Ukraine Clinical Project

CIFASD Conference Call

1/27/16

Maternal Samples

- Nested case/control sample
- Eligible subjects selected from the entire Ukraine cohort based on
  - Availability of two maternal blood samples (enrollment at 3rd trimester)
  - Live born infant with study related physical exam and neurobehavioral testing completed

Maternal Samples

- Cases and controls selected from eligibles; consist of three groups defined as
  - A) mother exposed to moderate to heavy amounts of alcohol in pregnancy; live born infant has facial features, growth deficiency, and lower scores on Bayley scales of infant development between 6 and 12 months of age
  - B) mother exposed to similar amounts of alcohol as (A); live born infant has no facial features, normal growth, and scores in the normal range on the Bayley between 6 and 12 months of age
  - C) mother reported no or low alcohol exposure (controls)

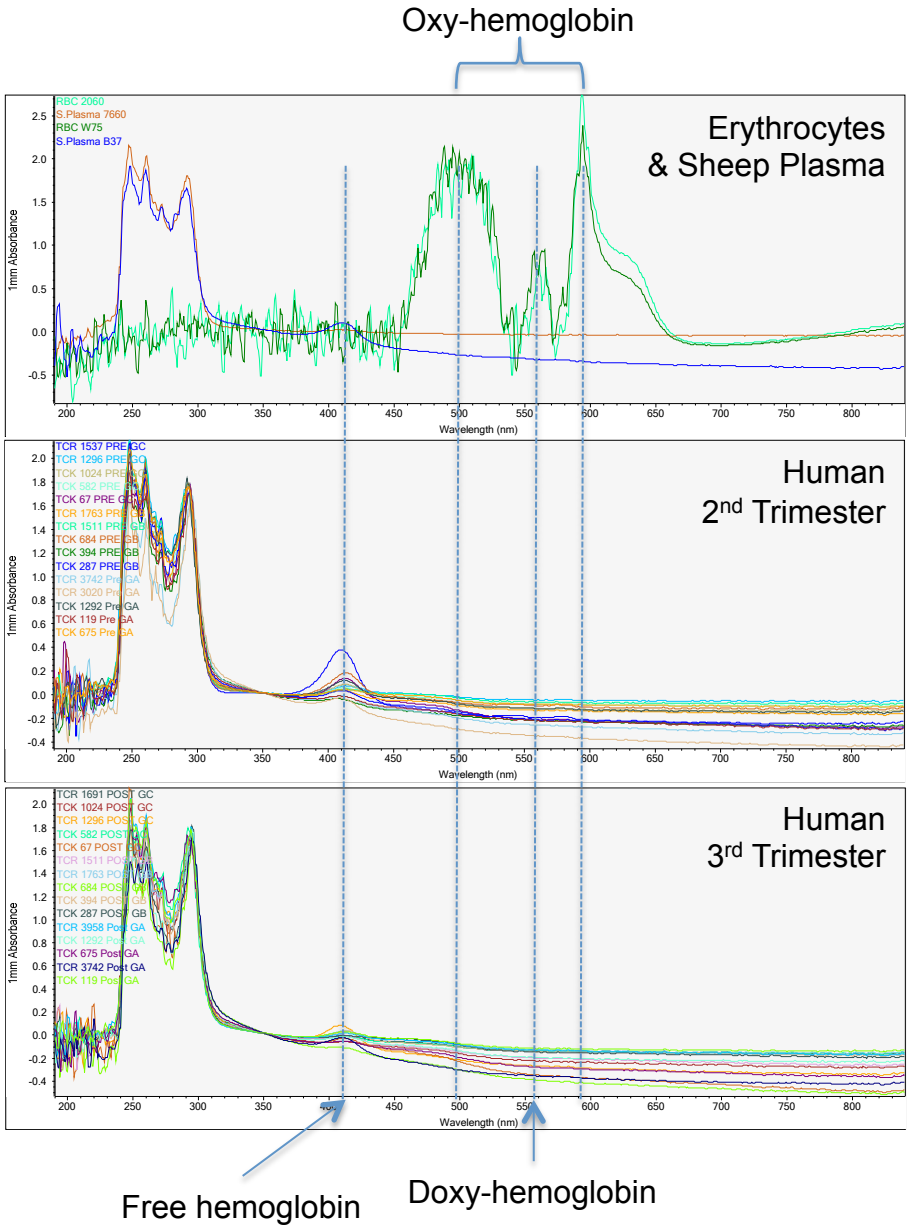
Maternal Samples

- Sample set meeting these criteria previously used
  - for broad-based methylation analysis
  - to measure epigenetic changes in PER2 and POMC genes for Dipak Sarkar's developmental project

Plasma miRNA project

Rajesh Miranda

Free Hemoglobin Analysis

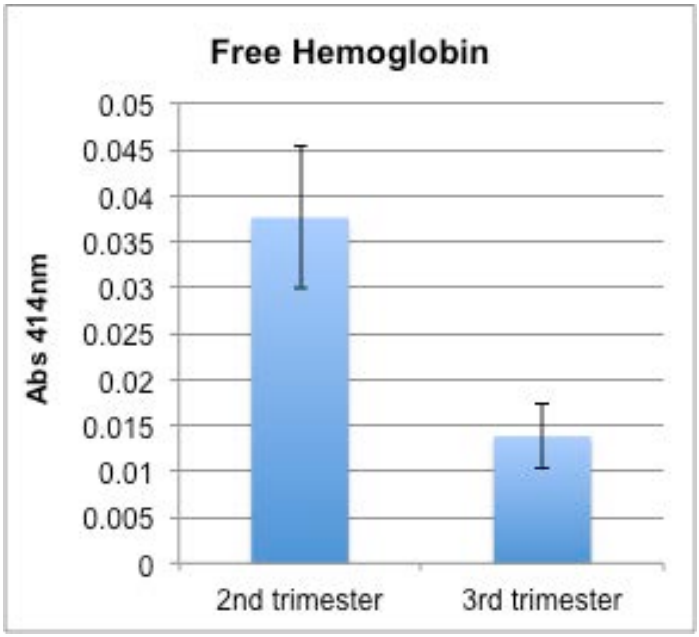


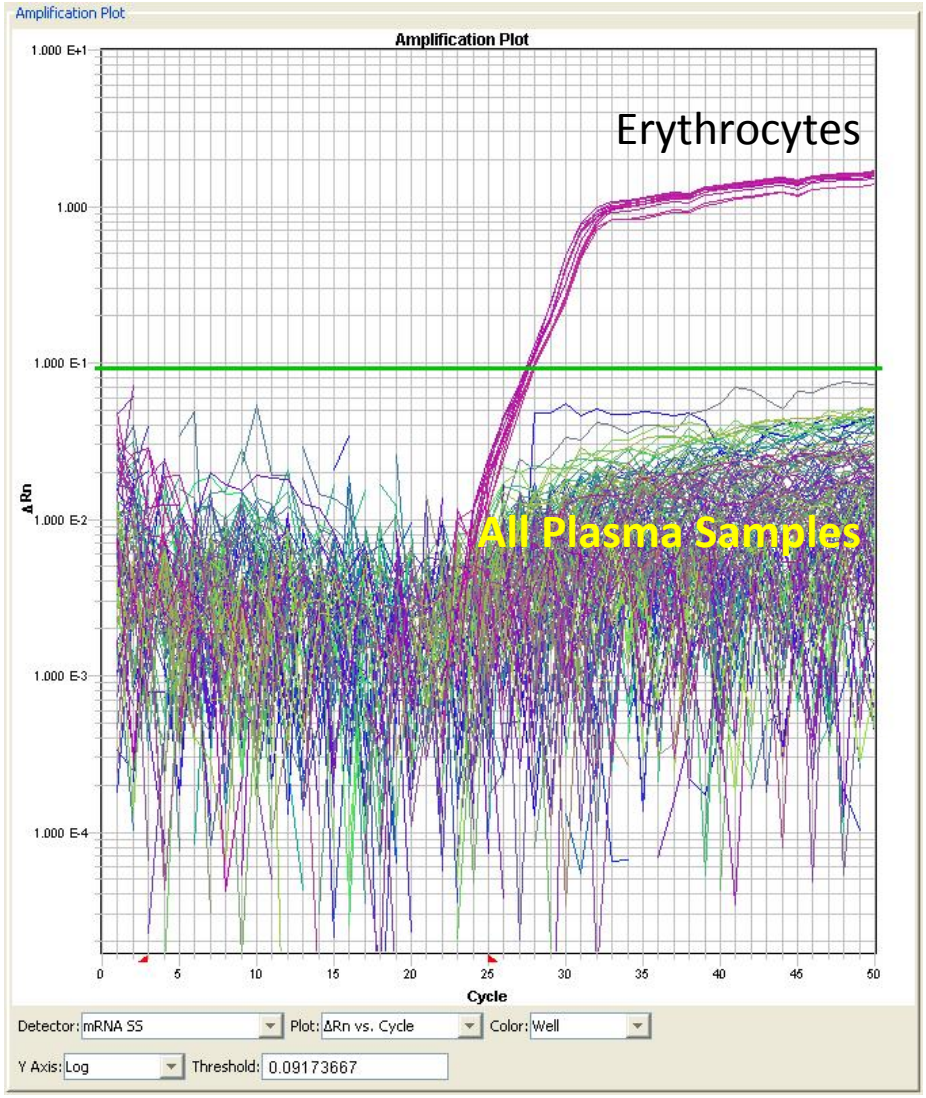
Tests of Between-Subjects Effects

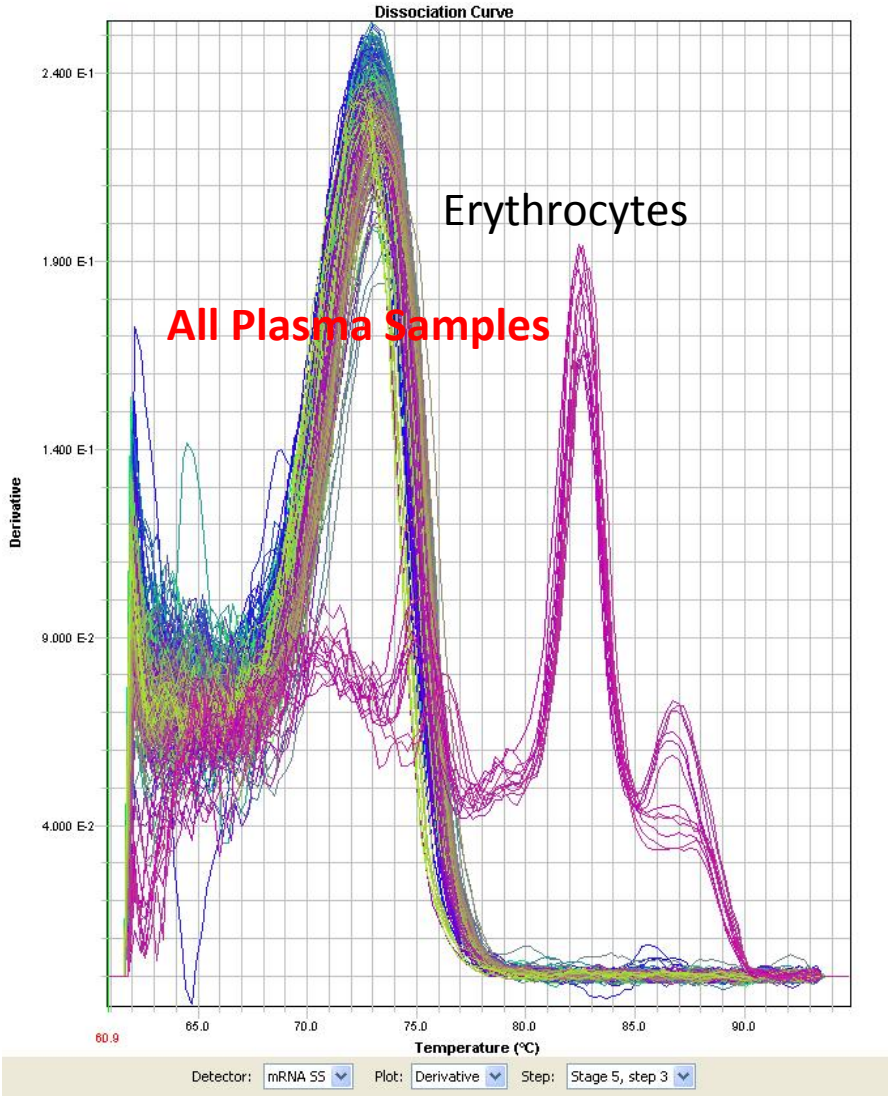
Dependent Variable: Corrected_Abs_414nm

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.037[a]	11	.003	1.332	.215
Intercept	.065	1	.065	25.810	.000
Recrutiment_site	5.028E-005	1	5.028E-005	.020	.888
Pregnancy_stage	.017	1	.017	6.666	.011
Exposure_Group	.001	2	.001	.271	.763
Recrutiment_site * Pregnancy_stage	.002	1	.002	.677	.412
Recrutiment_site * Exposure_Group	.000	2	.000	.098	.907
Pregnancy_stage * Exposure_Group	.010	2	.005	1.968	.144
Recrutiment_site * Pregnancy_stage * Exposure_Group	.000	2	.000	.099	.905
Error	.313	125	.003		
Total	.441	137			
Corrected Total	.350	136			

a. R Squared = .105 (Adjusted R Squared = .026)




Amplification Plot
Amplification Plot
Erythrocytes
All Plasma Samples
ΔRn
Cycle
1.000 E+1
1.000
1.000 E-1
1.000 E-2
1.000 E-3
1.000 E-4
0
5
10
15
20
25
30
35
40
45
50
Detector: mRNA SS
Plot: ΔRn vs. Cycle
Color: Well
Y Axis: Log
Threshold: 0.09173667


Dissociation Curve
Erythrocytes
All Plasma Samples
Derivative
Temperature (°C)
2.400 E-1
1.900 E-1
1.400 E-1
9.000 E-2
4.000 E-2
60.9
65.0
70.0
75.0
80.0
85.0
90.0
Detector: mRNA SS
Plot: Derivative
Step: Stage 5, step 3

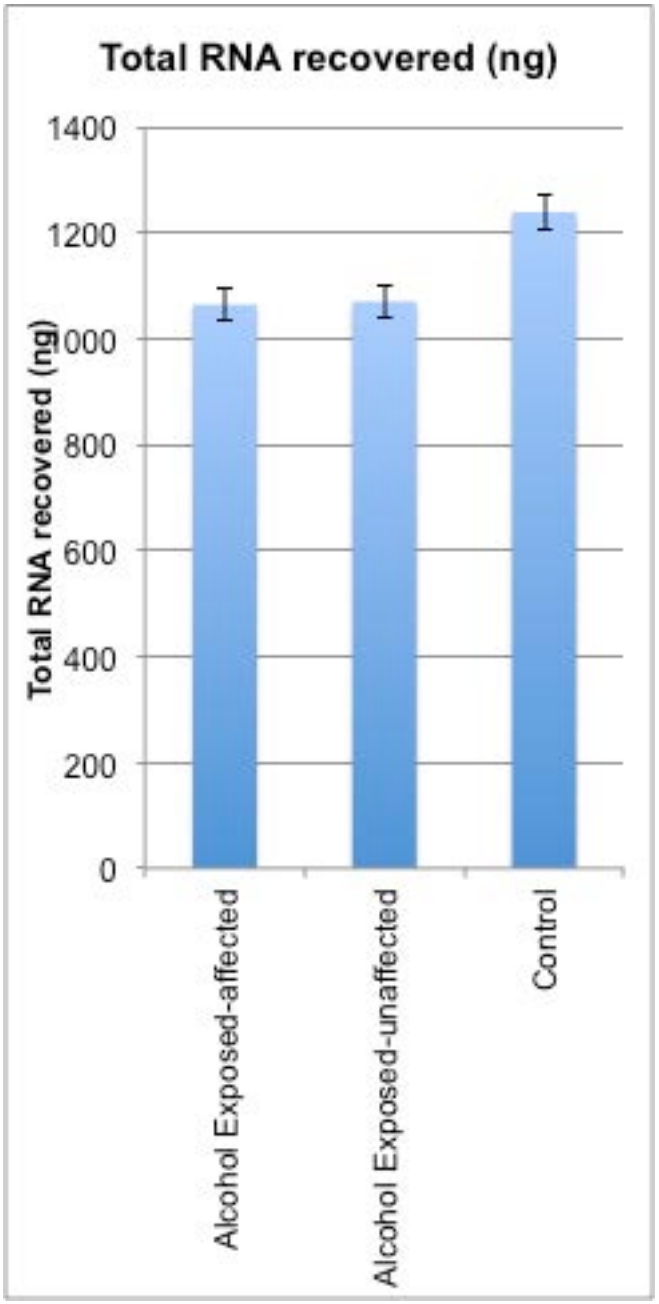
RNA Recovery

Tests of Between-Subjects Effects

Dependent Variable: Total_RNA_ng

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1270490.5[a]	11	115499.137	2.387	.010
Intercept	138226075	1	138226075	2857.214	.000
Recruitment_site	32932.021	1	32932.021	.681	.411
Trimester	187300.478	1	187300.478	3.872	.051
Exposure_Group	954265.369	2	477132.685	9.863	.000106
Recruitment_site * Trimester	132596.315	1	132596.315	2.741	.100
Recruitment_site * Exposure_Group	77630.203	2	38815.102	.802	.451
Trimester * Exposure_Group	12837.305	2	6418.653	.133	.876
Recruitment_site * Trimester * Exposure_Group	552.397	2	276.199	.006	.994
Error	5998862.44	124	48377.923		
Total	179574400	136			
Corrected Total	7269352.94	135			

a. R Squared = .175 (Adjusted R Squared = .102)



Tests of Between-Subjects Effects

Dependent Variable: Count

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33121.977[a]	11	3011.089	.532	.879
Intercept	9566727.52	1	9566727.52	1688.769	.000
Recruitment_site	645.923	1	645.923	.114	.736
Pregnancy_Stage	1.688	1	1.688	.000	.986
Exposure_group	4509.103	2	2254.551	.398	.673
Recruitment_site * Pregnancy_Stage	952.346	1	952.346	.168	.683
Recruitment_site * Exposure_group	12482.270	2	6241.135	1.102	.336
Pregnancy_Stage * Exposure_group	3660.352	2	1830.176	.323	.725
Recruitment_site * Pregnancy_Stage * Exposure_group	5312.563	2	2656.281	.469	.627
Error	702449.133	124	5664.912		
Total	12941395.0	136			
Corrected Total	735571.110	135			

a. R Squared = .045 (Adjusted R Squared = -.040)

Tests of Between-Subjects Effects

Dependent Variable: Average_Expression

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.687[a]	11	2.608	1.019	.434
Intercept	115090.371	1	115090.371	44972.515	.000
Recruitment_site	8.989	1	8.989	3.513	.063
Pregnancy_Stage	1.501	1	1.501	.587	.445
Exposure_group	3.228	2	1.614	.631	.534
Recruitment_site * Pregnancy_Stage	.626	1	.626	.245	.622
Recruitment_site * Exposure_group	5.290	2	2.645	1.034	.359
Pregnancy_Stage * Exposure_group	1.952	2	.976	.381	.684
Recruitment_site * Pregnancy_Stage * Exposure_group	5.724	2	2.862	1.118	.330
Error	317.332	124	2.559		
Total	150455.578	136			
Corrected Total	346.019	135			

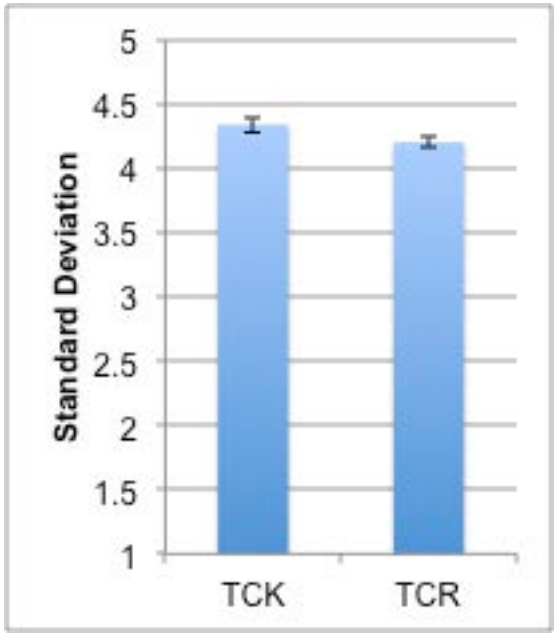
a. R Squared = .083 (Adjusted R Squared = .002)

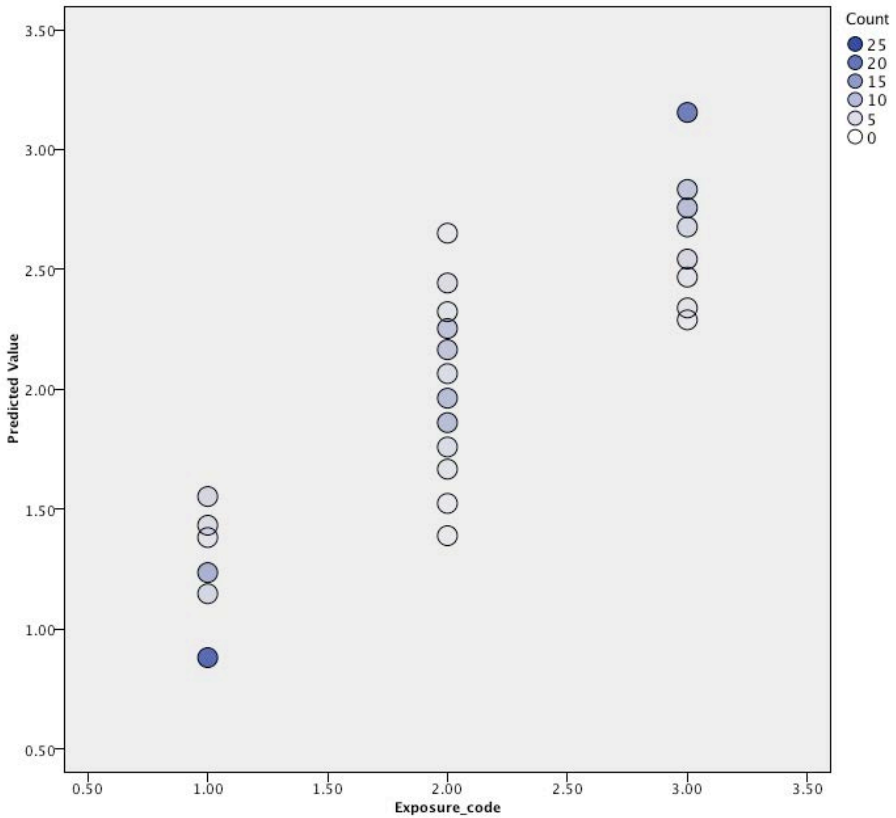
Tests of Between-Subjects Effects

Dependent Variable: SD_Variance_expression

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.672[a]	11	.152	.906	.536
Intercept	1923.331	1	1923.331	11470.792	.000
Recruitment_site	.836	1	.836	4.987	.027
Pregnancy_Stage	.020	1	.020	.119	.731
Exposure_group	.229	2	.115	.683	.507
Recruitment_site * Pregnancy_Stage	.092	1	.092	.551	.459
Recruitment_site * Exposure_group	.672	2	.336	2.003	.139
Pregnancy_Stage * Exposure_group	.210	2	.105	.627	.536
Recruitment_site * Pregnancy_Stage * Exposure_group	.028	2	.014	.084	.920
Error	20.791	124	.168		
Total	2477.421	136			
Corrected Total	22.463	135			

a. R Squared = .074 (Adjusted R Squared = -.008)



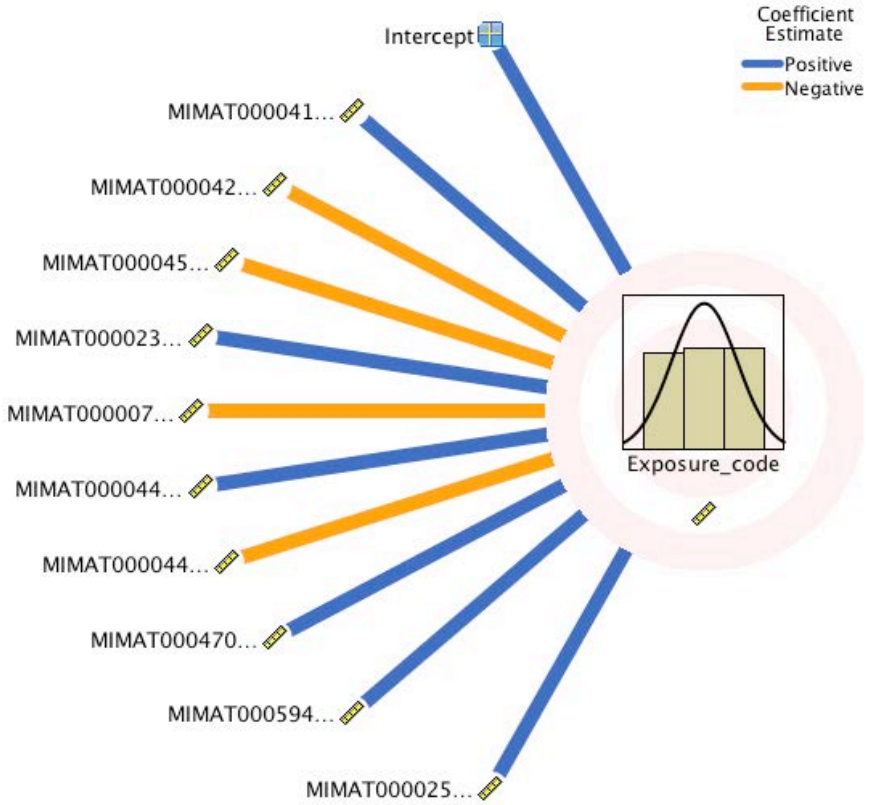


Alcohol-exposed Affected

Alcohol-exposed Unaffected

Control

Linear Classification model
46 miRNAs best predict class membership



Analysis of Top 14 candidate miRNA predictors

KEGG pathway	p-value	#genes	#miRNAs
Proteoglycans in cancer	2.11E-09	103	13
TGF-beta signaling pathway	7.20E-08	49	13
Glioma	1.76E-05	37	11
ErbB signaling pathway	0.00024632	50	12
Signaling pathways regulating pluripotency of stem cells	0.00031564	70	13
Hippo signaling pathway	0.00045902	70	13
Pathways in cancer	0.00045902	170	13
Axon guidance	0.00063219	58	11
FoxO signaling pathway	0.0006798	65	11
Thyroid hormone signaling pathway	0.0006798	56	13
Amphetamine addiction	0.00085416	33	10
Renal cell carcinoma	0.00116797	38	11
Focal adhesion	0.00130633	97	12
ECM-receptor interaction	0.00204389	39	11
Ubiquitin mediated proteolysis	0.00228231	69	11
PI3K-Akt signaling pathway	0.00233613	144	12
Transcriptional misregulation in cancer	0.00233613	79	12
cGMP-PKG signaling pathway	0.00536169	77	12
mTOR signaling pathway	0.00536169	34	12
Adrenergic signaling in cardiomyocytes	0.00595621	65	12
cAMP signaling pathway	0.00978237	89	12

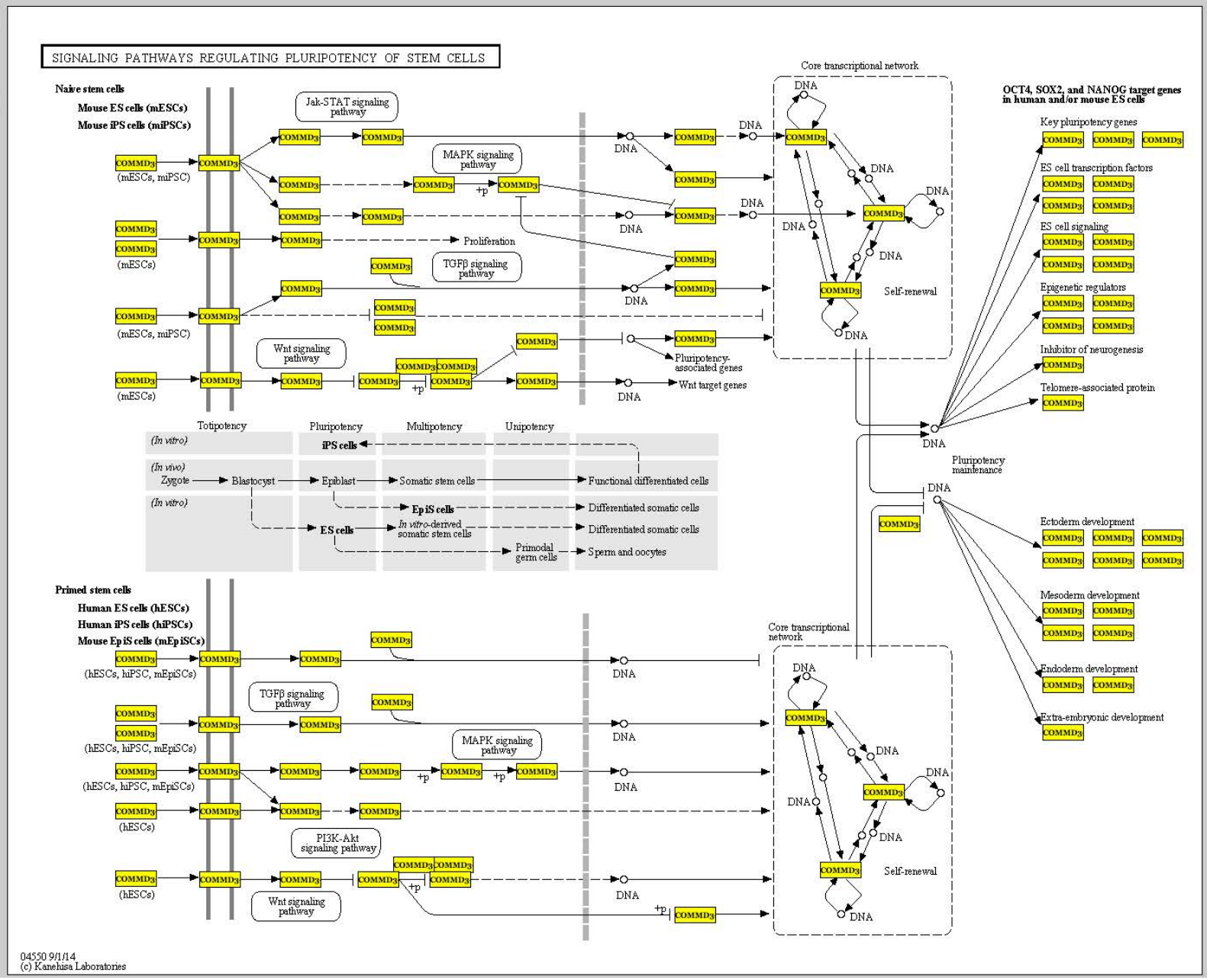
Pathway Viewer -- Pathway *'Signaling pathways regulating pluripotency of stem cells'* (hsa04550)

Notations

- : gene contained in 1 list
- : gene contained in > 1 lists
- : highlighted gene

Show/ hide genes

BMI1 (Homo sapiens)	Disable
JARID2 (Homo sapiens)	Disable
TCF3 (Homo sapiens)	Disable
GSK3B (Homo sapiens)	Disable
DVL3 (Homo sapiens)	Disable
WNT16 (Homo sapiens)	Disable
FZD5 (Homo sapiens)	Disable
ID2 (Homo sapiens)	Disable
WNT7A (Homo sapiens)	Disable
PAX6 (Homo sapiens)	Disable
SMAD2 (Homo sapiens)	Disable
NRAS (Homo sapiens)	Disable
INHBB (Homo sapiens)	Disable
SMAD9 (Homo sapiens)	Disable
APC (Homo sapiens)	Disable
HOXB1 (Homo sapiens)	Disable
PIK3CB (Homo sapiens)	Disable
REST (Homo sapiens)	Disable
ACVR1B (Homo sapiens)	Disable
BMPR1B (Homo sapiens)	Disable
MAPK14 (Homo sapiens)	Disable
TBX3 (Homo sapiens)	Disable
HAND1 (Homo sapiens)	Disable
FZD6 (Homo sapiens)	Disable
SMARCAD1 (Homo sapiens)	Disable
INHBA (Homo sapiens)	Disable
WNT4 (Homo sapiens)	Disable
WNT3 (Homo sapiens)	Disable
IGF1R (Homo sapiens)	Disable
ZFHX3 (Homo sapiens)	Disable
ID4 (Homo sapiens)	Disable
KRAS (Homo sapiens)	Disable
FZD3 (Homo sapiens)	Disable
POU5F1B (Homo sapiens)	Disable
ACVR1 (Homo sapiens)	Disable
ACVR2B (Homo sapiens)	Disable
PCGF5 (Homo sapiens)	Disable
LIF (Homo sapiens)	Disable
FZD4 (Homo sapiens)	Disable
RIF1 (Homo sapiens)	Disable
PIK3R3 (Homo sapiens)	Disable
JAK2 (Homo sapiens)	Disable
SMAD4 (Homo sapiens)	Disable
LIFR (Homo sapiens)	Disable
SKIL (Homo sapiens)	Disable
SMAD5 (Homo sapiens)	Disable
ID1 (Homo sapiens)	Disable
PIK3R1 (Homo sapiens)	Disable
PIK3CG (Homo sapiens)	Disable
ACVR2A (Homo sapiens)	Disable
FGF2 (Homo sapiens)	Disable
BMP2 (Homo sapiens)	Disable



Pathway Viewer -- Pathway *'Amphetamine addiction'* (hsa05031)

Notations

- : gene contained in 1 list
- : gene contained in > 1 lists
- : highlighted gene

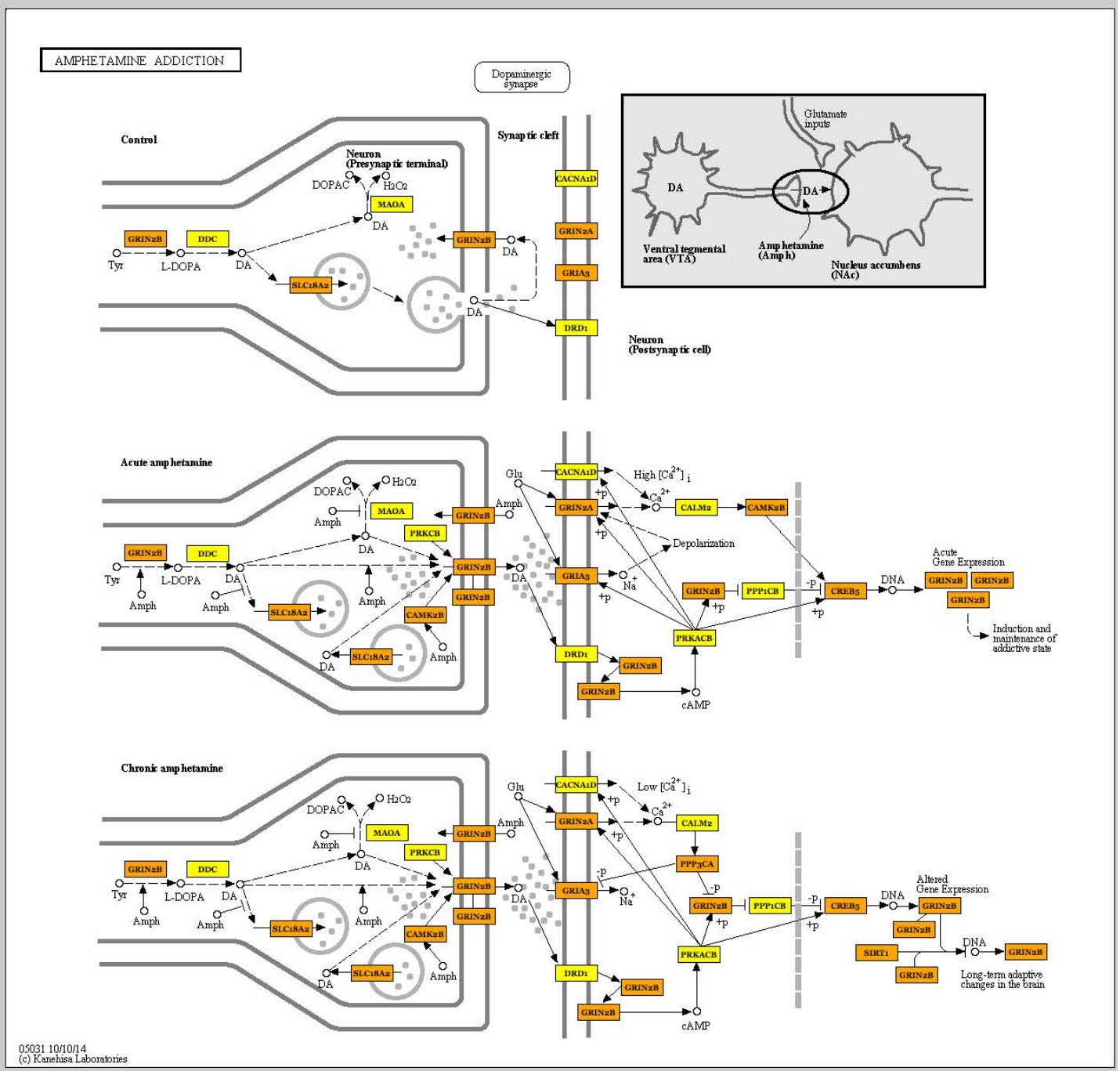
Show/ hide genes

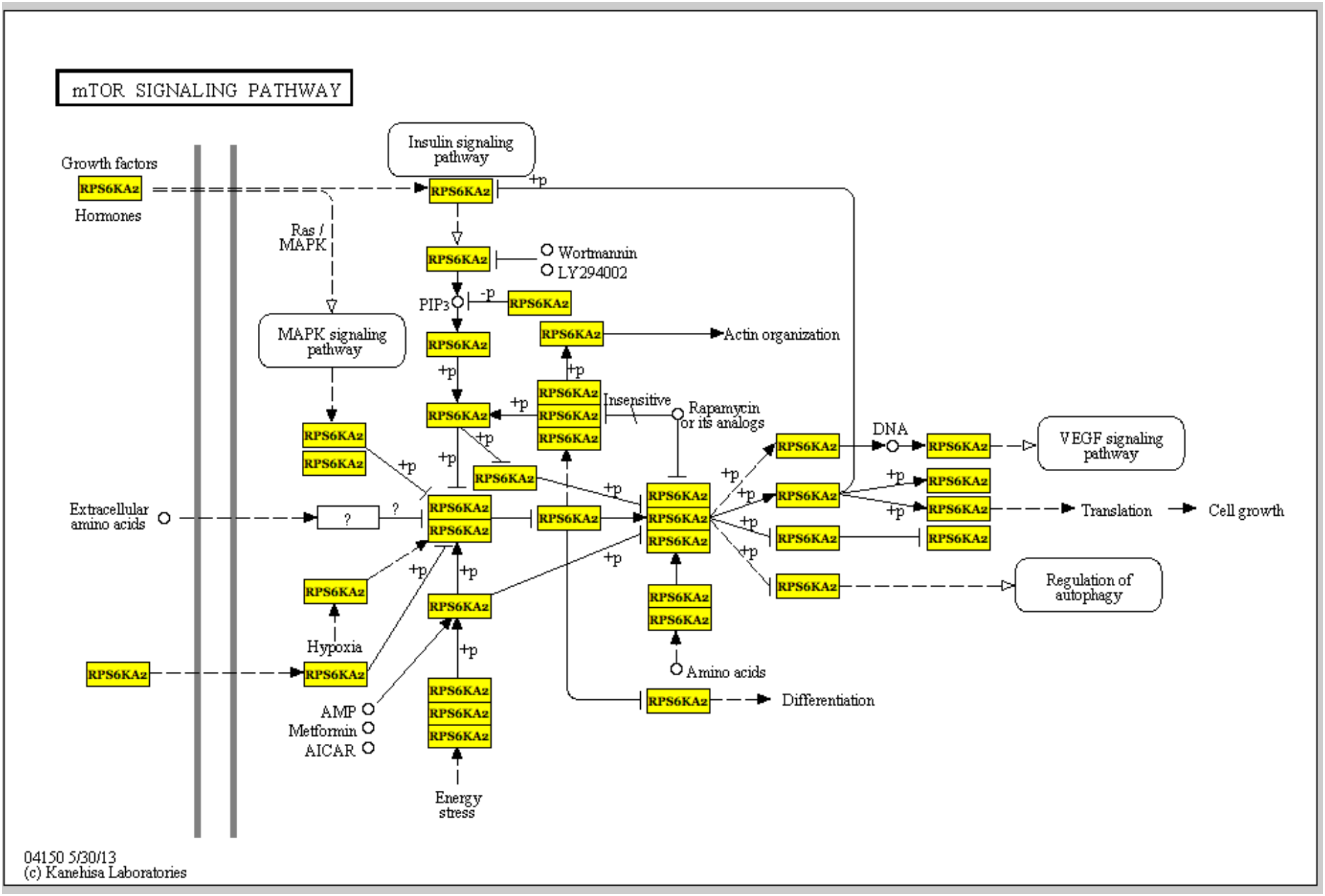
CAMK2D (Homo sapiens)	Disable
FOS (Homo sapiens)	Disable
PRKCA (Homo sapiens)	Disable
GRIN3A (Homo sapiens)	Disable
ATF2 (Homo sapiens)	Disable
CAMK2G (Homo sapiens)	Disable
CAMK4 (Homo sapiens)	Disable
CALM1 (Homo sapiens)	Disable
GRIN2B (Homo sapiens)	Disable
SIRT1 (Homo sapiens)	Disable
CREB5 (Homo sapiens)	Disable
PPP1CC (Homo sapiens)	Disable
DRD1 (Homo sapiens)	Disable
SLC18A2 (Homo sapiens)	Disable
GRIA1 (Homo sapiens)	Disable
DDC (Homo sapiens)	Disable
CALM2 (Homo sapiens)	Disable
GRIA2 (Homo sapiens)	Disable
PPP3CA (Homo sapiens)	Disable
MAOA (Homo sapiens)	Disable
PRKCB (Homo sapiens)	Disable
CAMK2B (Homo sapiens)	Disable
CACNA1D (Homo sapiens)	Disable
GRIN2A (Homo sapiens)	Disable
PRKACB (Homo sapiens)	Disable
PPP1CB (Homo sapiens)	Disable
GRIA3 (Homo sapiens)	Disable

Disable all

Highlight genes

CAMK2D (Homo sapiens)	Enable
FOS (Homo sapiens)	Enable
PRKCA (Homo sapiens)	Enable
GRIN3A (Homo sapiens)	Enable
ATF2 (Homo sapiens)	Enable
CAMK2G (Homo sapiens)	Enable
CAMK4 (Homo sapiens)	Enable
CALM1 (Homo sapiens)	Enable
GRIN2B (Homo sapiens)	Enable
SIRT1 (Homo sapiens)	Enable
CREB5 (Homo sapiens)	Enable
PPP1CC (Homo sapiens)	Enable
DRD1 (Homo sapiens)	Enable
SLC18A2 (Homo sapiens)	Enable
GRIA1 (Homo sapiens)	Enable
DDC (Homo sapiens)	Enable
CALM2 (Homo sapiens)	Enable
GRIA2 (Homo sapiens)	Enable
PPP3CA (Homo sapiens)	Enable
MAOA (Homo sapiens)	Enable
PRKCB (Homo sapiens)	Enable
CAMK2B (Homo sapiens)	Enable
CACNA1D (Homo sapiens)	Enable
GRIN2A (Homo sapiens)	Enable
PRKACB (Homo sapiens)	Enable
PPP1CB (Homo sapiens)	Enable
GRIA3 (Homo sapiens)	Enable




mTOR SIGNALING PATHWAY
Growth factors
RPS6KA2
Hormones
Insulin signaling pathway
Ras / MAPK
MAPK signaling pathway
Wortmannin
LY294002
PIP3
-p
Actin organization
Insensitive
Rapamycin or its analogs
DNA
VEGF signaling pathway
Translation
Cell growth
Regulation of autophagy
Differentiation
Amino acids
Extracellular amino acids
?
Hypoxia
AMP
Metformin
AICAR
Energy stress
04150 5/30/13
(c) Kanehisa Laboratories

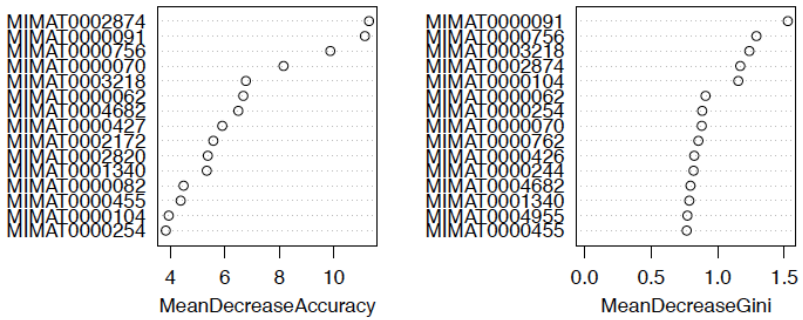
Random Forests Classification Model

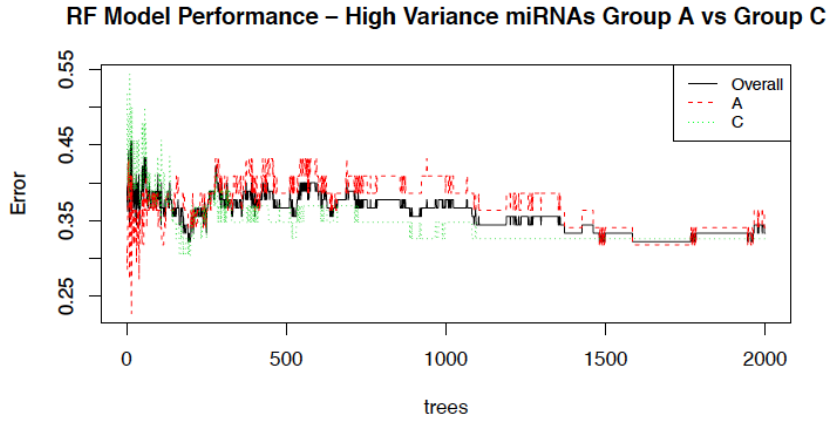
1.5 Random Forest - 75 High Variance miRNAs Group A vs Group C

[1] "Overall Error Rate: 33.33

	GA	GC	class.error
GA	29	15	0.341
GC	15	31	0.326

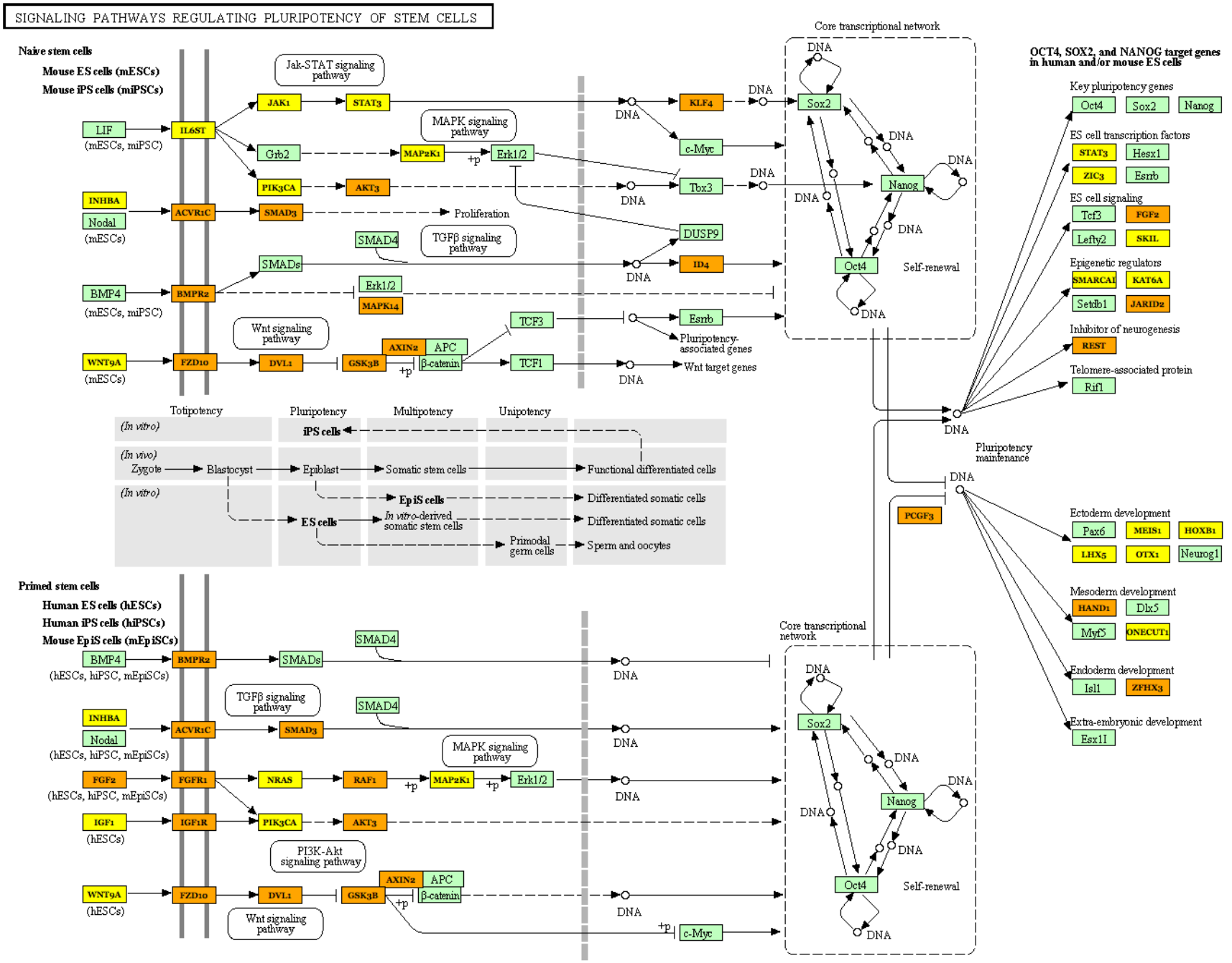
Table 5: Confusion Matrix for High Variance miRNAs Group A vs Group C

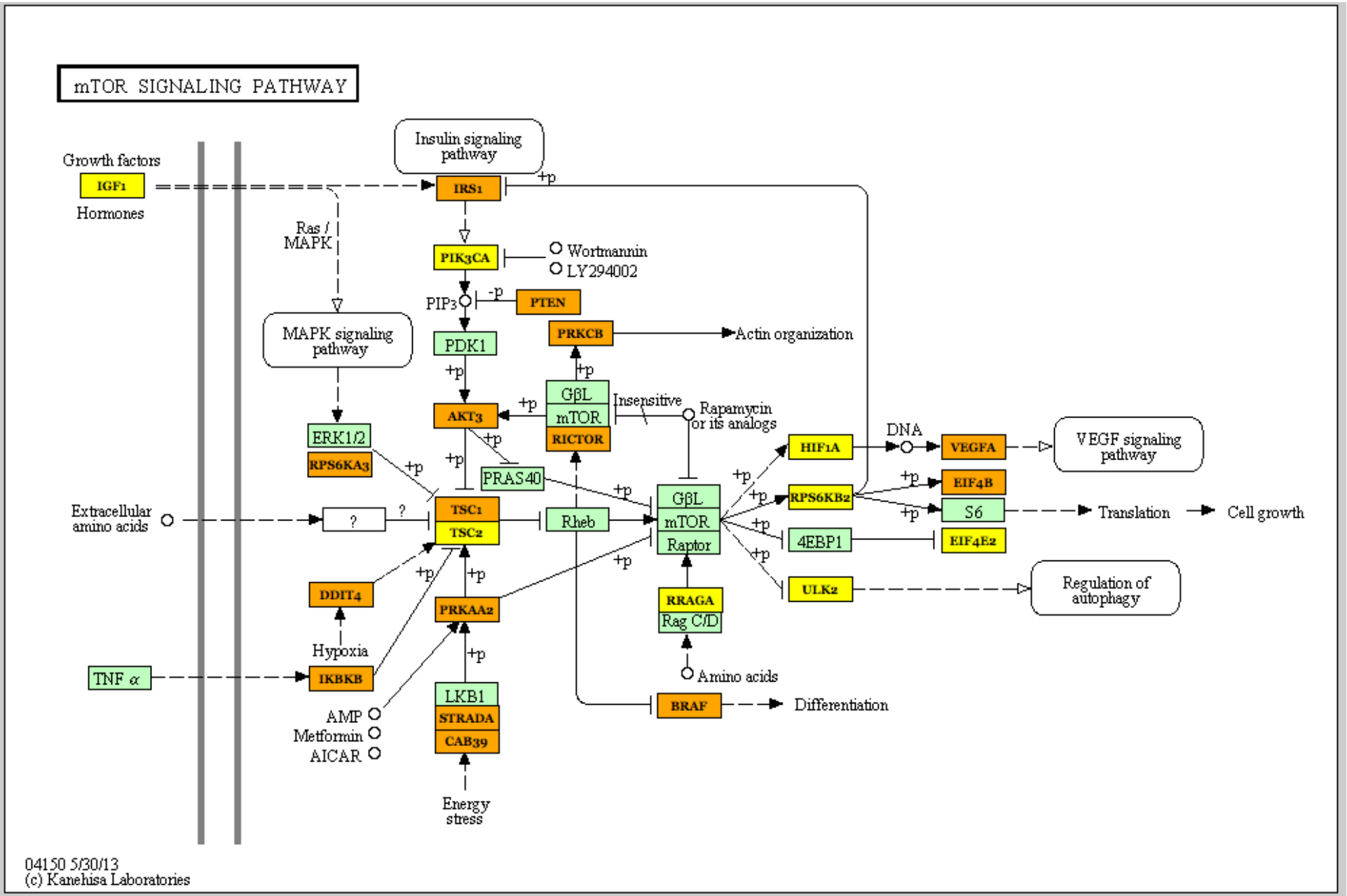




Analysis of top 15 candidate miRNA predictors

KEGG pathway	p-value	#genes	#miRNAs
Fatty acid biosynthesis	8.37E-15	7	5
Fatty acid metabolism	2.06E-11	22	11
Signaling pathways regulating pluripotency of stem cells	1.22E-06	66	14
Adrenergic signaling in cardiomyocytes	2.84E-05	59	14
Proteoglycans in cancer	0.00010235	85	14
Hippo signaling pathway	0.0005956	63	14
Thyroid hormone signaling pathway	0.0005995	47	12
Wnt signaling pathway	0.00073793	58	13
PI3K-Akt signaling pathway	0.00078408	127	14
AMPK signaling pathway	0.00392369	54	14
mTOR signaling pathway	0.00717255	31	13
FoxO signaling pathway	0.00742935	56	13
Neurotrophin signaling pathway	0.00742935	52	13
Regulation of actin cytoskeleton	0.00874377	79	13
MAPK signaling pathway	0.00874377	93	14
Pathways in cancer	0.00874377	137	14
Glioma	0.01055503	27	13
Glycosaminoglycan biosynthesis - heparan sulfate / heparin	0.01137512	11	8
Axon guidance	0.01137512	48	13
TGF-beta signaling pathway	0.01307434	30	12
Glutamatergic synapse	0.01498513	43	12
Endocytosis	0.01518223	77	13


SIGNALING PATHWAYS REGULATING PLURIPOTENCY OF STEM CELLS
Naive stem cells
Mouse ES cells (mESCs)
Mouse iPS cells (miPSCs)
LIF
(mESCs, miPSC)
IL6ST
JAK1
STAT3
Jak-STAT signaling pathway
Grb2
MAP2K1
Erk1/2
MAPK signaling pathway
PIK3CA
AKT3
INHBA
Nodal
(mESCs)
ACVR1C
SMAD3
Proliferation
SMAD4
TGFβ signaling pathway
SMADs
BMP4
(mESCs, miPSC)
BMPR2
Erk1/2
MAPK14
Wnt signaling pathway
TCF3
AXIN2
APC
WNT9A
(mESCs)
FZD10
DVL1
GSK3B
β-catenin
TCF1
KLF4
c-Myc
Tbx3
DUSP9
ID4
Esrrb
Pluripotency-associated genes
Wnt target genes
DNA
Core transcriptional network
Sox2
Nanog
Oct4
Self-renewal
OCT4, SOX2, and NANOG target genes in human and/or mouse ES cells
Key pluripotency genes
Oct4
Sox2
Nanog
ES cell transcription factors
STAT3
Hesx1
ZIC3
Esrrb
ES cell signaling
Tcf3
FGF2
Lefty2
SKIL
Epigenetic regulators
SMARCA1
KAT6A
Setdb1
JARID2
Inhibitor of neurogenesis
REST
Telomere-associated protein
Rif1
Totipotency
Pluripotency
Multipotency
Unipotency
(In vitro)
iPS cells
(In vivo)
Zygote
Blastocyst
Epiblast
Somatic stem cells
Functional differentiated cells
Epi5 cells
Differentiated somatic cells
ES cells
In vitro-derived somatic stem cells
Differentiated somatic cells
Primodal germ cells
Sperm and oocytes
Pluripotency maintenance
PCGF3
Ectoderm development
Pax6
MEIS1
HOXB1
LHX5
OTX1
Neurog1
Mesoderm development
HAND1
Dlx5
Myf5
ONECUT1
Endoderm development
Isl1
ZFHX3
Extra-embryonic development
Esx1l
Primed stem cells
Human ES cells (hESCs)
Human iPS cells (hiPSCs)
Mouse Epi5 cells (mEpi5Cs)
BMP4
(hESCs, hiPSC, mEpiSCs)
INHBA
Nodal
(hESCs, hiPSC, mEpiSCs)
FGF2
(hESCs, hiPSC, mEpiSCs)
IGF1
(hESCs)
IGF1R
NRAS
RAF1
PI3K-Akt signaling pathway
WNT9A
(hESCs)
+p
04550 9/1/14
(c) Kanehisa Laboratories


mTOR SIGNALING PATHWAY
Growth factors
IGF1
Hormones
Insulin signaling pathway
IRS1
+p
Ras / MAPK
PIK3CA
Wortmannin
LY294002
PIP3
-p
PTEN
MAPK signaling pathway
PDK1
PRKCB
Actin organization
+p
+p
GβL
mTOR
RICTOR
Insensitive
Rapamycin or its analogs
AKT3
+p
ERK1/2
RPS6KA3
+p
+p
PRAS40
+p
HIF1A
DNA
VEGFA
VEGF signaling pathway
+p
EIF4B
+p
RPS6KB2
+p
S6
Translation
Cell growth
Extracellular amino acids
?
?
TSC1
TSC2
Rheb
GβL
mTOR
Raptor
+p
4EBP1
EIF4E2
+p
ULK2
Regulation of autophagy
+p
+p
DDIT4
PRKAA2
+p
RRAGA
Rag C/D
Hypoxia
TNF α
IKBKB
Amino acids
LKB1
STRADA
CAB39
AMP
Metformin
AICAR
BRAF
Differentiation
Energy stress
04150 5/30/13
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WEINBERG CIFASD DEVELOPMENTAL PROJECT

With Tina Chambers and CIFASD team

Co-I: Dr. Charlis Raineki

PhD student: Tamara Bodnar

Identifying an immune signature characteristic of FASD: Implications for possible immune- based intervention strategies

Background

- CIFASD Developmental Project inspired by our recent animal work on neuroimmune function
  - Following an immune/inflammatory challenge in adulthood, PAE animals exhibit a more severe and prolonged course of inflammation
  - This exacerbated response may have its basis in a PAE-induced pro-inflammatory bias that is present from early life

CIFASD Proposal

- It is not known whether children with FASD exhibit a similar pro-inflammatory profile
  - Adverse early-life experiences (eg., prenatal infection, childhood trauma) are associated with increased cytokine expression, with detrimental long-term consequences
  - Altered cytokine profiles in children with ASD
  - Aberrant inflammatory mechanisms may be at the root of many physical and mental health disorders, and as such, may benefit from immune-based interventions.
- Specific Aims of our proposal:
  - Probe for a unique immune signature (early pro-inflammatory bias?) in children with FASD that may be linked to maternal immune function
  - Measure endocrine (HPA) function in parallel – index of endocrine-immune interaction
  - Both plasma and salivary markers – are there alcohol-specific alterations in saliva that parallel those in blood
  - Investigate whether nutritional supplementation can modulate the expected pro-inflammatory/endocrine profile

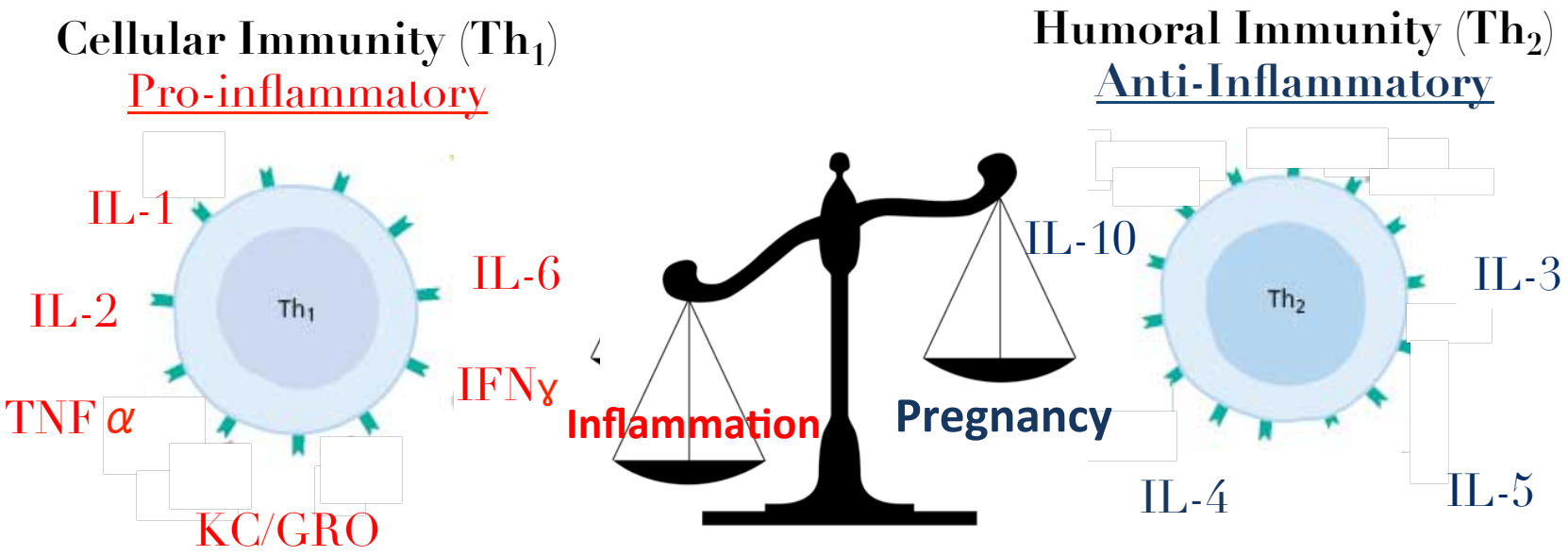
Methods

- Collaboration with Tina Chambers to analyze samples from mothers and children in CIFASD Ukraine cohorts
- Plasma samples from mothers – categorized by outcomes of the children:
  - A: Alcohol-consuming women whose children were affected (facial features, growth, neurobehavioral deficits)
  - B: Alcohol-consuming women whose children were not affected (no features, no growth or neurobehavioral deficits)
  - C: Women with low-no alcohol consumption with unaffected children
- Samples taken at enrollment (~second trimester) and 32 wk follow-up (third trimester)
- Samples analyzed using Human Biomarker 40-Plex Kit from MesoScale Discovery
  - Wide dynamic range, best available assay sensitivity
  - Pro- and anti-inflammatory cytokine and chemokine panels, angiogenesis panel, vascular injury panel - 40 analytes total

Cytokines and the Th_1/Th_2 Inflammatory Response

Cytokines:

- **Signaling molecules** of the immune system (the hormones of the immune system)
- **Immunomodulating agents** that result in:
  - Growth and differentiation of immune cells
  - Activation of immune cells
  - Fever, inflammation, etc.
- **Produced by**: cells of the immune system and others



Caveats:

- Data PRELIMINARY!
  - Data from only 6 of 8 plates processed and included in slides to date
  - Some patterns may become stronger or less strong with complete n/group
  - Further processing needed using PCA, network analysis, etc to see bigger picture of cytokine function
  - Need to relate results to: other biological measures (Rajesh, Dipak, etc) and to demographic, psychosocial, behavioral, etc measures

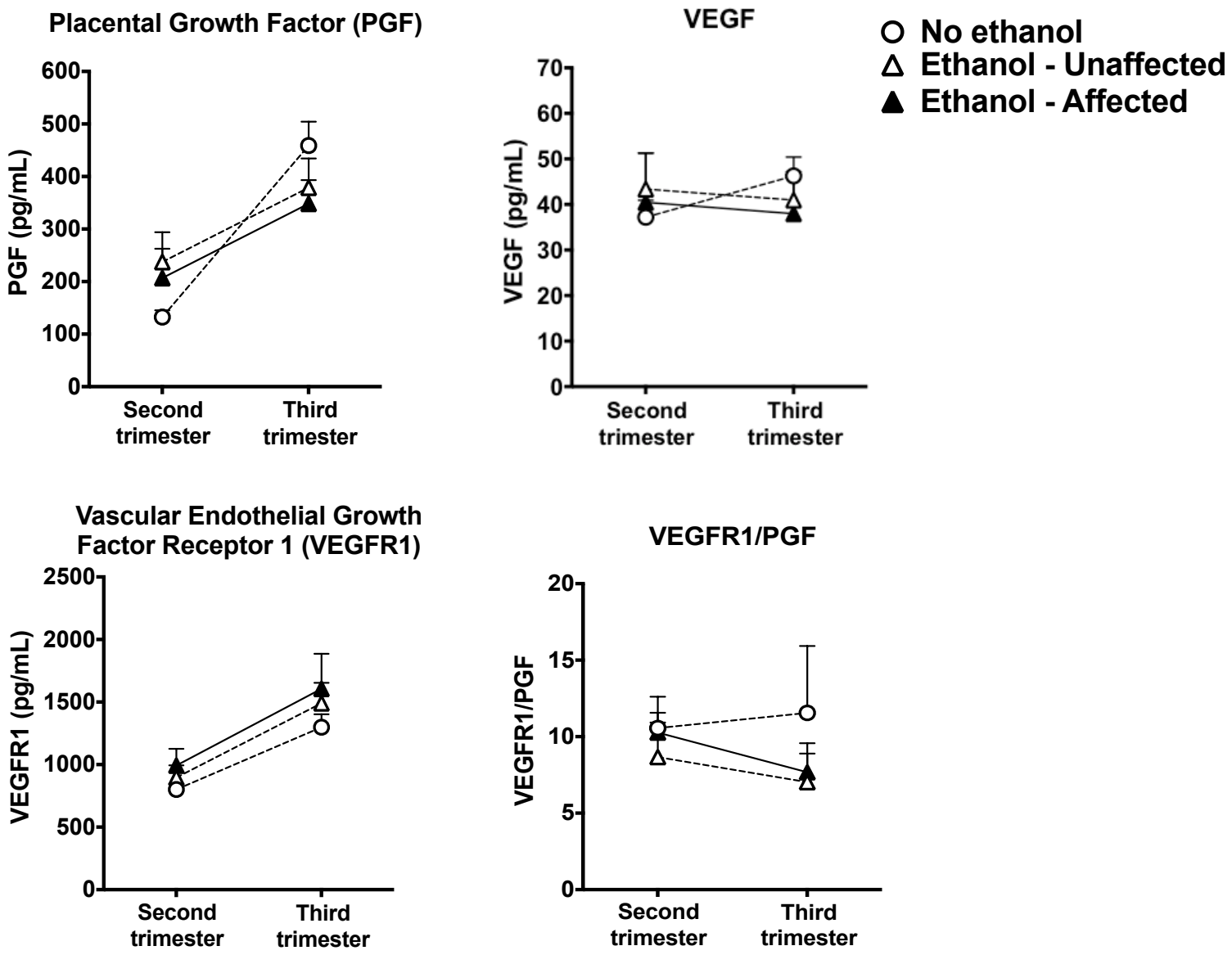
Ethanol-consuming moms, regardless of whether child is affected (A and B)

different from

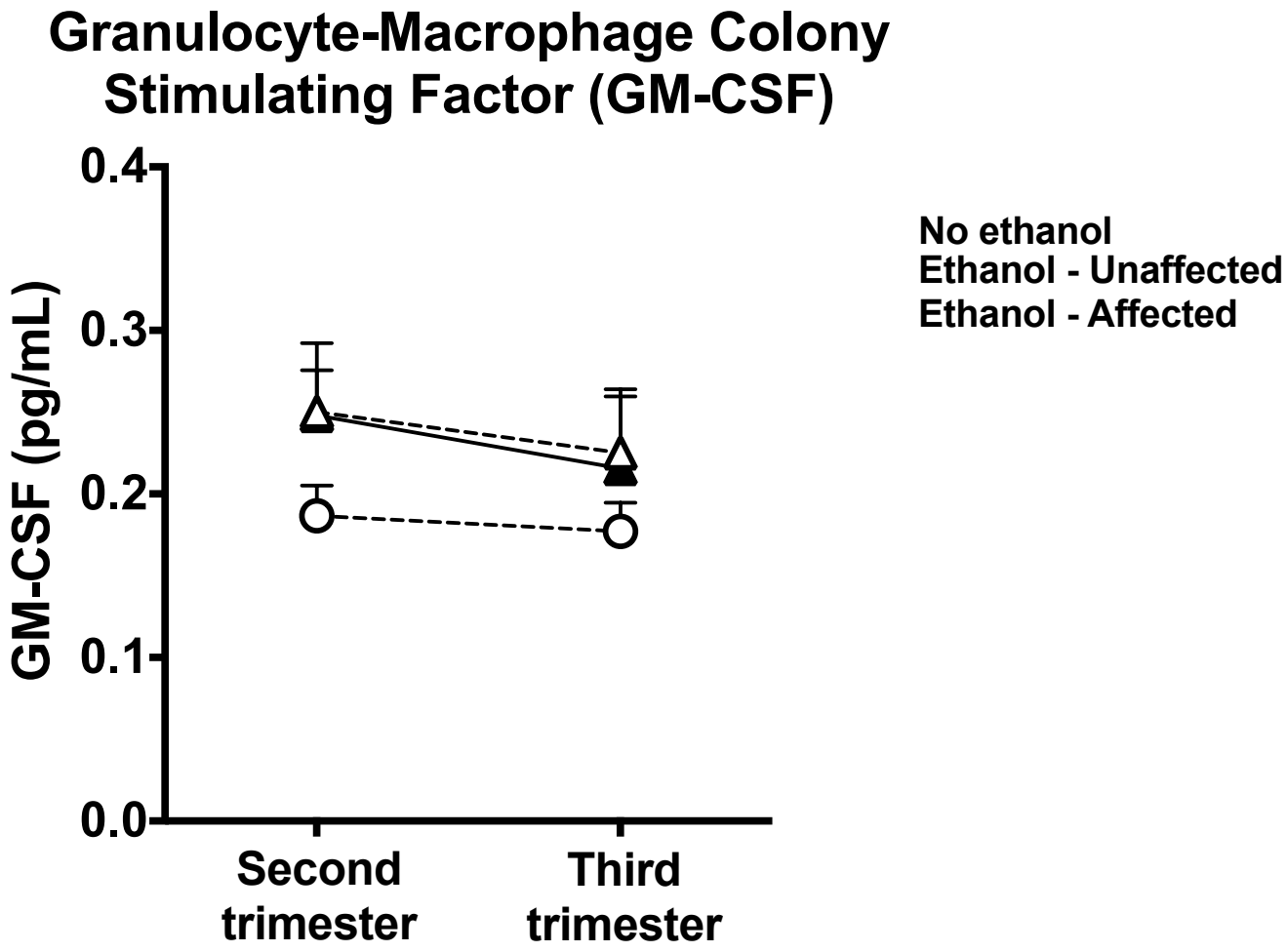
(C) Moms with low to no ethanol consumption –

Effect of ethanol consumption *per se*

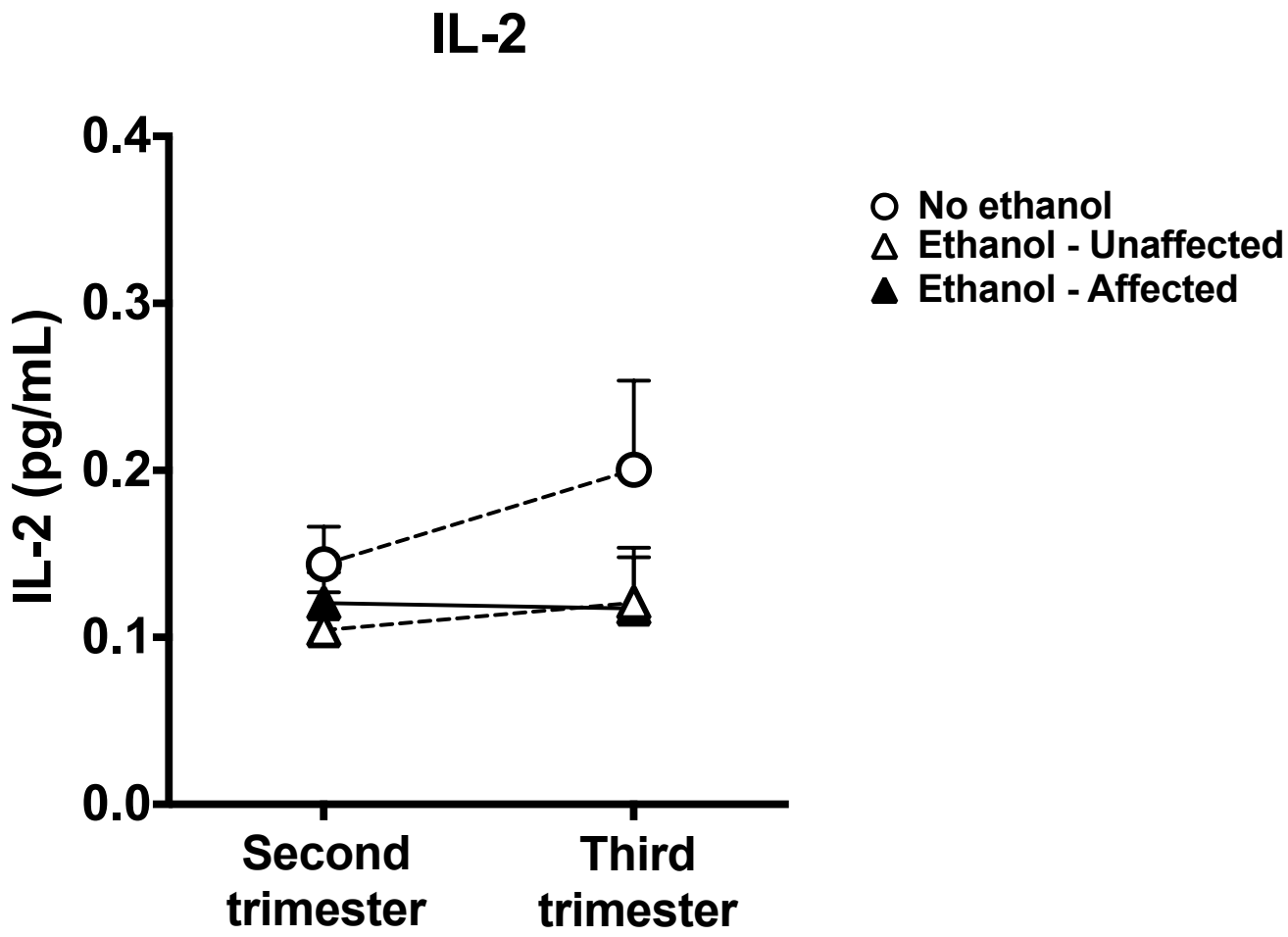
Pro-angiogenic proteins



GM-CSF is part of inflammatory cascade – Role in implantation and embryo development



Pro-inflammatory cytokine, may signal onset of labor

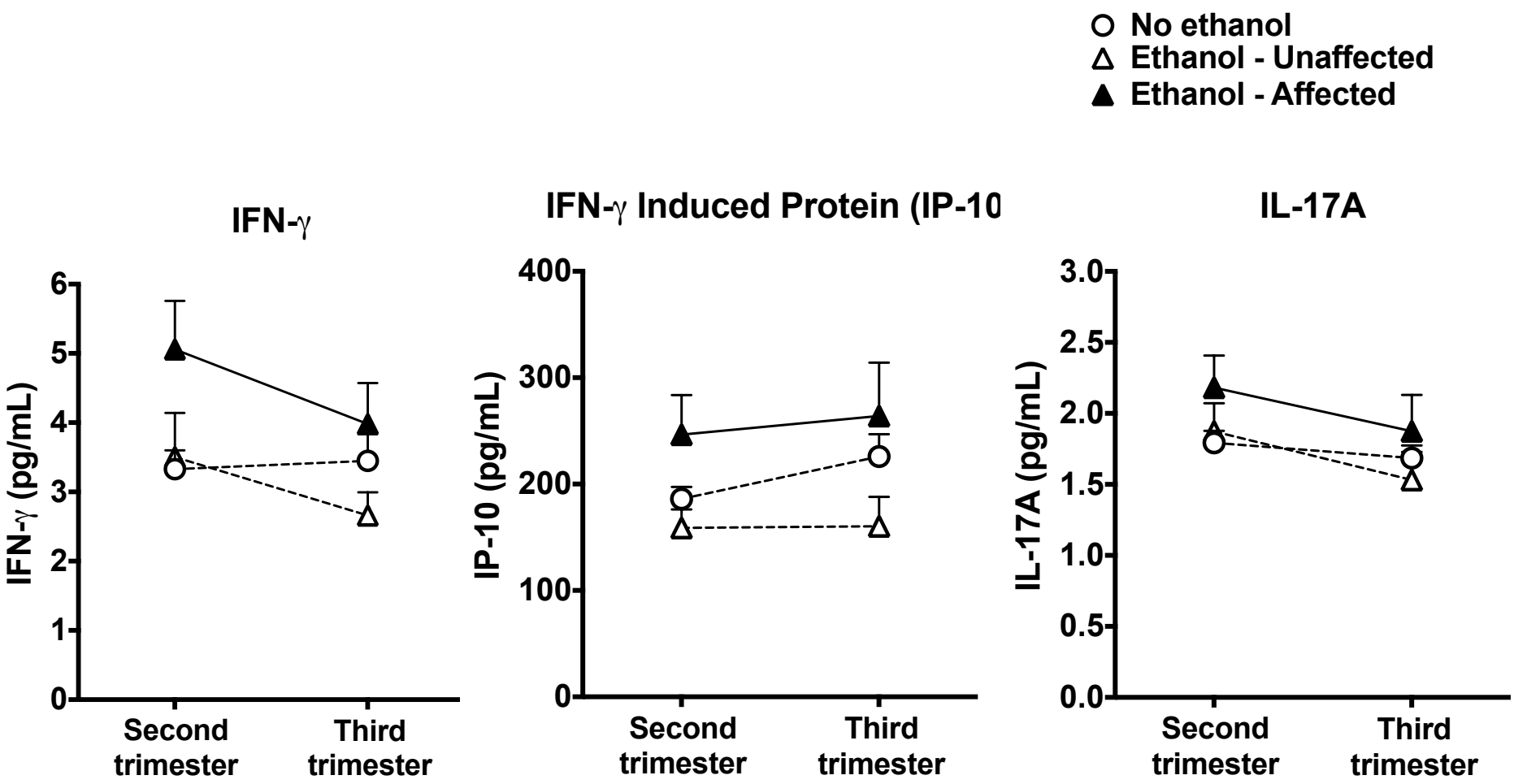


Ethanol-consuming moms with affected children (A)

different from

Ethanol-consuming moms whose children are not affected (B), and low-no ethanol consumption group (C)

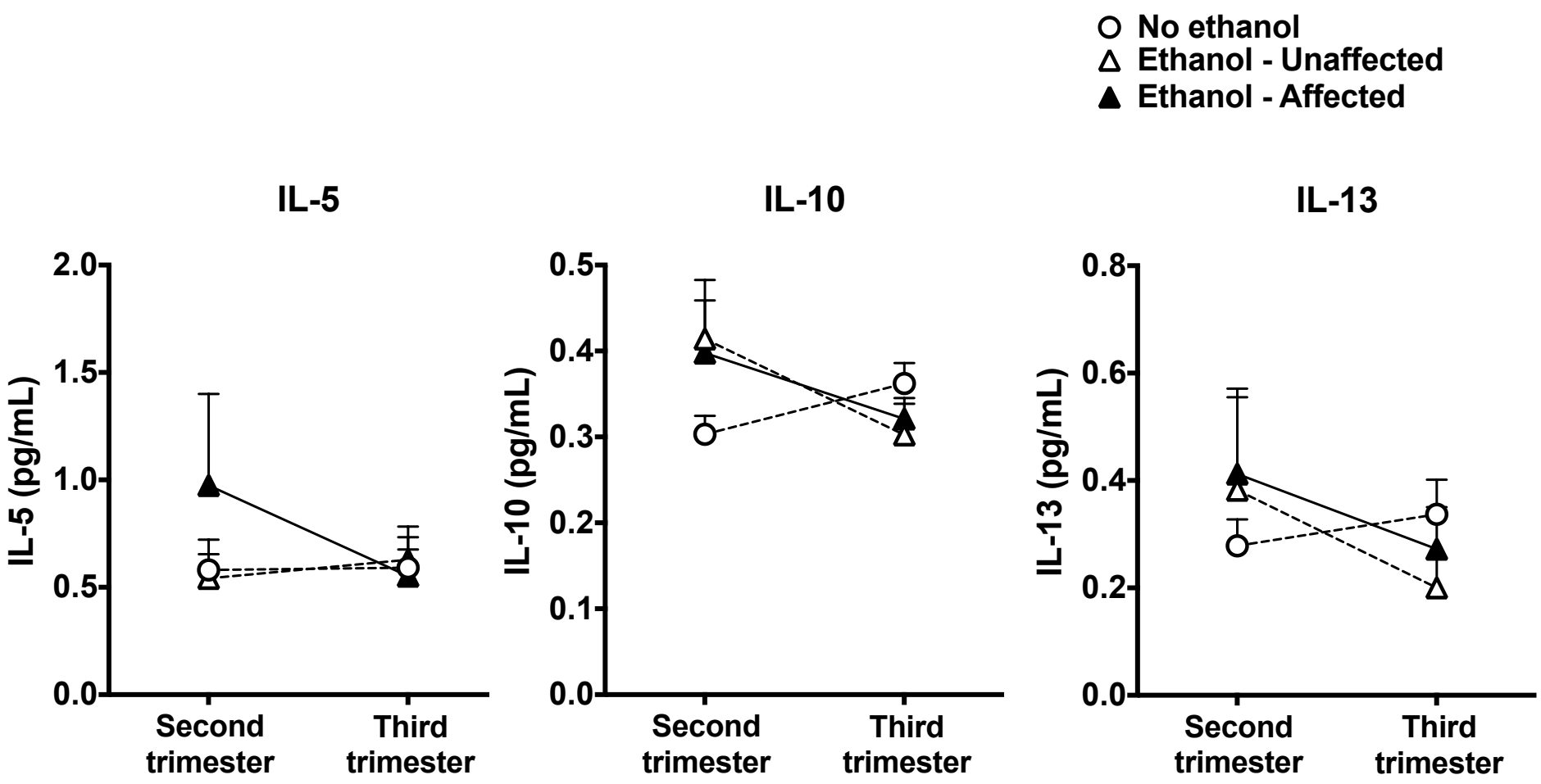
Pro-inflammatory cytokines



TENTATIVE Comment

- Ideas from the prenatal stress literature:
  - Increased pro-inflammatory Th1 cytokines in the mother (maternal-fetal interface) are linked to higher risk of allergy and other immune (particularly auto-immune) disturbances in the fetus
  - If we see increases in Th1 cytokines, we could link this to possible changes in the child's immune response

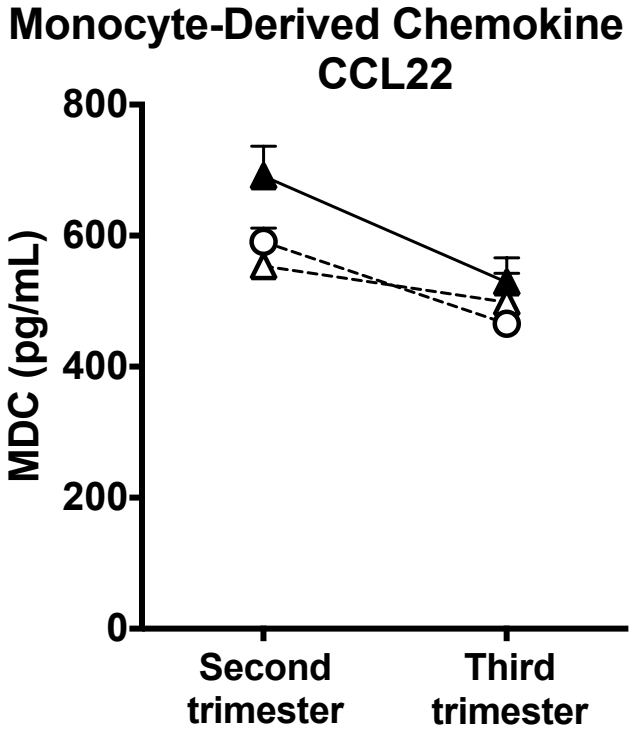
Anti-inflammatory cytokines

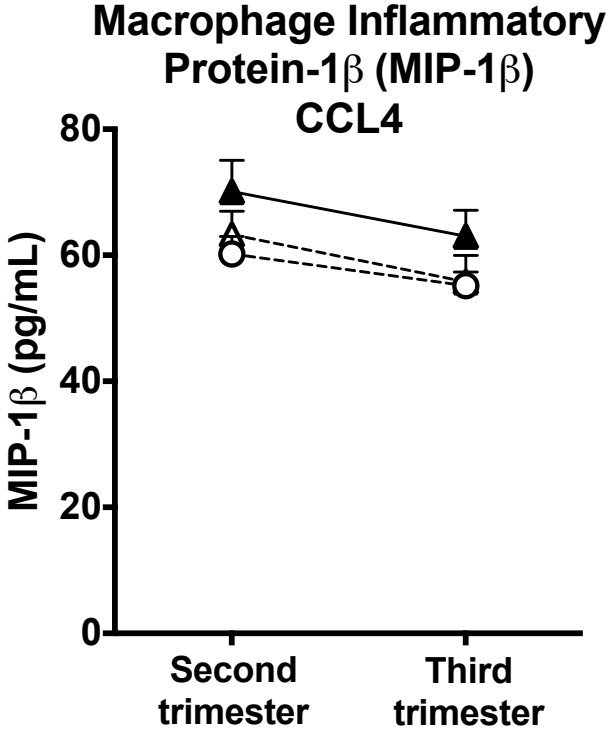


Chemokines
(chemotaxis, movement of cells)

○ No ethanol
△ Ethanol - Unaffected
▲ Ethanol - Affected

CCL22
Involved in maternal-fetal immune tolerance;
trafficking of activated T cells to inflammatory sites





Alcohol-consuming mom with unaffected children (B)

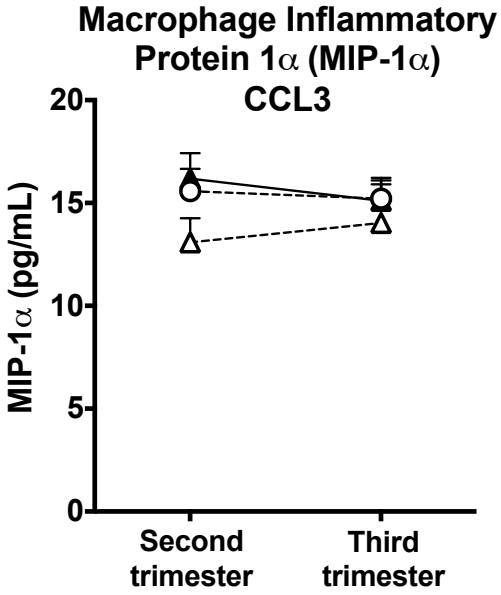
different from

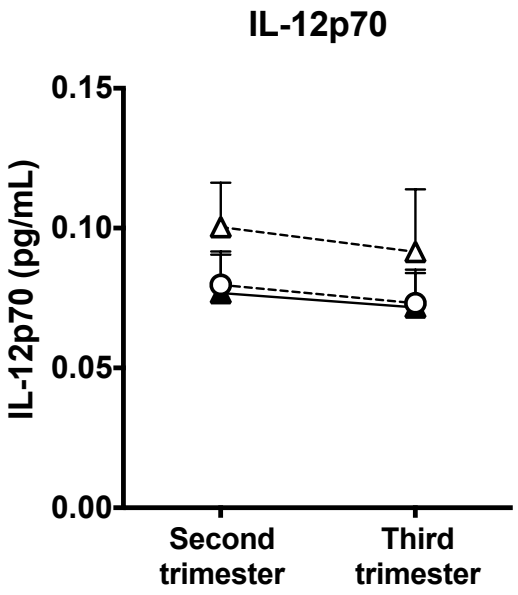
Alcohol-consuming moms with affected children (A) and moms with low-no ethanol consumption (C)

Chemokines

○ No ethanol
△ Ethanol - Unaffected
▲ Ethanol - Affected

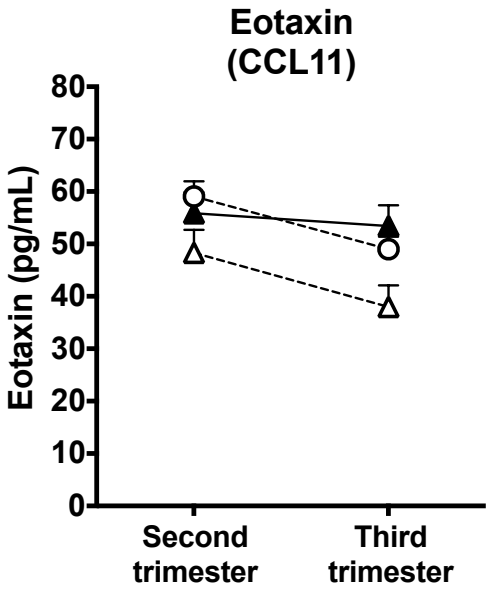
Chemokine with inflammatory and chemokinetic properties

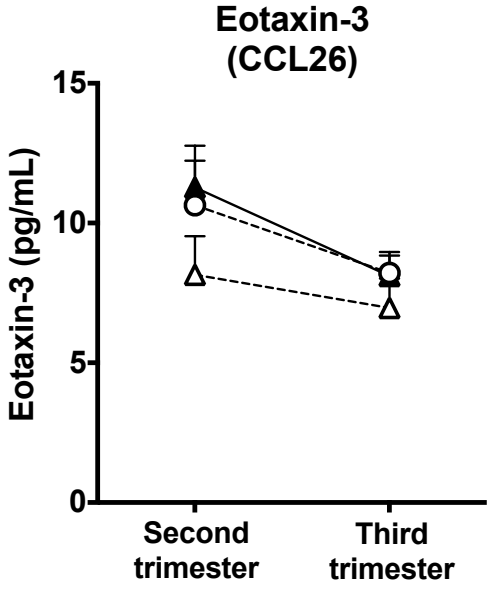




Activates T cells to stimulate IFN-Y production

Recruits eosinophils- involved in allergic processes





Chemotactic for eosinophils and basophils

Of relevance

Studies in the ASD field are showing links between early life cytokine profiles and the development and severity of ASD

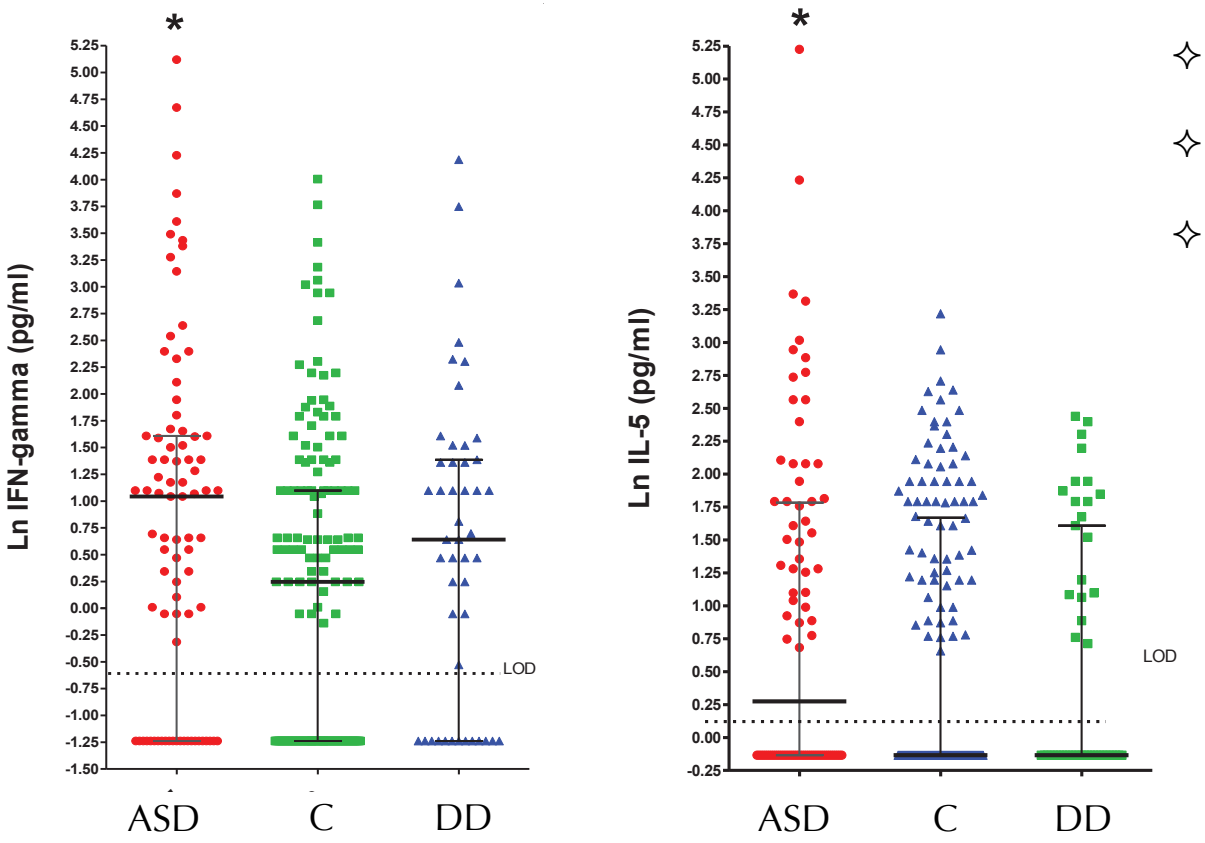
Goines et al. Molecular Autism 2011, 2:13
http://www.molecularautism.com/content/2/1/13

Molecular Autism

RESEARCH Open Access

Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study

Paula E Goines[1,2], Lisa A Croen[3], Daniel Braunschweig[1,2], Cathleen K Yoshida[3], Judith Grether[4], Robin Hansen[2,5], Martin Kharrazi[6], Paul Ashwood[2,7] and Judy Van de Water[1,2*]



- **ASD**: Autism Spectrum Disorder
- **C:** Control, general population
- **DD:** Developmental Delay

Archival Report

Biological Psychiatry

Neonatal Cytokine Profiles Associated with Autism Spectrum Disorder

Paula Krakowiak, Paula E. Goines, Daniel J. Tancredi, Paul Ashwood, Robin L. Hansen, Irva Hertz-Picciotto, and Judy Van de Water

- Examined blood spots from: 214 children with ASD, 62 typically developing controls, 27 children with developmental delay.
- Levels of 17 cytokines and chemokines compared across groups and in relation to developmental and behavioral domains
- **Peripheral cytokine profiles at birth associated with ASD later in childhood. Cytokine profiles vary depending on ASD severity.**
- Cytokines have complex roles in neurodevelopment; dysregulated levels may be indicative of genetic differences and environmental exposures, and/or their interactions, that relate to ASD

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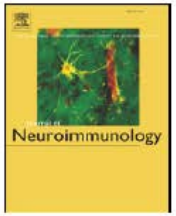
journal homepage: www.elsevier.com/locate/jneuroim



Increased production of IL-17 in children with autism spectrum disorders and co-morbid asthma

Marjannie Eloi Akintunde [a,b,*], Melissa Rose [b,c,d], Paula Krakowiak [c,d], Luke Heuer [a,c], Paul Ashwood [b,c,e], Robin Hansen [b,c,f], Irva Hertz-Picciotto [b,c,d], Judy Van de Water [a,b,c]

- Objective: to assess connection between inflammation and cytokine profiles in children with ASD
- Peripheral immune cells from children with ASD and TD controls isolated and stimulated with mitogens
- **IL-17 production higher following stimulation in children with ASD and in children with ASD plus co-morbid asthma compared to TD controls.**
- IL-17 may be linked to co-morbidity (asthma, allergies) often associated with ASD