Genetics of Maternal Psychological Symptoms and Impact on Neonatal Gene Expression

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Multi-scalar systems-level view of CANDLE



The phenotype: maternal mood and psychological symptoms during pregnancy

- Psychometric measures from ~1300 women using Brief Symptoms Inventory (BSI) (depression, anxiety, phobia, etc.)
- Collected at 3rd trimester of pregnancy:
 - Cross-talk between endocrine system and nervous system in modulating mood and depression in women
 - Marked elevation in gonadal steroid hormones (estrogen, progesterone)
 - Activation of HPA stress axis; elevated cortisol levels ¹
 - 12% depression rate at 3rd trimester (9% depression rate among adult women overall)²
- Psychological symptoms also measured at different time-points after childbirth for CANDLE

¹ Bloch et al., Compr Psychiatry. 2003; Lokuge et al., J Clin Psychiatry. 2011
 ² http://www.nimh.nih.gov/statistics/1mdd_adult.shtml

Predictors of maternal psychological symptoms



Education: F(4, 1312) = 4.89, p < 0.0007

Phenotype and Genotype

Phenotype data

Psychometric measures from ~1300 women using BSI (depression, anxiety, phobia, etc.)

<u>Genotype data</u>

- Genome-wide genotype data from 372 mother-child dyads
- Affymetrix SNP arrays: >800,000 SNPs

Genotype-Phenotype association

354 mothers with both BSI and genotype data

Genetic relatedness among CANDLE mothers

- Genome-wide identity-by-state (IBS) clustering in Plink: compare across >200,000 high quality autosomal SNPs
- Compute proportion of genetic regions identical between each pair; clustering based on genetic identity/distance
- Visualized by multidimensional scaling plot



- Highly stratified population as multiple ethnicities represented
- Genetic clusters largely overlap self-reported ethnicity

http://pngu.mgh.harvard.edu/ ~purcell/plink/

Genome-wide association study (GWAS) for maternal psychological symptom

- Global severity index (GSI): summarized index of overall mood symptoms measured by BSI; a quantitative trait (not case vs. control)
- Association test across 231,122 SNPs.
- Test corrected for population structure and covariates of GSI (maternal education, smoking, prior use of antidepressants)



Psychological phenotypes are complex traits

- Complex traits are summations of multiple factors:
 - Multiple genetic loci influence the trait
 - Each genetic variant has small effect size and contributes to only a portion of the phenotypic variance
 - Combined effect of multiple variants in biological pathways
- Power issue: Sample size too small to detect true positive effects at genomewide threshold using single SNP tests
- Solution >> increase sample size
- Hypothesis driven, gene-set (or pathway) based analysis of the genetics of maternal mood symptoms:
 - Estrogen system implicated in mood and depression in women, particularly during and after child birth
 - Estrogen mediates biological effect (including in the brain) by binding to estrogen receptors
 - Test if variants in multiple estrogen receptor genes have a cumulative effect on maternal mood during pregnancy

Biological function (gene ontology) based association test

Gene-set based test to look at cumulative effect of estrogen receptor genes on maternal psychological symptoms:

- Gene Ontology ¹ database: classifies genes into families based on function
- GO category 'estrogen receptor activity' (GO:0030284), and 'steroid hormone receptor activity' (GO:0003707))
- Family of genes with estrogen receptor activity:
 - ESR1 (estrogen receptor alpha)
 - ESR2 (estrogen receptor beta)
 - LEF1 (lymphoid enhancer-binding factor 1)
 - NKX3-1 (NK3 homeobox 1)
 - ESRRB (estrogen-related receptor beta)
 - ESRRG (estrogen-related receptor gamma)

¹ http://amigo.geneontology.org

Combined gene-set test for estrogen receptors



¹ Method adapted from Ersland et al. PLoS One. 2012; Peng et al. Eur J Hum Genet. 2010

Combined gene-set test for estrogen receptors

Gene level p adjusted for haplotype structure and number of independent tests

Most significant p at **SNP** level

Gene	#SNP ¹	#Tests ²	SNP ³	Feature	Chr	Mb	Alleles	MAF	HWE P	Beta	Р	Sidak	
ESRRG	116	57	rs946438	intron	1	217.185	A/G	0.47	0.60	1.53	0.01	0.51	l
LEF1	11	6	rs7698367	intron	4	109.030	C/T	0.40	0.52	-1.26	0.05	0.26	
ESR1	38	23	rs3798577	3'UTR	6	152.421	A/G	0.44	0.21	-1.68	0.01	0.22	
NKX3-1	6	2	rs907584	intergenic	8	23.520	C/T	0.12	0.80	1.56	0.12	0.22	
ESR2	6	2	rs960070	intron	14	64.745	C/G	0.44	0.83	-1.84	0.004	0.007	
ESRRB	30	17	rs7155312	inergenic	14	76.776	A/G	0.22	0.23	2.58	0.0007	0.01	
									Fisher's	combine	p-values	0.004	



Multi-scalar systems-level view of CANDLE



Does the mother's phenotype and maternal factors have an impact on child state?

 Newborn gene expression as a molecular proxy of child state

New born cord blood transcriptome

- Global gene expression measured in the cord blood of 122 newborns
- Illumina microarray platform with >48,000 probes that measures abundance of mRNA transcripts
- Quantifies the amount of mRNA produced by a gene



Functional organization of cord blood gene expression

- Construct gene co-expression networks based on expression correlation and adjacency patterns across samples
- Topological overlap map shows hotspots of interconnected transcripts
- Define gene modules by clustering transcripts with high topological overlap into distinct modules

- Organizes transcriptome into functionally cohesive gene networks: From thousands of transcripts to few gene modules
- Eigengene definition: Networks of tightly correlated transcripts summarized by a single eigenvectors (PC1); a.k.a. Eigengene







Associating cord blood gene modules to maternal factors and child traits

Cord blood gene expression organized into 16 modules. This covariance matrix shows how module eigengenes relate to each other



Classified as M1 (>3000 members) to M16 (46 members)

Which of these gene modules are most sensitive to (1) maternal mood,(2) smoking?And what can this tell us about the impact of maternal state on child?

Transcript network in newborn associated with maternal mood trait



- Network of 91 transcript (ME14) enriched in metabolic genes (e.g., SELP, F13A1, GP1BA, ITGB3, CCL5)
- Maternal GSI negatively correlated with ME14 (R = -0.189; p = 0.04)

Transcript network in newborn associated with smoking



- Smoking may have effect on the ME3 module (1487 members); enriched in regulators of gene expression
- Maternal smoking and ME3 (F(1, 115) = 4.9, p = 0.028)
- Heightened expression in newborns with mothers who smoked

Maternal smoking may influence expression of nicotine dependence transcripts in newborns

Correlation subnetwork extracted out of ME3: CHRNB3 (nicotine receptor) and DRD2 (dopamine receptor)



- DRD2 and CHRNB3 are candidate for nicotine dependence
- In utero exposure to nicotine associated with impulsivity in children (Fitzpatrick et al., Int J Psychophysiol. 2012)
- Also links between dopamine receptors and impulsivity.
- Is this effect on gene expression a mechanism for the trans-generational effect of smoking on child behavior? Explore this in CANDLE?

Do expression of DRD2 and CHRNB3 in cord blood predict child behavior later in life?

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Get Any:	Databases marked with ** suffix are not public yet. Access requires user login . Enter list here (APOE, APOA, etc.): logical OR Enter terms, genes, ID numbers in the Get Any field.	 Select Species (or select All) Select Group (a specific sample) Select Type of data: Phenotype (traits) Genotype (markers) Expression (mRNAs)
Combined:	Use * or ? wildcards (Cyp*a?, synap*). Use Combined for terms such as <i>tyrosine kinase</i> . Enter terms to combine (blood pressure): logical AND Search Make Default Advanced Search	 Select a Database Enter search terms in the Get Any or Combined field: words, genes, ID numbers, probes, advanced search commands Click on the Search button Optional: Use the Make Default button to save your preferences
Quick HELP E You can also u into the Get A	xamples and User's Guide use advanced commands. Copy these simple examples uny or Combined search fields:	How to Use GeneNetwork Take a 20-40 minute GeneNetwork Tour that includes screen shots and typical steps in the analysis.

GeneNetwork: data repository and analytical interface for CANDLE for systems level analysis

Correlation between DRD2 expression in cord blood (X-axis) and parent-child interaction on teaching scale (NCAST) at 24 months



Correlation between CHRNB3 expression in cord blood (X-axis) and parent-child interaction on teaching scale (NCAST) at 24 months (Y-axis)



To conclude...



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