Transcriptomic evidence for dysregulation of retrotransposition and microglial signaling in a non-human primate model of maternal immune activation

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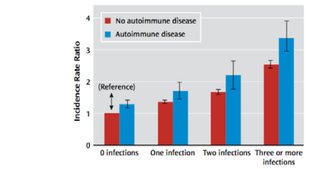
Abstract

Maternal immune activation (MIA) is an established risk factor for multiple neurodevelopmental and psychiatric disorders including schizophrenia. However, the molecular and neurobiological mechanisms through which MIA imparts risk for these disorders remain poorly understood. A recently developed nonhuman primate model of exposure to the viral mimic poly:ICLC during pregnancy shows abnormal social and repetitive behaviors, providing an unprecedented opportunity for mechanistic dissection. Here, we performed RNA-sequencing on homogenate brain tissue across four brain regions from 4-year old MIA-exposed (n=9) or saline-exposed (n=4) offspring. We used weighted gene co-expression network analysis (WGCNA) to identify modules of co-expressed genes, which we further characterize by gene ontology and cell-type specific marker enrichments. We identify 140 genes differentially expressed across all brain regions (prefrontal cortex, anterior cingulate, hippocampus, and primary visual cortex) in MIA-exposed offspring. Differential expression was observed for multiple major histocompatibility complex genes as well as known regulators of retrotransposition, and we further observe upregulation of the ERV1 class of endogenous retroviruses. Gene-set enrichment analyses highlight significant upregulation of extracellular matrix and glial differentiation pathways and downregulation of DNA repair pathways and microglial marker genes. Finally, co-expression networks identify region-specific dysregulation of oligodendrocyte and BDNF signaling. Together, these results begin to elucidate the brain level molecular mechanisms through which maternal immune activation imparts risk for psychiatric disease.

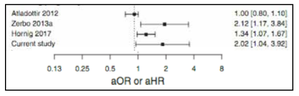
Maternal Immune Activation & Model

MIA: a Risk Factor for Neurodevelopmental and Psychiatric Disorders

Schizophrenia (Benros ME et al *AJP* 2011)



Autism & Prenatal Fever (Brucato M et al *Autism Res* 2017)



Non-human Primate Model of MIA

- polyICLC during 1st or 2nd trimester
- Behavioral Changes
  - Decreased affiliative vocalizations
  - Increased repetitive behavior
  - Increased reactivity
  - Abnormal social behaviors
  - Decreased social attention

Maternal Immune Activation in Nonhuman Primates Alters Social Attention in Juvenile Offspring

Christopher J. Machado, Alexander M. Whitaker, Stephen E.P. Smith, Paul H. Patterson, and Melissa D. Bauman

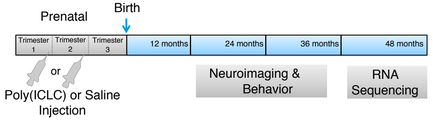
ARCHIVAL REPORT

Activation of the Maternal Immune System During Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring

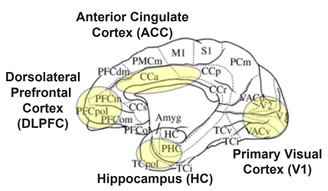
Melissa D. Bauman, Ana-Maria Iosif, Stephen E.P. Smith, Catherine Bregere, David G. Amaral, and Paul H. Patterson

Experimental Design

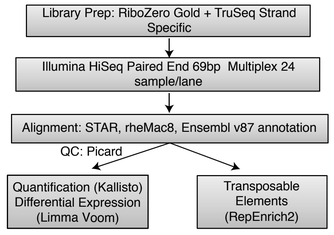
Experimental Timeline



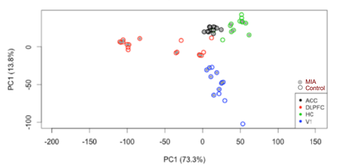
Brain Regions Profiled



RNA-Sequencing Pipeline

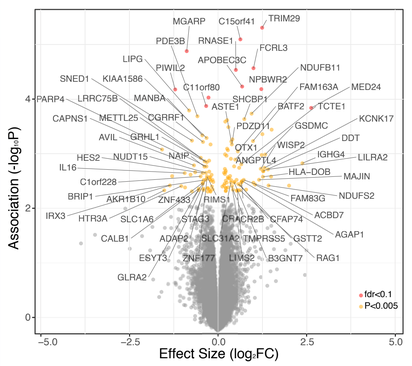


Quality Control

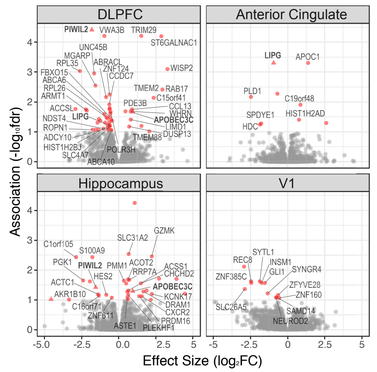


Differential Gene Expression (DGE)

A) Global differential expression



B) Regional differential expression



C) Gene ontology enrichment analyses

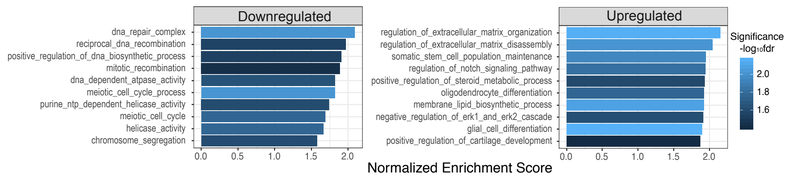
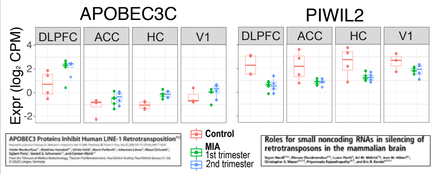


Fig 1. MIA induces lasting gene expression alterations in NHP cortex.
(A) A volcano plot of differential gene expression (DGE) across all four cortical regions in MIA vs control exposed offspring. (B) Region-specific DGE associations are shown. Three genes exhibit DE across multiple regions: *PIWIL2, APOBEC3C,* and *LIPG*. (C) Gene set enrichment analyses (GSEA) highlight extracellular matrix, oligodendrocyte and glial pathways among upregulated genes and DNA repair pathways among downregulated genes.

Dysregulation of Retrotransposition

A) Top DGE genes regulate retrotransposition



B) Protein-level changes in PIWIL2

C) Upregulation of transposable elements ERV1-class endogenous retroviruses

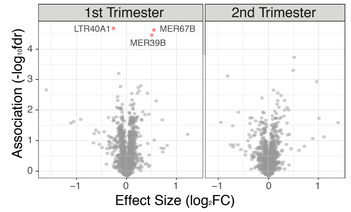
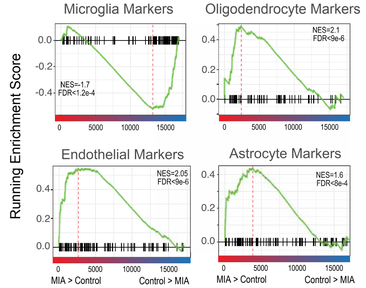


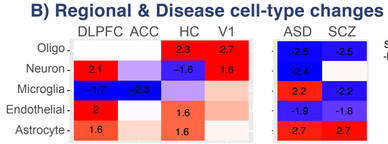
Fig 2. Transcriptomic evidence for dysregulation of retrotransposition following MIA
(A) Top genes exhibiting DGE -- *PIWIL2* and *APOBEC3C* -- are known regulators of retrotransposition. (B) Differential expression of *PIWIL2* is confirmed at the protein level via western blot. (C) Upregulation of MER39B and MER67B -- two ERV1-class endogenous retroviruses -- is observed following MIA, particularly for first-trimester exposure.

Cell-Type Specific Gene Expression Changes

A) Global cell-type enrichment



B) Regional & Disease cell-type changes



C) Microglial association with ERV1 levels

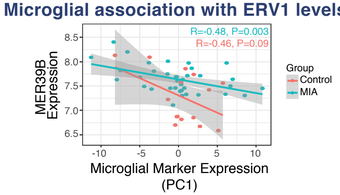
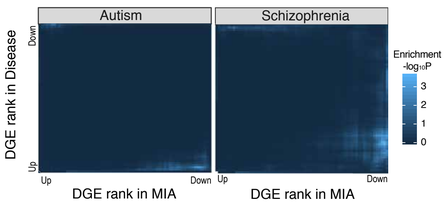


Fig 3. Cell-type specific gene expression alterations following MIA and in psychiatric disease
(A) Gene-set enrichment analysis was performed using cell-type specific marker genes (Zhang et al., *Neuron* 2015). Markers of microglia were enriched among genes downregulated following MIA, whereas oligodendrocyte, astrocyte, and enthothelial markers were enriched among upregulated genes.
(B) Left, cell-type specific gene set enrichment was performed within each brain region separately. Right, for comparison, analogous enrichments were assessed using transcriptome data from cortex in ASD and SCZ as part of the PsychENCODE project. MIA recapitulates astrocyte changes seen in ASD & SCZ and microglial alterations seen in SCZ.
(C) MER39B is associated with microglial marker gene expression, summarized by its first principle component.

Limited Global Overlap with ASD, SCZ Transcriptomes

A) No global overlap with transcriptomes in ASD and SCZ



B) Individual genes differentially expressed in MIA and ASD or SCZ

Gene	MIA logFC	MIA P	SCZ logFC	SCZ fdr	ASD logFC	ASD fdr
PIWIL2	-1.2	6.60E-05	0.14	0.014	0.22	0.38
IL16	**-0.94**	**0.0023**	**-0.12**	**0.0012**	**0.063**	**0.77**
MGARP	-0.88	1.30E-05	0.081	0.039	0.33	0.0093
CAL1B	-0.54	0.0044	0.18	8.20E-09	0.055	0.77
C1orf228	-0.49	0.0035	0.11	0.00094	0.094	0.58
MANBA	-0.44	0.00046	0.045	0.00079	0.04	0.55
ZNF423	-0.43	0.0036	0.057	0.004	0.019	0.88
ADAP2	**-0.41**	**0.003**	**0.087**	**0.021**	**-0.044**	**0.84**
BRIP1	-0.41	0.0043	-0.13	0.0016	0.15	0.42
ESYT3	-0.35	0.003	0.092	0.00034	0.046	0.76
RHBT1	**-0.31**	**0.0026**	**-0.048**	**0.0042**	**-0.025**	**0.79**
OPTN	0.29	0.00032	-0.004	0.84	-0.094	0.043
ETV6	**0.34**	**0.0026**	**0.064**	**0.0003**	**-0.055**	**0.67**
ASTE1	0.37	0.00059	-0.038	0.049	-0.091	0.21
CRACR2B	0.42	0.0045	-0.11	0.002	-0.013	0.97
TMEM38B	0.45	0.0017	-0.17	0.0022	-0.27	0.25
RRAS2	0.5	2.90E-05	-0.18	0.00012	-0.42	0.018
TMPRSS5	**0.54**	**0.0046**	**0.14**	**0.0035**	**-0.15**	**0.53**
ANGPTL4	**0.6**	**0.0018**	**0.4**	**7.70E-07**	**0.38**	**0.34**
CFAP70	0.63	0.0037	-0.077	0.011	-0.059	0.72
C1Sorf41	0.63	8.00E-06	0.04	0.034	-0.08	0.27
NHLRC4	0.67	0.0033	-0.19	0.00011	-0.12	0.66
FAM83G	**0.73**	**0.003**	**0.24**	**2.70E-06**	**0.4**	**0.042**
FAM163A	**0.99**	**0.00058**	**0.16**	**0.00018**	**-0.14**	**0.53**
WISP2	**1.2**	**0.0019**	**0.2**	**0.0027**	**-0.12**	**0.75**
NPBWR2	1.2	6.50E-05	-0.18	0.047	-0.31	0.4

Fig 4. Comparison with adult ASD, SCZ transcriptomic signatures
(A) Rank-rank hypergeometric overlap plots compare MIA differential expression changes with those observed in ASD and SCZ cortex, from PsychENCODE. Genes are ranked by association p-value signed by the direction of effect (up or downregulated). No global overlap between MIA and ASD or SCZ transcriptomes was observed.
(B) Table of genes DE in MIA and SCZ or ASD. Bold represents concordant direction of effect in MIA and disease.

Conclusions

1. **Maternal immune activation causes lasting transcriptomic changes in primate cortex, characterized by alterations in regulators of retrotransposition, MHC genes, and extracellular matrix pathways**
2. **MIA is associated with increased expression of ERV1-class endogenous retroviruses**
3. **Microglial marker genes were downregulated, which correlated with increased ERV1 expression**
4. **Limited global overlap is observed with disease transcriptomes in ASD, SCZ**
5. **Future work is needed to probe the timing of changes and impact of lasting ERV1 activation**

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