Developmental effects of maternal smoking during pregnancy on the human frontal cortex transcriptome

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Background: Cigarette smoking during pregnancy is a major public health concern. While there are well-described consequences in early child development, there is very little known about the effects of maternal smoking on human cortical biology during prenatal life.

Methods: We performed a genome-wide differential gene expression analysis using RNA sequencing (RNA-seq) on prenatal (N=33; 16 smoking-exposed) as well as adult (N=207; 57 active smokers) human post-mortem prefrontal cortices.

Findings: Smoking exposure during the prenatal period was directly associated with differential expression of 14 genes; in contrast, during adulthood, despite a much larger sample size, only 2 genes showed significant differential expression (FDR<10%). Moreover, 1,315 genes showed significantly different exposure effects between maternal smoking during pregnancy and direct exposure in adulthood (FDR<10%) – these differences were largely driven by prenatal differences that were enriched for pathways previously implicated in addiction and synaptic function. Furthermore, prenatal and age-dependent differentially expressed genes were enriched for genes implicated in non-syndromic autism spectrum disorder (ASD) and were differentially expressed as a set between patients with ASD and controls in post-mortem cortical regions.

Interpretation: These results underscore the enhanced sensitivity to the biological effect of smoking exposure in the developing brain and offer novel insight into the effects of maternal smoking during pregnancy on the prenatal human brain. They also begin to address the relationship between *in utero* exposure to smoking and the heightened risks for the subsequent development of neuropsychiatric disorders.

Leveraging Postmortem Human Brain Samples

We have generated transcriptome (polyA+ RNAseq) data from the DLPFCs of 240 samples with smoking information across the lifespan:

standard deviation or % is in parentheses	Prenatal		Adult Non-Psychiatric Controls	
	Smoking Unexposed	Smoking Exposed	Non-Smoker	Smoker
N	17	16	150	57
Age	17.4 (2.20)	19.8 (5.19)	41.5 (15.8)	42.5 (14.9)
Race				
Caucasian	0 (0.0%)	3 (18.8%)	76 (50.7%)	22 (38.6%)
African American	17 (100%)	13 (81.2%)	74 (49.3%)	35 (61.4%)
Male	10 (58.8%)	5 (31.2%)	108 (72.0%)	40 (70.2%)
Source				
NIMH	0 (0%)	0 (0%)	124 (82.7%)	55 (96.5%)
Stanley	0 (0%)	0 (0%)	12 (8.0%)	2 (3.5%)
UMB	17 (100%)	16 (100%)	14 (9.3%)	0 (0%)
RIN	8.82 (1.35)	8.94 (0.99)	8.33 (0.67)	8.52 (0.52)
pH	6.21 (NA)	6.06 (0.04)	6.55 (0.27)	6.57 (0.25)
PMI (hours)	2.53 (1.18)	2.44 (2.03)	28.52 (13.8)	30.60 (15.9)
Mitochondrial Mapping Rate	0.01 (0.00)	0.02 (0.01)	0.13 (0.07)	0.12 (0.06)

References/Links

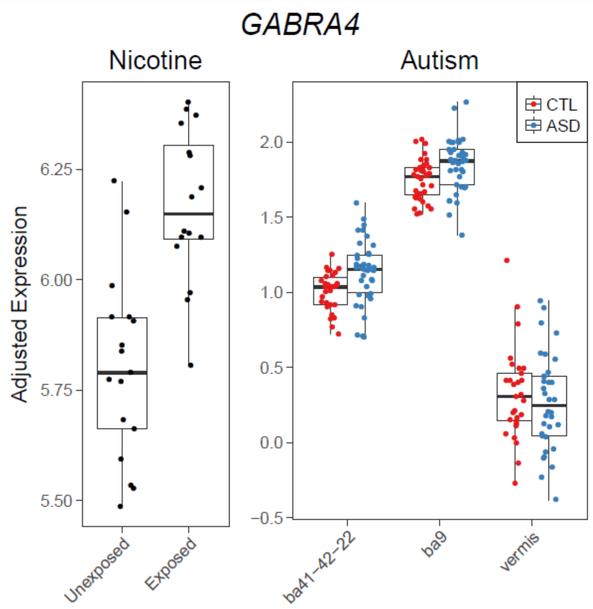
a. Corresponding pre-print:
Semick et al., bioRxiv 2017 [ID: 236968]

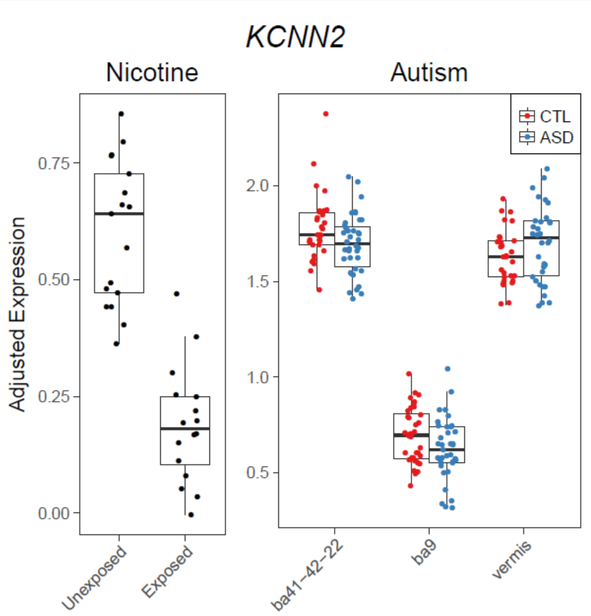
b. Autism case-control differences:
Parikshak et al., 2016 [PMID: 27919067]

c. Neuropsychiatric disease gene sets:
Birnbaum et al., 2014 [PMID: 24874100]

			Prenatal		Adult		
Cohort	**Symbol**	**Feature Level**	**Log_2 Fold Change**	**FDR**	**Log_2 Fold Change**	**FDR**	**Interaction P**
Prenatal	*PCDH10*	Expressed Region	-1.4	0.026	-0.11	1	5.9×10^{-4}
Prenatal	*KCNN2*	Gene	-0.69	0.047	-0.015	0.99	1.7×10^{-5}
Prenatal	*EPHA8*	Gene	1.5	0.048	-0.019	0.99	2.5×10^{-5}
Prenatal	*TENM3*	Gene	0.80	0.048	-0.006	1.0	2.8×10^{-11}
Prenatal	*IL1RAPL2*	Gene	-1.0	0.048	-0.06	0.99	2.4×10^{-4}
Prenatal	*MPPED1*	Gene	0.39	0.051	-0.005	1.0	2.0×10^{-5}
Prenatal	*GABRA4*	Gene	1.1	0.056	0.031	0.90	8.8×10^{-16}
Prenatal	*ECHDC2*	Gene	0.86	0.056	0.015	0.99	3.2×10^{-7}
Prenatal	*SDC1*	Gene	0.37	0.056	0.092	0.98	0.46
Prenatal	*CNTN4*	Gene	0.68	0.056	0.003	1.0	1.6×10^{-8}
Prenatal	*CHSY3*	Gene	0.55	0.063	0.02	0.99	2.8×10^{-8}
Prenatal	*RNF13*	Gene	-0.28	0.063	0.015	0.99	0.19
Prenatal	*ZNF608*	Gene	0.34	0.067	0.035	0.99	0.013
Prenatal	*NRCAM*	Gene, Junction	-0.57	0.1	0.014	0.99	1.3×10^{-5}
Adult	*MARCO*	Gene	0.90	0.43	-1.6	8.4×10^{-5}	1.5×10^{-4}
Adult	*CEP85*	Junction	-0.05	0.94	-0.25	0.061	0.042

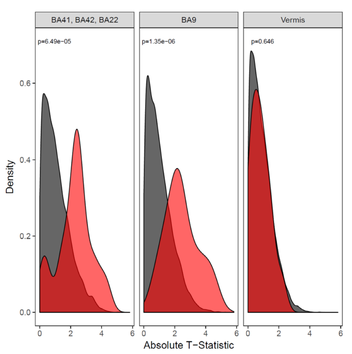
14 genes associated with smoking exposure within the prenatal cohort.



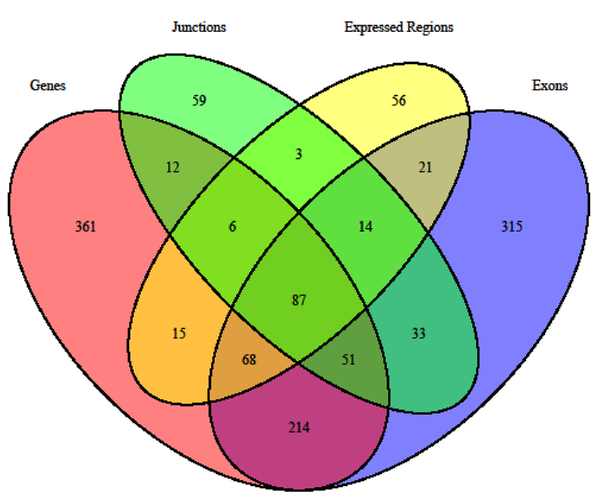


SFARI Gene
(OR=10.9, $p=3.8\times10^{-4}$)

	DE	Not DE
ASD	5	794
Not ASD	9	15,558
Overlap	56%	5.1%

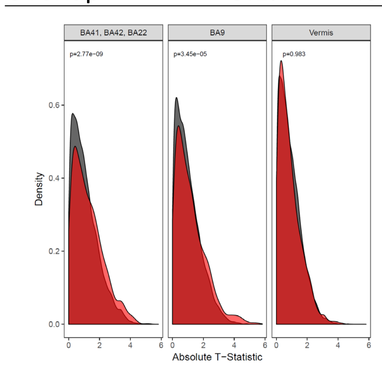


Prenatal differential expression is more similar to autism spectrum disorder case-control differences than expected.



SFARI Gene
(OR=1.9, $p=7.2\times10^{-8}$)

	DE	Not DE
ASD	101	698
Not ASD	1,108	14,465
Overlap	9.1%	4.8%



Gene Set	P	Odds Ratio
SFARI Gene	7.2×10^{-8}	1.9
AutDb	1.2×10^{-7}	1.9
ASD CNV	0.43	1.2
ASD DATABASE	3.8×10^{-4}	2.1
BPAD GWAS	0.71	1.1
ID	0.012	2.25
NDD	0.017	3.3
Neurodegenerative	0.37	0.30
SCZ CNV	0.85	1.1
SCZ Meta-analysis	0.066	2.5
SCZ PGC GWAS	0.84	1.0
SCZ SNV	0.0014	2.1

1,315 genes exhibited an interaction between smoking exposure and developmental stage. These interaction genes were enriched for autism spectrum disorder genes.