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Effects of prenatal pyrethroid exposure on placental transcriptomes in a Northern Thailand agricultural cohort study

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Abstract

Effects of prenatal pyrethroid exposure on placental transcriptomes in a Northern Thailand agricultural cohort study

By Jacqueline A. Holstein

Background: Prenatal pyrethroid exposure has been linked to adverse postnatal neurodevelopmental outcomes. Animal models have found that the disruption of dopamine expression in the placenta may be linked to postnatal behavioral characteristics of neurobehavioral disorders such as ADHD, autism, and neuromotor conditions.

Objective Our goal was to assess the placental transcriptome to examine the hypothesis that dopamine biomarkers, specifically D5, D1, D2, dopamine active transporter (DAT), Tyrosine hydroxylase (TH), Calmodulin-dependent Protein Kinase IIα (CAMK2A), monoamine oxidase A (MAOA), voltage-gated sodium channel type Iα (VSSC), catechol O-methyltransferase (COMT), and vesicular monoamine transporter (VMAT) are associated with pyrethroid exposure and postnatal neurodevelopmental scales in the Study of Asian Women and their Offspring's Development and Environmental Exposures (SAWASDEE) cohort.

Methods: This study is making use of existing data from the SAWASDEE cohort. For pyrethroid exposure models a composite score of log-transformed 3-phenoxybenzoic acid (3-PBA) across all trimesters was used. At 4 months after birth, nurses certified in use of the NICU Network Neurobehavioral Scale (NNNS) captured neurobehavioral outcomes. For 254 participants, we profiled the term placental transcriptome using RNA-Seq. Generalized linear models were used to examine the association between average 3-PBA across trimester and the transcriptome; overall and stratified by infant sex, and between eight neurobehavioral scales and the transcriptome. Differentially expressed genes were subjected to gene ontology enrichment analysis as well as ConsensusPathDB over-representation.

Results: No hypothesized dopaminergic pathway genes met FDR threshold (<0.05) for differential expression with pyrethroid exposure. When looking at the pyrethroid models stratified by infant sex we did observe two genes that were differentially expressed in males: *IGKC* and *KRT24*. After performing pathway analysis on NNNS data we found that similar to previous animal research; *metabolism of RNA, ETC: OXPHOS system in mitochondria, Oxidative phosphorylation, Respiratory electron transport,* and *mRNA Splicing* pathways were all significantly expressed.

Conclusion: Our study findings suggest that exposure to pyrethroid during pregnancy may disrupt placental gene networks. Gene network disruption may lead to down-stream changes in placental function and ultimately affect the developing fetus.

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Introduction

The incidence rates of Attention-deficit hyperactivity disorder (ADHD) have been rising across the world[1]. Only part of this increase can be explained by an increasing focus on mental health, better ability to diagnostic tools, and access to diagnostic services[2,3]. The environment is likely also a key contributor to this increase, and specifically prenatal exposure to insecticides, which have been linked to postnatal outcomes of neurodevelopmental disorders[4].

Pyrethroids are a synthetic analog of pyrethrin, a pesticide found naturally in chrysanthemums, that is used in both agricultural and residential settings[5]. Pyrethroids are primarily used to control insect vectors and are found in consumer products such as garden insecticides, pet shampoos, lice treatments and mosquito repellents applied to clothing[5,6]. They are a rapidly growing alternative to organophosphates and currently account for 30% of the global insecticide market[5–7]. Due to their increasing popularity and use, pyrethroid exposure in the general population has demonstrated widespread exposure across the world in both adults and children[6,8–12]. Exposures levels have steadily increased since the early 2000's; in the recent 2011-2013 NHANES data analysis the detection rate for pyrethroid was 78.1% in adults and 79.3% in children[6]. Since pyrethroids account for such a large portion of the pesticide market, one particularly susceptible population to exposure is agricultural workers who are routinely exposed at work[13–15].

Inhalation, dermal absorption, and ingestion are the three most common exposure routes for pyrethroids[16]. Once they enter the body, pyrethroids are quickly metabolized by cytochrome P450 and depending on the parent compound can form multiple

metabolites including: including 3-phenoxybenzoic acid (3-PBA), 3-(2,2-dichlorovinyl)-2,2-dimethylcylopropane carboxylic acid (DCCA), 4-fluoro-3-phenoxybenzoic acid (4F-PBA), and (2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA)[17]. In agricultural workers the most commonly excreted metabolite is 3-PBA[18,19].

As a pesticide, pyrethroids work in two ways on insects, one by inhibiting the closure of voltage-gated sodium channels in axonal membranes, causing axonal depolarization; and two by inhibiting γ-Aminobutyric acid (GABA) and voltage-gated chloride channels increasing neuronal excitability[5,20]. Pyrethroids are able do to this because they are lipophilic, allowing them to be quickly absorbed and distributed to the lipid membrane of nerve cells[21]. In biomonitoring studies the level of pyrethroid exposure in humans is below the concentrations that affects activity on voltage channels, however animal and biomonitoring research suggests that exposure to pyrethroids is linked to maternal to infant pyrethroid transfer as well as other similar toxicity pathways[22–24]. Further research has found that gestational exposure to pyrethroids is linked to postnatal neurobehavioral, neuromotor, and neurocognitive deficit outcomes[7,25,26]. Given the long-term impacts of prenatal exposure on offspring health, it is important to consider how pyrethroids can impact the mechanistic pathways of pyrethroid exposure in humans.

Throughout pregnancy the placenta serves as the primary connection between fetus and pregnant person, allowing for gas exchange, metabolism, nutrient transfer, growth hormone production, and immunologic control[27,28]. External environmental stressors, such as pesticide exposure, can cause the placenta to make changes to the intrauterine environment[27,28]. During all three trimesters the placenta produces varying levels of neurotransmitters such as dopamine which influence brain development[28,29]. The

lipophilicity of pyrethroids also allows pyrethroids to accumulate in and transfer through the placenta via passive diffusion[29–31]. Recent animal models have found that the disruption of dopamine expression in the placenta may be linked to postnatal diagnosis of neurobehavioral disorders such as ADHD, autism, and neuromotor conditions[32–34]. Postnatally the overexpression of dopamine active transporter (DAT) and D5 have been linked to ADHD.

Analysis of transcriptome-wide profiles of human term placenta may assist in identifying the presence of unique dopamine pathway biomarkers to further understand future postnatal risk and our understanding of placental development[35]. The identification of specific dopamine biomarkers may not only assist in early identification of neurobehavioral disorders, but may decrease the time to intervention. This analysis looked at the association of placental expressed genes from RNAseq studies with 3-PBA, and with NNNS outcomes, hypothesizing that dopamine biomarkers, specifically D5, D1, D2, dopamine active transporter (DAT), Tyrosine hydroxylase (TH), Calmodulin-dependent Protein Kinase II3α (CAMK2A), monoamine oxidase A (MAOA), voltage-gated sodium channel type Iα (VSSC), catechol O-methyltransferase (COMT), and vesicular monoamine transporter (VMAT) will be associated with pyrethroid exposure and postnatal neurodevelopmental scales in a longitudinal birth cohort.

Methods

Study Population

This study is making use of existing data on the Study of Asian Women and their Offspring's Development and Environmental Exposures (SAWASDEE) cohort, a study that enrolled pregnant women from Fang and Chom Thong, two districts in the Chiang

Mai province, Thailand. Enrolled participants were engaged in either rice or tangerine farm work where organophosphorus insecticides were being used. Exclusion criteria included non-singleton pregnancies and major pregnancy complications that could affect fetal growth and development. All study protocols were approved by the Institutional Review Board at Emory University and the ethics review committee at the Research Institute for Health Sciences, Chiang Mai University. Informed consent was obtained from all study participants prior to enrollment. The analytic sample includes 254 SAWASDEE cohort participants of mother-infant dyads with urinary pyrethroid pesticide metabolites and placenta RNA-Seq data and corresponding infants with NICU Network Neurobehavioral Scales (NNNS) data.

Urine Samples

Urine samples were collected throughout first, second, and third trimester to assess pyrethroid exposure. Samples were analyzed for 3-phenoxybenzoic acid (3-PBA), the metabolite of the pyrethroid Esfenvalerate to assess exposure. Urine samples were composited using equal volumes to create early-, mid- and late-pregnancy samples that roughly correspond to trimesters. For chemical analysis, all samples were randomized using a Fisher-Yates shuffling algorithm prior to analysis to reduce any potential batch effects[36,37]. A log-transformed 3PBA (ng/mL) composite controlled for urinary creatinine, as a dilution factor, was created across all trimesters for each participant which was used for analysis.

NNNS

The NNNS was performed on all newborns, 4 months of age, to assess neurobehavioral outcomes by trained psychometrists using standardized procedures[38,39].

The NNNS is a tool developed to assess at-risk infants, typical premature infants or infants prenatally exposed to neurotoxic substances. It has strong psychometric qualities with good internal and concurrent validity[40]. NNNS scores for attention, arousal, excitability, handling, lethargy, quality of movement, self-regulation, and stress abstinence were utilized in this analysis.

RNA sequencing

RNA-Seq libraries were prepared with the RNAse H protocol for rRNA depletion (New England Biolabs (NEB), Ipswich, MA, USA). Sequencing was performed using DNBseq™ Technology (MGI, Shenzhen, China) with a target of 20 million pair-end 100 bp reads per sample. RNA-Seq was performed at the Beijing Genomics Institute. All samples (n=264) had more on average more than 25 million reads with > 85% of the reads mapped to the human genome (GRCh38). RNA-Seq reads were characterized using MultiQC, aligned and quantified using STAR and GenCode v33 annotation (GRCh38)[41,42]. FeatureCounts was used to summarize mapped read counts to genes[43].

Statistical analysis

All statistical analyses were conducted using RStudio version 4.2.2. The distribution of pyrethroid outcomes and NNNS outcomes are summarized as means, standard deviations, and percentiles. To analyze the gene associations, we used DESeq2 R package[44]. After removing 6 replicates and 4 poor performing samples, and gene filtering, the final datasets included 254 samples. Generalized linear models were used to examined overall association for each gene and average 3-PBA level across trimester adjusting for infant sex and maternal gestational age at delivery; we then stratified by infant sex. Generalized linear models were also used to examine overall association for

each gene and eight NNNs scales adjusting for infant sex and gestational age at delivery. Genes with low expression (< 2 counts in at least 20% of samples) were excluded before gene network analyses. We constructed our exposure-transcriptome data set with 3-PBA as a continuous variable averaged across the three trimesters.

NNNS-transcriptome data sets were constructed with each score as a z-score. Significantly expressed genes from the eight models that were associated with more than one score were combined for further analysis. The habituation NNNS package was administered to < 50% of out sample (n= 100) due to the requirement that the infant must be asleep for examination, scores for habituation were eliminated from analysis[39,45]. For significantly expressed genes in the neurobehavioral outcome models we completed a Gene ontology enrichment analysis using *Generally Applicable Gene Set Enrichment for Pathways Analysis (*GAGE)[46]. For the same neurobehavioral outcome genes a pathway analysis was completed using ConsensusPathDB over-representation analysis[47,48]. Findings were considered statistically significant if the *q*-value was < 0.05.

Results

Pyrethroid Metabolites

The demographic characteristics of our study population are shown in Table 1. Maternal age ranged from 18 to 39 with mean age at enrollment of 25.1 years (SD = 5.4). The average gestational age at birth was 38 weeks (SD = 1.4). More than half of the participants were from Chom Thong (n = 182, 72%). There was about equal number of male and female infants (n=124, 49.2% females).

Table 1. Descriptive characteristics of the study population (n = 254)

Characteristics	n (%) / Mean ± SD
Study location (Fang (%))	72 (28.3)
Age of mother at enrollment (years, mean ± SD)	25.1 ± 5.4
Gestational age at delivery (weeks, mean ± SD)	38 ± 1.4
Infant sex (Female (%))	125 (49.2)

The mean and median 3-PBA level across all pregnancy time points were 0.82 ng/mL and 0.5 ng/mL (interquartile range [IQR] 0.30-0.93 ng/mL). Distribution of 3-PBA metabolite concentrations at each trimester (early, mid, and late) and the average across all trimesters are shown in Table 2.

Table 2. Distribution of urinary 3-PBA metabolites in early, mid and late pregnancies

	N	Minimum (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Maximum (ng/mL)
3-PBA					
Early-Pregnancy	235	0.22	0.85	0.44	18.15
Mid-Pregnancy	253	0.22	0.77	0.37	9.44
Late-Pregnancy	251	0.22	0.84	0.32	17.72
Average across pregnancy	254	0.21	0.82	0.5	7.27

3-PBA: 3-phenoxybenzoic acid

Differential expression analysis of the pyrethroid exposure model revealed 2 upregulated pseudo genes meeting a threshold of FDR<0.05. When stratified by infant sex, 2 genes were considered differentially expressed in males; keratin 24 (*KRT24*) and immunoglobulin kappa constant (*IGKC*) while the same two pseudo genes as the overall model were considered differentially expressed for females Table 3.

Table 3. Pyrethroid Model Expressed Genes by Infant Sex

Males			Females		
Gene	log_2 (Fold Change)	p-adj.*	Gene	log_2 (Fold Change)	p-adj.*
IGKC	1.56	$p < 0.001$	RP11_274B2112‡	1.48	$p < 0.001$
KRT24	-2.21	$p = 0.05$	RP11_291L226‡	0.97	$p < 0.001$

p values were from DESeq2 results
‡ Pseudo genes

No hypothesized dopaminergic pathway genes met FDR threshold for differential expression (Table 4).

Table 4. Pyrethroid Exposure - Transcriptome Dopamine Pathway Results

Gene	base mean	log_2 (fold change)	p-adj
Dopamine receptor D5	no results	no results	no results
Dopamine receptor D1	33.12	0.13	0.99
Dopamine receptor D2	no results	no results	no results
Dopamine active transporter (DAT)	no results	no results	no results
Tyrosine Hydroxylase	no results	no results	no results
Calmodulin-dependent Protein Kinase IIα (CAMK2A)	62.01	-0.046	0.99
Monoamine oxidase A (MAOA)	6197.36	0.018	0.99
Voltage-gated sodium channel type Iα (VSSC),	210.66	0.087	0.99
Catechol O-methyltransferase (COMT)	2103.71	0.0037	0.99
Vesicular monoamine transporter (VMAT)	no results	no results	no results

NNNS Data

Distribution of the NNNS scores are shown in Table 5

Table 5. Descriptive characteristics of the study population

Subs Score	N	Description[45]	Score Range	Minimum	Mean	Median	Maximum
Attention	240	Response to animate and inanimate auditory and visual stimuli	1-9	2.14	5.71	5.71	7.29
Arousal	246	Level of arousal including state and motor activity	1-9	2.71	3.69	3.57	5.29
Excitability	247	Measure of high levels of motor, state, and physiologic reactivity	0-15	0.0	1.27	0	7.0
Handling	242	Handling strategies used during orientation to maintain alert state	0-1	0.0	0.19	0.13	1.0
Lethargy	247	Measure of low levels of motor, state, and physiologic reactivity	0-15	1.0	3.19	3.0	8.0
Quality of Movement	246	Measure of motor control including smoothness, maturity, lack of startles and tremors	1-9	4.6	5.52	5.5	6.2
Self-Regulation	246	Capacity to organize motor activity, physiology, and state during the examination and to respond to cuddling, consoling, and negative stimuli	1-9	4.58	6.07	6.15	7.33
Stress Abstinence	247	Amount of stress and abstinence signs observed	0-1	0.0	0.06	0.06	0.16

Differential expression analysis was conducted on NNNS models for attention, arousal, excitability, handling, lethargy, quality of movement, self-regulation, and stress abstinence scores. No genes demonstrated differential expression based on a threshold of *FDR<0.05* for excitability scores. For the other scales, the genes that demonstrated differential expression (FDR<0.05) and were associated with multiple scales were combined for further analysis (1805 overlapping genes). We performed a gene ontology (GO) enrichment analysis to gain further biological process insight into genes that were

differentially expressed across behavioral outcomes models. The top identified GO terms were *RNA splicing*, *peptidyl-lysine modification*, *ribosome biogenesis, oxidative phosphorylation,* and *histone acetylation* (Figure 1). A ConsensusPathDB over-representation analysis was conducted to further analyze significant pathways within the NNNS data (Table 6). The dopaminergic synapse pathway was not found to be expressed in our analysis, however similar to previous animal research *metabolism of RNA, ETC: OXPHOS system in mitochondria, Oxidative phosphorylation, Respiratory electron transport,* and *mRNA Splicing* were expressed in the NNNS data (Figure 2). No hypothesized dopaminergic pathway genes met FDR threshold (Table 6).

Table 6. NNNS Attention - Transcriptome Dopamine Pathway Results

Gene	base mean	$\log_2$ (fold change)	p-adj
Dopamine receptor D5	no results	no results	no results
Dopamine receptor D1	33.12	-0.03	NA
Dopamine receptor D2	no results	no results	no results
Dopamine active transporter (DAT)	no results	no results	no results
Tyrosine Hydroxylase	no results	no results	no results
Calmodulin-dependent Protein Kinase IIα (CAMK2A)	62.75	-0.032	NA
Monoamine oxidase A (MAOA)	6000.05	-0.013	0.92
Voltage-gated sodium channel type Iα (VSSC),	211.34	-0.063	0.66
Catechol O-methyltransferase (COMT)	2099.47	-0.048	0.13
Vesicular monoamine transporter (VMAT)	no results	no results	no results

Discussion

The hypothesis of this analysis was that select genes in the dopaminergic synapse pathway would show differential expression with 3-PBA and neurobehavioral

performance; none of the pre-selected genes were differentially expressed. We observed, however, significant differential gene expression among male infants with in-utero pyrethroid exposure and a high level of gene expression in neurobehavioral model outcomes.

Pyrethroid

In males there was a 1.5 $\log_2$(fold change) in *IGKC* and a -2.21 $\log_2$(fold change) in *KRT24* expression with increasing 3-PBA concentrations. *IGKC* plays a key role during development in forming the adaptive immune system. Preliminary animal models suggest that exposure to pyrethroid reduces the proliferation of peripheral blood leukocytes further reducing the concentration of IgG immunoglobulins[49,50]. Our findings in male infants are consistent with preliminary animal models and suggest that further research would be beneficial.

KRT24 encodes a type I keratin family belonging to the superfamily of intermediate filament proteins, in conjunction with actin microfilaments and microtubules they compose the cytoskeleton of epithelial cells[51]. There is minimal research related to pyrethroid exposure and *KRT24* exposure, however, current research on the under expression of *KRT24* and neonatal development has found that down regulation of *KRT24* may result in infant skin conditions as well as autoimmune diseases[52,53].

NNNS Data

We observed multiple RNA pathways, including mRNA splicing and metabolism of RNA, that were statistically significantly over-represented when looking at genes that overlapped for differential expression across NNNS. This is congruent with observational

research which has found placental mRNA splicing is associated with infant neurodevelopmental outcomes[54].

The pathway results are consistent with previous findings of oxidative stress biomarkers after prenatal exposure to pyrethroid. Mouse models and recent biomonitoring data reported that neonatal exposure to pyrethroid induced oxidative stress and dysregulated immune responses[55–58]. These findings reported primarily adverse pregnancy outcomes such as premature birth and low birth weight but recent animal models have cited neurobehavioral outcomes such as Parkinson's disease and ASD. These results are also consistent with previous rodent model placenta transcriptome published earlier this year[59]. Although this analysis does not address correlation it provides evidence of possible NNNS score association to gene expression and lays the foundation for future analysis.

Limitations

We acknowledge several limitations to this study. Our sample size of the study was limited (n=254) which limits the statistical power of the study. The placenta is a complex organ, consisting of a heterogenous mix of cell sub-types with distinct transcriptional profiles. Our placenta biopsies are collected using a standardized protocol to reduce this variability[60] but there still may be residual confounding by cell composition that is not accounted. This analysis focuses on agricultural workers whom have routine direct exposure to pesticides; this limits the generalizability of our findings. Our analysis utilized NNNS data for behavioral outcomes, the SAWASDEE cohort data collection is still ongoing therefore final neurobehavioral outcomes for infant participants have not yet

been collected. Previously cited neurobehavioral outcomes in observational studies do not manifest until preschool age, so it is possible that our NNNS models under report.

Conclusion

Our study findings suggest that exposure to pyrethroid during pregnancy may disrupt placental gene networks particularly in male infants. Gene network disruption may lead to down-stream changes in placental function and ultimately affect the developing fetus. Furthermore our findings suggest that genes disruptions may affect neurodevelopmental outcomes in overlapping ways that are captured in NNNS scores. Further research is recommended to explore the effects of pyrethroid exposure on neurobehavioral outcomes, specifically oxidative stress and immune responses.

Figures

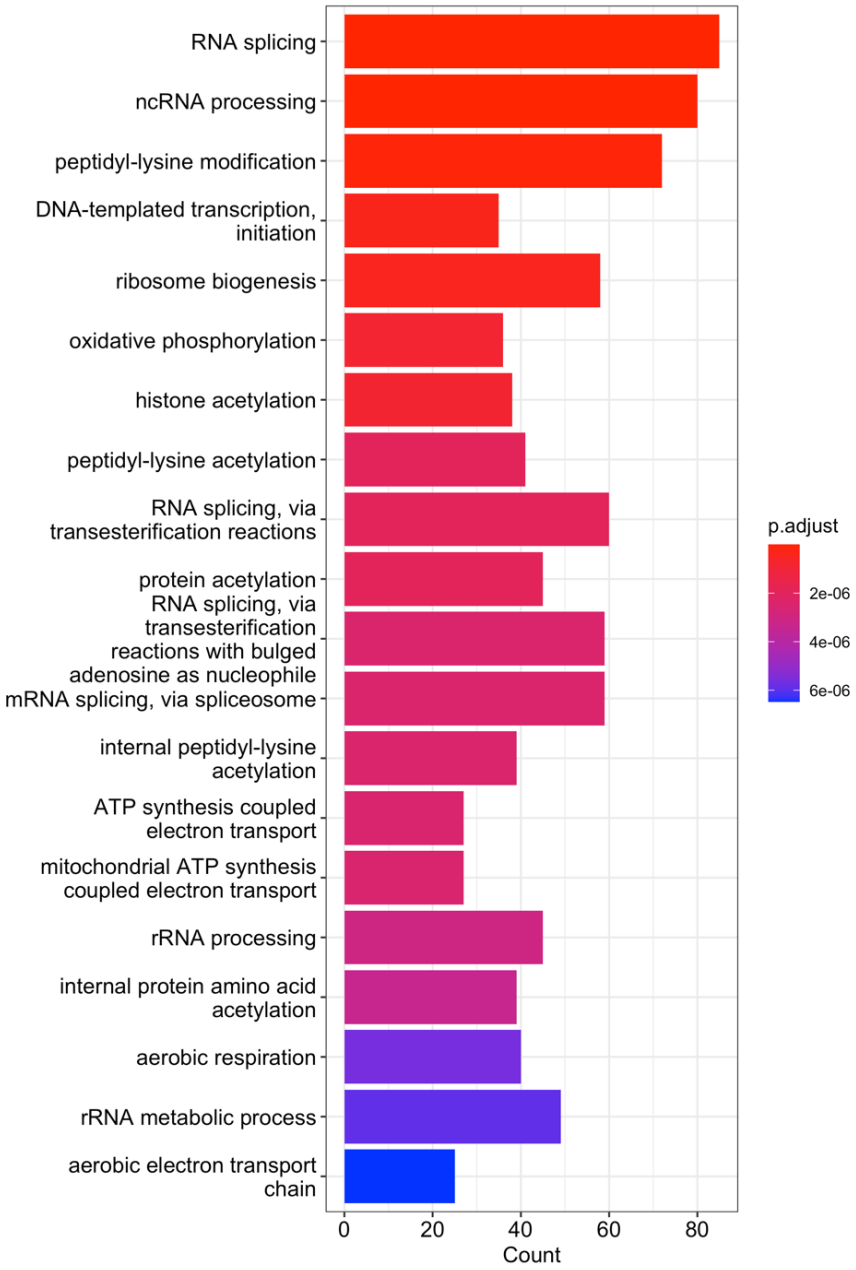

Figure 1. Top 20 Gene Ontology Enrichment Terms from NNNS analysis

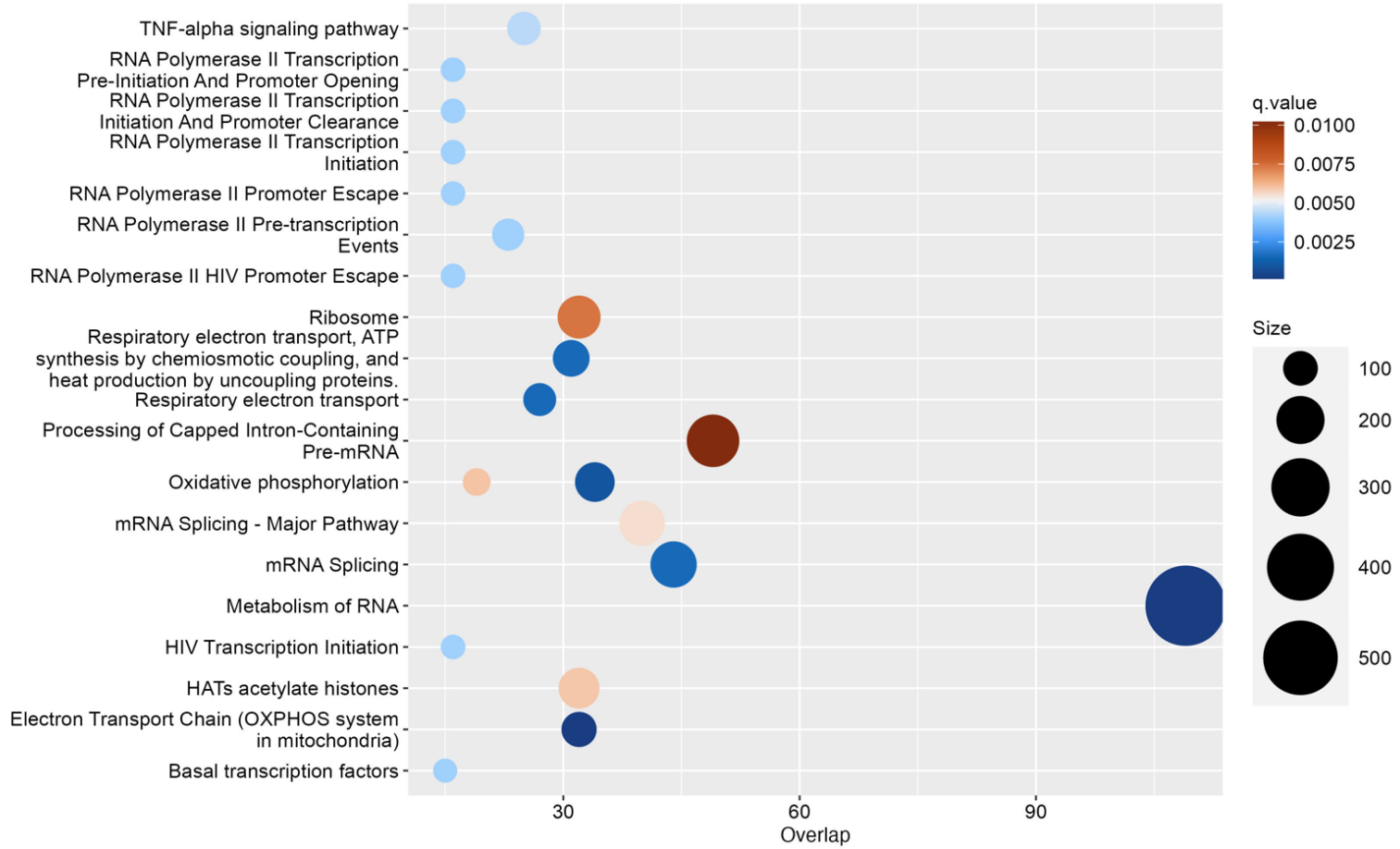

Figure 2. Top 20 significant pathways from NNNS ConsensusPathDB over-representation analysis where number of genes overlapping among different gene sets is represented on the x-axis and dots represent the total count of genes per pathway.

References:

1. Faraone SV, Sergeant J, Gillberg C, Biederman J. The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry Off J World Psychiatr Assoc WPA*. 2003;2(2):104-113.
2. Conrad P, Bergey MR. The impending globalization of ADHD: Notes on the expansion and growth of a medicalized disorder. *Soc Sci Med*. 2014;122:31-43. doi:10.1016/j.socscimed.2014.10.019
3. Visser SN, Danielson ML, Bitsko RH, et al. Trends in the Parent-Report of Health Care Provider-Diagnosed and Medicated Attention-Deficit/Hyperactivity Disorder: United States, 2003–2011. *J Am Acad Child Adolesc Psychiatry*. 2014;53(1):34-46.e2. doi:10.1016/j.jaac.2013.09.001
4. Qi Z, Song X, Xiao X, et al. Effects of prenatal exposure to pyrethroid pesticides on neurodevelopment of 1-year- old children: A birth cohort study in China. *Ecotoxicol Environ Saf*. 2022;234:113384. doi:10.1016/j.ecoenv.2022.113384
5. Gajendiran A, Abraham J. An overview of pyrethroid insecticides. *Front Biol*. 2018;13(2):79-90. doi:10.1007/s11515-018-1489-z
6. Lehmler HJ, Simonsen D, Liu B, Bao W. Environmental exposure to pyrethroid pesticides in a nationally representative sample of U.S. adults and children: The National Health and Nutrition Examination Survey 2007–2012. *Environ Pollut*. 2020;267:115489. doi:10.1016/j.envpol.2020.115489
7. Babina K, Dollard M, Pilotto L, Edwards JW. Environmental exposure to organophosphorus and pyrethroid pesticides in South Australian preschool children: A cross sectional study. *Environ Int*. 2012;48:109-120. doi:10.1016/j.envint.2012.07.007
8. Roca M, Miralles-Marco A, Ferré J, Pérez R, Yusà V. Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. *Environ Res*. 2014;131:77-85. doi:10.1016/j.envres.2014.02.009
9. van Wendel de Joode B, Mora AM, Lindh CH, et al. Pesticide exposure and neurodevelopment in children aged 6-9 years from Talamanca, Costa Rica. *Cortex J Devoted Study Nerv Syst Behav*. 2016;85:137-150. doi:10.1016/j.cortex.2016.09.003
10. Ding G, Shi R, Gao Y, et al. Pyrethroid Pesticide Exposure and Risk of Childhood Acute Lymphocytic Leukemia in Shanghai. *Environ Sci Technol*. 2012;46(24):13480-13487. doi:10.1021/es303362a
11. Rodríguez T, van Wendel de Joode B, Lindh CH, Rojas M, Lundberg I, Wesseling C. Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. *Occup Environ Med*. 2012;69(2):119-125. doi:10.1136/oem.2010.062539
12. Motsoeneng P, Dalvie M. Relationship between Urinary Pesticide Residue Levels and Neurotoxic Symptoms among Women on Farms in the Western Cape, South Africa. *Int J Environ Res Public Health*. 2015;12(6):6281-6299. doi:10.3390/ijerph120606281
13. Tang W, Wang D, Wang J, et al. Pyrethroid pesticide residues in the global environment: An overview. *Chemosphere*. 2018;191:990-1007. doi:10.1016/j.chemosphere.2017.10.115
14. Maggi F, Tang FHM, la Cecilia D, McBratney A. PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. *Sci Data*. 2019;6(1):170. doi:10.1038/s41597-019-0169-4

15. de-Assis MP, Barcella RC, Padilha JC, Pohl HH, Krug SBF. Health problems in agricultural workers occupationally exposed to pesticides. *Rev Bras Med Trab*. 2020;18(03):352-363. doi:10.47626/1679-4435-2020-532
16. US Department of Health and Human Services. Toxicological Profile for Pyrethrins and Pyrethorids. Published online 2003. https://www.atsdr.cdc.gov/toxprofiles/tp155.pdf
17. Bao W, Liu B, Simonsen DW, Lehmler HJ. Association Between Exposure to Pyrethroid Insecticides and Risk of All-Cause and Cause-Specific Mortality in the General US Adult Population. *JAMA Intern Med*. 2020;180(3):367. doi:10.1001/jamainternmed.2019.6019
18. López-Gálvez N, Wagoner R, Beamer P, de Zapien J, Rosales C. Migrant Farmworkers' Exposure to Pesticides in Sonora, Mexico. *Int J Environ Res Public Health*. 2018;15(12):2651. doi:10.3390/ijerph15122651
19. Trunnelle KJ, Bennett DH, Ahn KC, et al. Concentrations of the urinary pyrethroid metabolite 3-phenoxybenzoic acid in farm worker families in the MICASA study. *Environ Res*. 2014;131:153-159. doi:10.1016/j.envres.2014.03.003
20. Burr SA. Structure-Activity and Interaction Effects of 14 Different Pyrethroids on Voltage-Gated Chloride Ion Channels. *Toxicol Sci*. 2004;77(2):341-346. doi:10.1093/toxsci/kfh027
21. Plumlee KH. Insecticides and Molluscicides. In: *Clinical Veterinary Toxicology*. Elsevier; 2004:177-192. doi:10.1016/B0-32-301125-X/50024-8
22. Richardson JR, Fitsanakis V, Westerink RHS, Kanthasamy AG. Neurotoxicity of pesticides. *Acta Neuropathol (Berl)*. 2019;138(3):343-362. doi:10.1007/s00401-019-02033-9
23. Aznar-Alemany Ò, Eljarrat E. Bioavailability and Bioaccumulation of Pyrethroid Insecticides in Wildlife and Humans. In: Eljarrat E, ed. *Pyrethroid Insecticides*. Vol 92. The Handbook of Environmental Chemistry. Springer International Publishing; 2020:205-225. doi:10.1007/698_2020_466
24. Shafer TJ, Meyer DA. Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. *Toxicol Appl Pharmacol*. 2004;196(2):303-318. doi:10.1016/j.taap.2003.12.013
25. Lucero B, Muñoz-Quezada MT. Neurobehavioral, Neuromotor, and Neurocognitive Effects in Agricultural Workers and Their Children Exposed to Pyrethroid Pesticides: A Review. *Front Hum Neurosci*. 2021;15:648171. doi:10.3389/fnhum.2021.648171
26. Badaeva LN, Nedorenko NI. [Placental histogenesis and the cardiotoxic effect in rat progeny under the influence of the synthetic pyrethroid Decis]. *Vrach Delo*. 1991;(10):68-71.
27. Sandovici I, Hoelle K, Angiolini E, Constância M. Placental adaptations to the maternal–fetal environment: implications for fetal growth and developmental programming. *Reprod Biomed Online*. 2012;25(1):68-89. doi:10.1016/j.rbmo.2012.03.017
28. Hsiao EY, Patterson PH. Placental regulation of maternal-fetal interactions and brain development. *Dev Neurobiol*. 2012;72(10):1317-1326. doi:10.1002/dneu.22045

29. Alonso MB, Feo ML, Corcellas C, et al. Pyrethroids: A new threat to marine mammals? *Environ Int*. 2012;47:99-106. doi:10.1016/j.envint.2012.06.010
30. Alonso MB, Feo ML, Corcellas C, et al. Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil. *Environ Pollut*. 2015;207:391-402. doi:10.1016/j.envpol.2015.09.039
31. Mathiesen L, Buerki-Thurnherr T, Pastuschek J, Aengenheister L, Knudsen LE. Fetal exposure to environmental chemicals; insights from placental perfusion studies. *Placenta*. 2021;106:58-66. doi:10.1016/j.placenta.2021.01.025
32. Money KM, Stanwood GD. Developmental origins of brain disorders: roles for dopamine. *Front Cell Neurosci*. 2013;7. doi:10.3389/fncel.2013.00260
33. Madras BK, Miller GM, Fischman AJ. The Dopamine Transporter and Attention-Deficit/Hyperactivity Disorder. *Biol Psychiatry*. 2005;57(11):1397-1409. doi:10.1016/j.biopsych.2004.10.011
34. Maitra S, Sarkar K, Sinha S, Mukhopadhyay K. The Dopamine Receptor D5 May Influence Age of Onset: An Exploratory Study on Indo-Caucasoid ADHD Subjects. *J Child Neurol*. 2016;31(10):1250-1256. doi:10.1177/0883073816652233
35. Yong HEJ, Chan SY. Current approaches and developments in transcript profiling of the human placenta. *Hum Reprod Update*. 2020;26(6):799-840. doi:10.1093/humupd/dmaa028
36. Fisher, Ronald A., Yates, Frank. Statistical tables for biological, agricultural and medical research. In: 6th ed. Oliver & Boyd; 1948:26-27.
37. Knuth, Donald E. Seminumerical algorithms. In: *The Art of Computer Programing*. 2. Addison-Wesley; 1969:139-140.
38. Baumert BO, Fiedler N, Prapamontol T, et al. Investigation of Prenatal Pesticide Exposure and Neurodevelopmental Deficits in Northern Thailand: Protocol for a Longitudinal Birth Cohort Study. *JMIR Res Protoc*. 2022;11(2):e31696. doi:10.2196/31696
39. Sittiwang S, Nimmapirat P, Suttiwan P, et al. The relationship between prenatal exposure to organophosphate insecticides and neurodevelopmental integrity of infants at 5-weeks of age. *Front Epidemiol*. 2022;2:1039922. doi:10.3389/fepid.2022.1039922
40. Noble Y, Boyd R. Neonatal assessments for the preterm infant up to 4 months corrected age: a systematic review: Review. *Dev Med Child Neurol*. 2012;54(2):129-139. doi:10.1111/j.1469-8749.2010.03903.x
41. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*. 2016;32(19):3047-3048. doi:10.1093/bioinformatics/btw354
42. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15-21. doi:10.1093/bioinformatics/bts635
43. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 2014;30(7):923-930. doi:10.1093/bioinformatics/btt656
44. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550. doi:10.1186/s13059-014-0550-8

45. Appendix 2: Neonatal Intensive Care Unit Network Neurobehavioral Scale Summary Score Definitions. *Pediatrics*. 2004;113(Supplement_2):694-694. doi:10.1542/peds.113.S2.694
46. Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics*. 2009;10(1):161. doi:10.1186/1471-2105-10-161
47. Kamburov A, Pentchev K, Galicka H, Wierling C, Lehrach H, Herwig R. ConsensusPathDB: toward a more complete picture of cell biology. *Nucleic Acids Res*. 2011;39(suppl_1):D712-D717. doi:10.1093/nar/gkq1156
48. Kamburov A, Wierling C, Lehrach H, Herwig R. ConsensusPathDB—a database for integrating human functional interaction networks. *Nucleic Acids Res*. 2009;37(suppl_1):D623-D628. doi:10.1093/nar/gkn698
49. Skolarczyk J, Pekar J, Nieradko-Iwanicka B. Immune disorders induced by exposure to pyrethroid insecticides. *Postępy Hig Med Dośw*. 2017;71(1):0-0. doi:10.5604/01.3001.0010.3827
50. Einarsdottir HK, Stapleton NM, Scherjon S, et al. On the Perplexingly Low Rate of Transport of IgG2 across the Human Placenta. Bayry J, ed. *PLoS ONE*. 2014;9(9):e108319. doi:10.1371/journal.pone.0108319
51. KRT24 keratin 24 [Homo sapiens (human)] - Gene - NCBI. Accessed April 8, 2023. https://www.ncbi.nlm.nih.gov/gene/192666
52. Cooper GM. Intermediate Filaments. *Cell Mol Approach 2nd Ed*. Published online 2000. Accessed April 8, 2023. https://www.ncbi.nlm.nih.gov/books/NBK9834/
53. Visscher MO, Hu P, Carr AN, et al. Newborn infant skin gene expression: Remarkable differences versus adults. Simon M, ed. *PLOS ONE*. 2021;16(10):e0258554. doi:10.1371/journal.pone.0258554
54. Ruano CSM, Apicella C, Jacques S, et al. Alternative splicing in normal and pathological human placentas is correlated to genetic variants. *Hum Genet*. 2021;140(5):827-848. doi:10.1007/s00439-020-02248-x
55. Sferruzzi-Perri AN, Higgins JS, Vaughan OR, Murray AJ, Fowden AL. Placental mitochondria adapt developmentally and in response to hypoxia to support fetal growth. *Proc Natl Acad Sci*. 2019;116(5):1621-1626. doi:10.1073/pnas.1816056116
56. Elser BA, Kayali K, Dhakal R, et al. Combined Maternal Exposure to Cypermethrin and Stress Affect Embryonic Brain and Placental Outcomes in Mice. *Toxicol Sci*. 2020;175(2):182-196. doi:10.1093/toxsci/kfaa040
57. De Felice A, Greco A, Calamandrei G, Minghetti L. Prenatal exposure to the organophosphate insecticide chlorpyrifos enhances brain oxidative stress and prostaglandin E2 synthesis in a mouse model of idiopathic autism. *J Neuroinflammation*. 2016;13(1):149. doi:10.1186/s12974-016-0617-4
58. Pallotta M, Ronca R, Carotenuto R, et al. Specific Effects of Chronic Dietary Exposure to Chlorpyrifos on Brain Gene Expression—A Mouse Study. *Int J Mol Sci*. 2017;18(11):2467. doi:10.3390/ijms18112467
59. Lesseur C, Kaur K, Kelly SD, et al. Effects of prenatal pesticide exposure on the fetal brain and placenta transcriptomes in a rodent model. *Toxicology*. 2023;490:153498. doi:10.1016/j.tox.2023.153498

60. Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. *Placenta*. 2005;26(5):372-379. doi:10.1016/j.placenta.2004.07.003