicine, University of Otago, Christchurch, New Zealand.

*Correspondence to: Alexandra J. Noble School of Biological Sciences, University of Canterbury, Christchurch, New Zealand Email: alexandra.noble@pg.canterbury.ac.nz

Supplementary Table 1 - Forward and reverse primers (5' – 3') used to target potential candidates of in utero tobacco exposure. Primers for CpG sites of interest include the Illumina overhang sequence at the 5' end.

Primer name	Illumina Probe ID	Bisulfite converted primer (including the Illumina overhang sequence)
AHRR_F	cg05575921	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTTTTTGGTGTGGTTTAA
AHRR_R	cg05575921	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ACCACCATCTTATCTTATTT
CNTNAP2_F	cg2594950	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTGTTTTGGAGTAGTTTAA
CNTNAP2_R	cg2594950	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATAATCTTCACTTTTCATTAC
CYP1A1_F	cg05549655	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATAGTAGTTTGTGGTAAA
CYP1A1_R	cg05549655	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGRATACAAAAAATCTAAATCTAC
GFI1_F	cg09935388	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGGGAAGGAATGAGTAGAT
GFI1_R	cg09935388	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTAAAACTAATAACCCCAA
GFI1_F	cg09662411	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATAGTAGTTTTYGATTTTATTTTGA
GFI1_R	cg09662411	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACCCTCCCCCTACCTTTC
DUSP6_F	Promoter region	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTAAATAGAGTTGGGTTTT
DUSP6_R	Promoter region	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAAACAACTACAACAAC
BDNFpro1_F	Promoter region 1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAAAGGGAAAGTTGTTGGGTT
BDNFpro1_R	Promoter region 1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTAAAAAATCTTACTTATC
BDNFpro2_F	Promoter region 2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTTTTATTTTTTTTTTTGTT
BDNFpro2_R	Promoter region 2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTTCTAAAACCTTCTAAC
BDNFpro3_F	Promoter region 3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTTTTATTTTTTTTTGGGAAT
BDNFpro3_R	Promoter region 3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGRTCTCCCAACAATACTAAA
PRDM8pro1_F	Promoter region 1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGTTGAAGTAGTTGTTTT
PRDM8pro1_R	Promoter region 1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAATATATAAAAATCATAAC
PRDM8pro2_F	Promoter region 2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATTTTTTATATTATTTTTTTT
PRDM8pro2_R	Promoter region 2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAACCTATAAAAACCTTCC
MEF2Cpro1_F	Promoter region	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGGAAAGATTGATTTATTAAG
MEF2Cpro1_R	Promoter region	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTTATCTTACCTTTACTT
ASH2L_F	Promoter region	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGYGGGTAGGGAGTGTTAGATTTTA
ASH2L_R	Promoter region	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTAAAAAACATAATTCCAC
GRIN2Bpro2_F	Promoter region	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGTGGGAAATGCGGGGTTT
GRIN2Bpro2_R	Promoter region	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAAGGTAATTCAGGGTATG

Supplementary Table 2 - Top CpG sites found to be nominally significantly differentially methylated (unadjusted P < 0.05) in response to in utero tobacco exposure (Model 1). Asterisk, *, indicates CpG sites in genes identified as differentially

methyated in response to adult smoking status (Supplementary Table 3). Abbreviations: FC, fold change; CPM, counts per million; FDR, FDR-corrected P value.

Gene	Illumina ID, CpG site location	Log FC	Average Log CPM	P Value	FDR
* <i>AHRR</i>	Chr5, 373398	-0.369	12.699	0.0009	0.187
* <i>GFI1</i>	Chr1, 92946546	-0.588	12.284	0.002	0.192
* <i>BDNF</i>	Chr11, 27743856	-1.323	10.237	0.004	0.192
* <i>GRIN2b</i>	Chr12, 14133243	2.100	10.113	0.004	0.192
* <i>GFI1</i>	Chr1, 92947559	-0.507	9.0675	0.005	0.192
* <i>GFI1</i>	Chr1, 92947752	-0.433	9.8441	0.006	0.192
<i>GRIN2b</i>	Chr12, 14133359	1.789	10.523	0.007	0.192
* <i>GFI1</i>	Chr1, 92946452	-0.374	12.211	0.008	0.192
* <i>GIF1</i>	Chr1, 92946429	-0.558	12.163	0.009	0.192
<i>BDNF</i>	Chr11, 27743594	-0.773	11.078	0.010	0.192
<i>GFI1</i>	Chr1, 92946514	-0.477	10.053	0.011	0.200
* <i>BDNF</i>	Chr11, 27743729	-1.266	8.550	0.016	0.262
<i>GFI1</i>	Chr1, 92946568	-0.339	12.218	0.019	0.284
* <i>AHRR</i>	cg05575921	-0.270	12.687	0.022	0.291
<i>AHRR</i>	Chr5, 373355	-0.228	12.749	0.022	0.291
* <i>GIF1</i>	Chr1, 92946418	-0.512	12.160	0.030	0.365
<i>DUSP6</i>	Chr12, 89746641	-0.635	10.060	0.033	0.371
<i>GFI1</i>	Chr1, 92946434	-0.314	12.193	0.035	0.371
<i>GFI1</i>	Chr1, 92946340	-0.368	12.360	0.047	0.413
* <i>GFI1</i>	Chr1, 92946132	-0.420	12.295	0.048	0.413
<i>DUSP6</i>	Chr12, 89746479	0.813	10.285	0.049	0.413
<i>ASH2L</i>	Chr8, 37962720	0.692	11.626	0.049	0.413

Supplementary Table 3 – Top CpG sites found to be nominally significantly differentially methylated (unadjusted $P < 0.05$) in response to adult smoking. Abbreviations: FC, fold change; CPM, counts per million; FDR, FDR-corrected P value.

Gene	CpG site location	Log FC	Average Log CPM	P value	FDR
<i>AHHR</i>	Chr5, 373398	-0.343	12.699	0.002	0.273
<i>GFI1</i>	cg09662411	-0.444	12.314	0.005	0.273
<i>GFI1</i>	Chr1, 92946923	-0.372	12.378	0.007	0.273
<i>GFI1</i>	Chr1, 92946222	-0.492	12.299	0.007	0.273
<i>GFI1</i>	cg09935388	-0.458	9.2268	0.008	0.273
<i>GFI1</i>	Chr1, 92946429	-0.560	12.163	0.008	0.273
<i>ASH2L</i>	Chr8, 37962657	-0.129	11.333	0.010	0.273
<i>GFI1</i>	Chr1, 92947752	-0.422	12.093	0.012	0.273
<i>GFI1</i>	Chr1, 92947586	-0.445	9.229	0.013	0.273
<i>GRIN2b</i>	Chr12, 14133243	2.388	10.113	0.015	0.273
<i>GFI1</i>	Chr1, 92946270	-0.315	12.363	0.018	0.273
<i>ASH2L</i>	Chr8, 37962793	-0.674	11.685	0.021	0.273
<i>GFI1</i>	Chr1, 92946452	-0.336	12.125	0.022	0.273
<i>GFI1</i>	Chr1, 92 947581	-0.332	9.2268	0.022	0.273
<i>GFI1</i>	Ch1, 92946415	-0.303	12.160	0.022	0.273
<i>GFI1</i>	Chr1, 92946620	-0.263	12.195	0.022	0.273
<i>BDNF</i>	Chr11, 27743452	-0.674	10.381	0.026	0.286
<i>GFI1</i>	cg06338710	-0.402	12.198	0.029	0.286
<i>GFI1</i>	Chr1, 92946434	-0.327	12.193	0.029	0.286
<i>BDNF</i>	Chr11, 27743729	-1.214	8.550	0.030	0.286
<i>GFI1</i>	Chr1, 92946418	-0.500	12.160	0.031	0.286
<i>GFI1</i>	Chr1, 92946235	-0.428	12.311	0.034	0.295
<i>GFI1</i>	Chr1, 92947559	-0.336	12.356	0.041	0.337
<i>GFI1</i>	Chr1, 92946132	-0.436	12.295	0.043	0.337
<i>GFI1</i>	Chr1, 92946452	-0.287	12.211	0.045	0.337
<i>AHRR</i>	cg05575921	-0.233	12.687	0.048	0.337