Epigenetic biomarker for prenatal fipronil exposure and health outcomes in newborns

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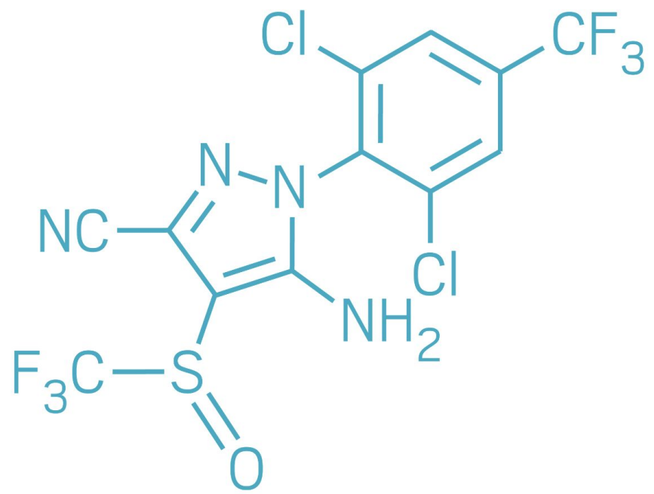
Fipronil



- A broad-spectrum phenylpyrazole insecticide

- Acts as a noncompetitive blocker of GABA-gated chloride channels in the central nervous system
  - ✔ Selective toxicity toward insect GABA receptors

- A widely used in many settings, including agricultural, commercial, industrial, and residential applications
  - ✔ Approx. 10% of the global pesticide market
  - ✔ A widespread environmental contaminant

Health effects of Fipronil

- Undesirable effects on non-target organisms and concern about the potential for adverse health effects in humans

- Several *in vitro* and animal studies
  - ✔ Cytotoxicity or cell death in human cell lines
  - ✔ Disturbed behavior
  - ✔ Thyroid, endocrine, or reproductive dysfunction in rats

- Human
  - ✔ Acute poisoning -- headache, dizziness, sweating, nausea, vomiting, agitation, and seizures

- Classification
  - ✔ A possible human carcinogen (EPA)
  - ✔ A Class II moderately hazardous pesticide (WHO)

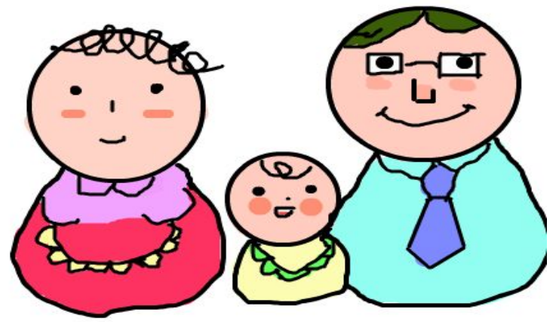
Fipronil-contaminated eggs

- In 2017, fipronil-contaminated eggs were distributed to several European and Asian countries (i.e., Germany, Switzerland, France, Hong Kong, and South Korea)
  - ✔ Fipronil-contaminated eggs may have been consumed for a long time before the discovery
  - ✔ Exposure levels may have exceeded the threshold, posing a threat to human health
- Limited human data regarding the distribution of fipronil and its health effects after chronic exposure

Why *in utero* exposure is important?

- Most sensitive and vulnerable population
- Early life exposure can be programed and affects health in offspring
- Limited data on risk assessment of prenatal fipronil exposure

Previous publication



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Distribution of fipronil in humans, and adverse health outcomes of *in utero* fipronil sulfone exposure in newborns

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ABSTRACT

Fipronil is a highly effective insecticide with extensive usages; however, its distribution and toxic/health effects in the human population after chronic exposure have not yet been clearly identified. Our objectives were to determine the levels of serum fipronil and fipronil sulfone, a primary fipronil metabolite, in a general and sensitive human population using a birth cohort of parent-infant triads in Korea. We further investigated whether *in utero* exposure to fipronil and fipronil sulfone can affect health outcomes in newborn infants.

Blood and umbilical cord blood from 169 participants, 59 mother-neonate pairs and 51 matching biological fathers, were collected; serum fipronil and fipronil sulfone (both blood and cord blood) and serum thyroid hormones (cord blood) were measured. Demographic, physiological, behavioral, clinical, and socioeconomic data for each participant were collected via a one-on-one interview and a questionnaire survey.

Fipronil sulfone was detected in the serum of mothers, fathers, and infantile cord blood, while fipronil itself was not. Maternal fipronil sulfone levels were correlated to those of matched biological fathers and newborn infants. Adjusted analyses identified significant associations between parental fipronil sulfone levels and household income. Infantile fipronil sulfone levels were significantly associated with both maternal and paternal levels as well as maternal pre-pregnant BMI. Furthermore, infantile fipronil sulfone levels were inversely associated with cord blood T3 and free T3 levels as well as 5-min Apgar scores of newborn infants.

Serum fipronil sulfone was detected in a specific population of mother-neonate pairs and their matched biological fathers in a manner suggestive of regular exposure to fipronil among urban residents. The findings also suggest that serum fipronil sulfone placentally transfers to the fetus and affects infantile adverse health outcomes. This is a first of its kind study; therefore, future studies are warranted.

- Serum fipronil sulfone was detected in general and sensitive populations
- Fipronil sulfone placentally transfers to the fetus
- *In utero* exposure to fipronil sulfone affects adverse health outcomes in newborns (i.e., Apgar score at 5 minutes and thyroid hormones' levels)

Aim and hypothesis

- Key mechanisms underlying *in utero* fipronil exposure and thyroid dysfunction in newborns are unclear

- Environmental factors can regulate epigenetic marks as well as serve as triggers for disease

  ✔ *In utero* -- most crucial time when epigenetic factors are most important and susceptible to change

  ✔ Use of epigenetics as biomarkers to better understand the early-stage biological response and molecular mechanisms by which environmental exposures to fipronil lead to thyroid dysfunction in newborns

- Hypothesis -- exposure to *in utero* fipronil will result in epigenetic alterations that are associated with an increased risk of thyroid dysfunction and birth outcomes in newborns

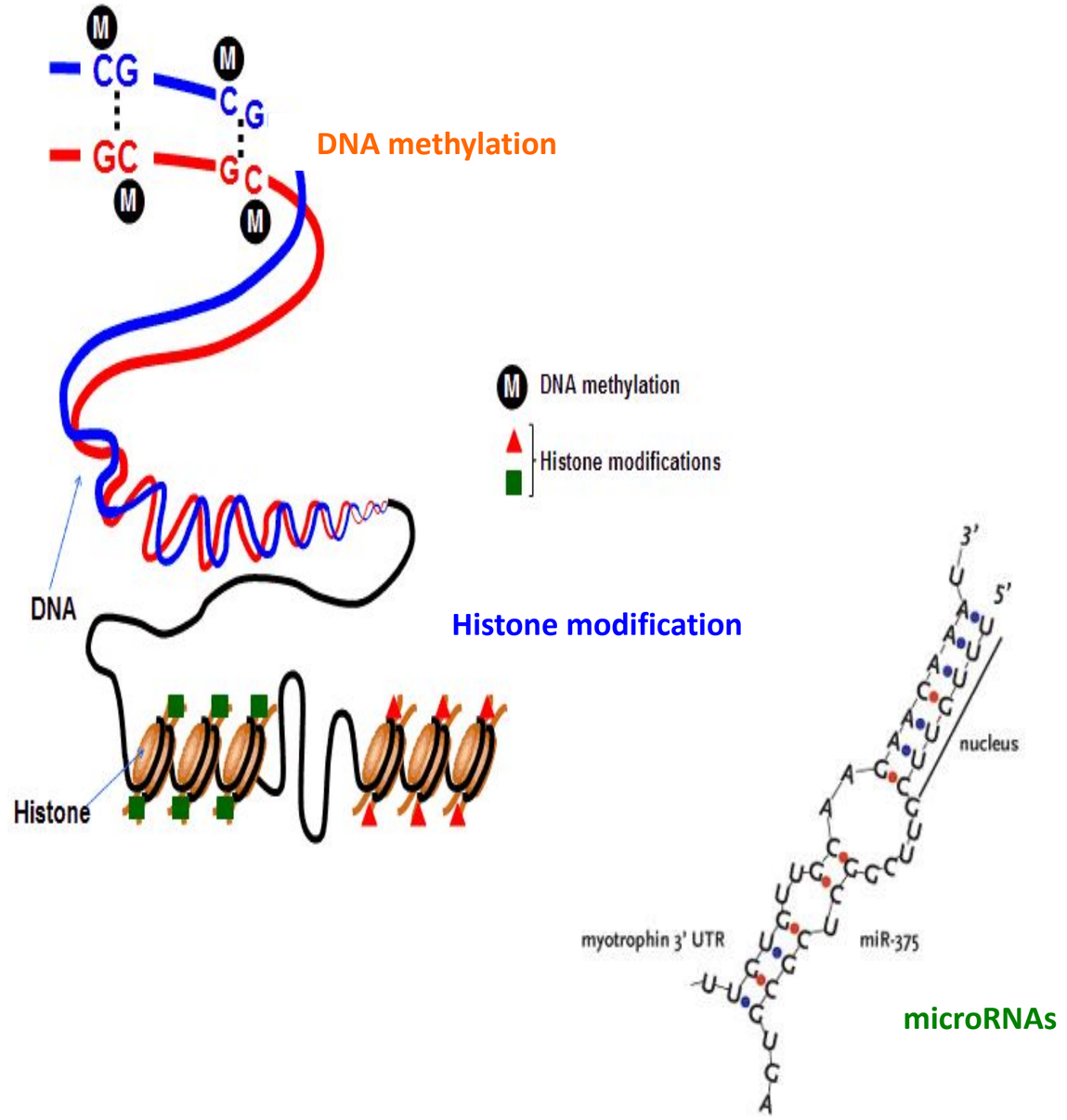
Epigenetic programming

- *epi* - Greek: “upon” or “above”

- 1942 - Conrad Waddington

  ✔ The branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being

- Heritable, but reversible, changes in gene expression without DNA sequence change.



http://www.ncc.go.jp/en/nccri/divisions/14carc/14carc01.html

Methods

- Demographic, physiological, behavioral, and socioeconomic data
- Fipronil exposure data
- Infantile birth outcomes and thyroid hormone levels

39 Maternal Fipronil sulfone levels in serum

miRNAs profiling in 39 placenta RNAs (800miRNAs using Nanostring)

Thyroid hormones in cord blood and birth outcomes in 39 newborns

Identification of specific miRNAs

Prediction of Target genes

Analysis of molecular network and functional pathway

Table 1. Characteristics of the study population

Variable	Mean ± SD (range)	Number (%)
Maternal characteristics (N = 39)		
Fipronil sulfone level (ng/mL) [a]	0.87 ± 0.49 (0.23 – 2.91)	
Age at delivery (years)	31.33 ± 3.16 (26 – 41)	
Pre-pregnant BMI (kg/m^2)	21.40 ± 3.705 (16.61 – 32.19)	
Parity		
Primipara		23 (58.97)
Multipara		16 (41.03)
Second-hand smoking status		
No		27 (69.23)
Yes		12 (30.77)
Education		
< University		5 (12.82)
≥ University		34 (87.18)
Household income (KRW/month) [b]		
< 3,000,000		22 (56.41)
≥ 3,000,000		17 (43.59)
Infants' characteristics (N = 39)		
Fipronil sulfone level (ng/mL) [c]	0.59 ± 0.31 (0.25 – 1.75)	
Sex		
Male		21 (53.85)
Female		18 (46.15)
Gestational age (weeks)	37.18 ± 2.75 (30.6– 40.7)	
Birth weight (g)	2905.38 ± 582.81 (1710 – 3880)	
Birth length (cm)	48.71 ± 3.04 (41.5 – 53.5)	
Head circumference (cm)	33.29 ± 2.15 (28.5 – 36.5)	
Ponderal index (g/cm^3) [d]	2.48 ± 0.16 (2.16 – 2.79)	
Apgar score at 5-minute	9.15 ± 0.594 (8 – 10)	
Thyroid hormones		
T3 (ng/mL) [e]	0.57 ± 0.08 (0.41 – 0.76)	
T4 (ug/dL) [f]	7.79 ± 1.25 (5.55 – 10.34)	
Free T3 (ng/dL) [g]	0.12 ± 0.02 (0.08 – 0.16)	
Free T4 (ng/dL) [h]	1.26 ± 0.16 (0.99 – 1.58)	
TSH (uIU/mL) [i]	11.73 ± 7.58 (2.97 – 40.55)	

[a] Level measured in serum, geometric mean ± geometric standard deviation
[b] Thousand South Korean Won (KRW) is approximately equivalent to U$ 1.00
[c] Level measured in cord blood, geometric mean ± geometric standard deviation
[d] Ponderal index, birth weight (gram) divided by third power of body length (centimeter), then multiplied by 100.
[e] T3, Triiodothyronine; [f] T4, Thyroxine; [g] Free T3, Free triiodothyronine; [h] FreeT4, Free Thyroxine
[i] TSH, Thyroid-stimulating hormone

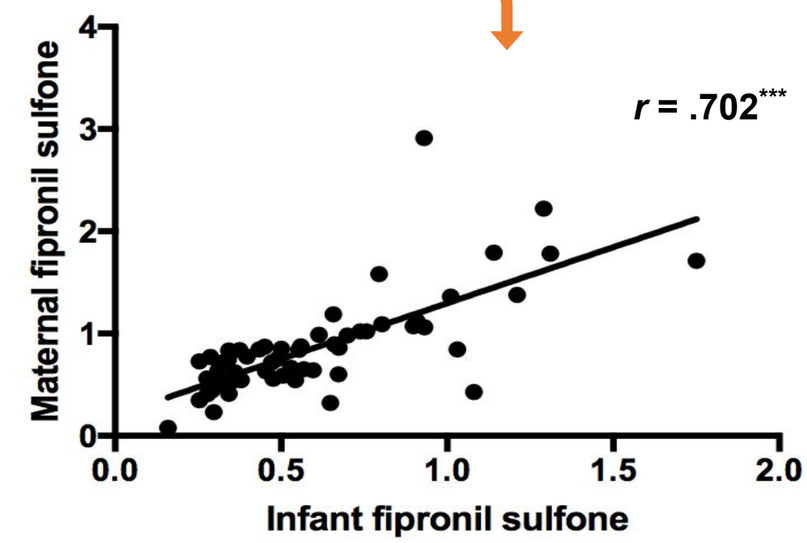


Figure 1. Correlation between maternal fipronil sulfone level and infantile fipronil sulfone level

Table 2. Association between maternal fipronil sulfone level and birth outcomes in newborns

	Maternal fipronil sulfone			
	β	Lower CI	Upper CI	p-Value
Birth weight (g)	0.094	-310.024	529.398	0.598
Birth length (cm)	0.189	-1.034	3.339	0.291
Head circumference (cm)	0.096	-1.170	1.997	0.599
Ponderal index (g/cm^3) [a]	-0.251	-0.196	0.033	0.158
Apgar score at 5-minute	0.148	-0.240	0.590	0.857
Thyroid hormones				
T3 (ng/mL) [b]	**-0.331**	**-0.108**	**0.002**	**0.057**
T4 (ug/dL) [c]	-0.100	-1.152	0.656	0.580
Free T3 (ng/dL) [d]	**-0.399**	**-0.028**	**-0.003**	**0.014**
Free T4 (ng/dL) [e]	-0.050	-0.129	0.096	0.767
TSH (uIU/mL) [f]	-0.069	-6.731	4.650	0.712

β and *p*-value were obtained after adjustment for maternal age, parity, Pre-pregnant BMI, and house-hold income.

Table 3. List of miRNAs associated with maternal fipronil sulfone exposure

- MicroRNAs are arranged in descending order based on beta values.
- β and *p*-value were obtained after adjustment for maternal age, parity, Pre-pregnant BMI, and house-hold income.

miRNAs	β	p-Value
hsa-miR-1307-3p	0.664	0.000
hsa-miR-1203	0.663	0.000
hsa-miR-941	0.663	0.000
hsa-miR-367-3p	0.663	0.000
hsa-miR-3613-3p	0.638	0.000
hsa-miR-3136-5p	0.634	0.000
hsa-miR-410-3p	0.586	0.000
hsa-miR-4455	0.585	0.000
hsa-miR-873-3p	0.580	0.000
hsa-miR-346	0.578	0.001
hsa-miR-6721-5p	0.569	0.001
hsa-miR-1915-3p	0.563	0.000
hsa-miR-1306-3p	0.562	0.000
hsa-miR-329-3p	0.554	0.001
hsa-miR-656-3p	0.536	0.002
hsa-miR-128-1-5p	0.514	0.002
hsa-miR-323a-3p	0.475	0.006
hsa-miR-575	0.442	0.011
hsa-miR-548g-3p	0.435	0.009
hsa-miR-660-5p	0.428	0.015
hsa-miR-1973	0.423	0.012
hsa-miR-499a-5p	0.414	0.013
hsa-miR-218-5p	0.410	0.019
hsa-miR-638	0.406	0.019
hsa-miR-130a-3p	0.377	0.026
hsa-miR-590-5p	0.373	0.031
hsa-miR-4532	0.372	0.022
hsa-miR-363-5p	0.369	0.035
hsa-miR-1-3p	0.365	0.037
hsa-miR-1255b-5p	0.356	0.034
hsa-miR-3180	0.355	0.047
hsa-miR-605-5p	0.355	0.040
hsa-miR-222-3p	0.354	0.048
hsa-miR-320e	0.353	0.037
hsa-miR-129-2-3p	0.352	0.042
hsa-miR-143-3p	0.347	0.049
hsa-miR-188-5p	0.344	0.035
hsa-miR-513c-5p	0.342	0.037
hsa-miR-4516	0.342	0.047

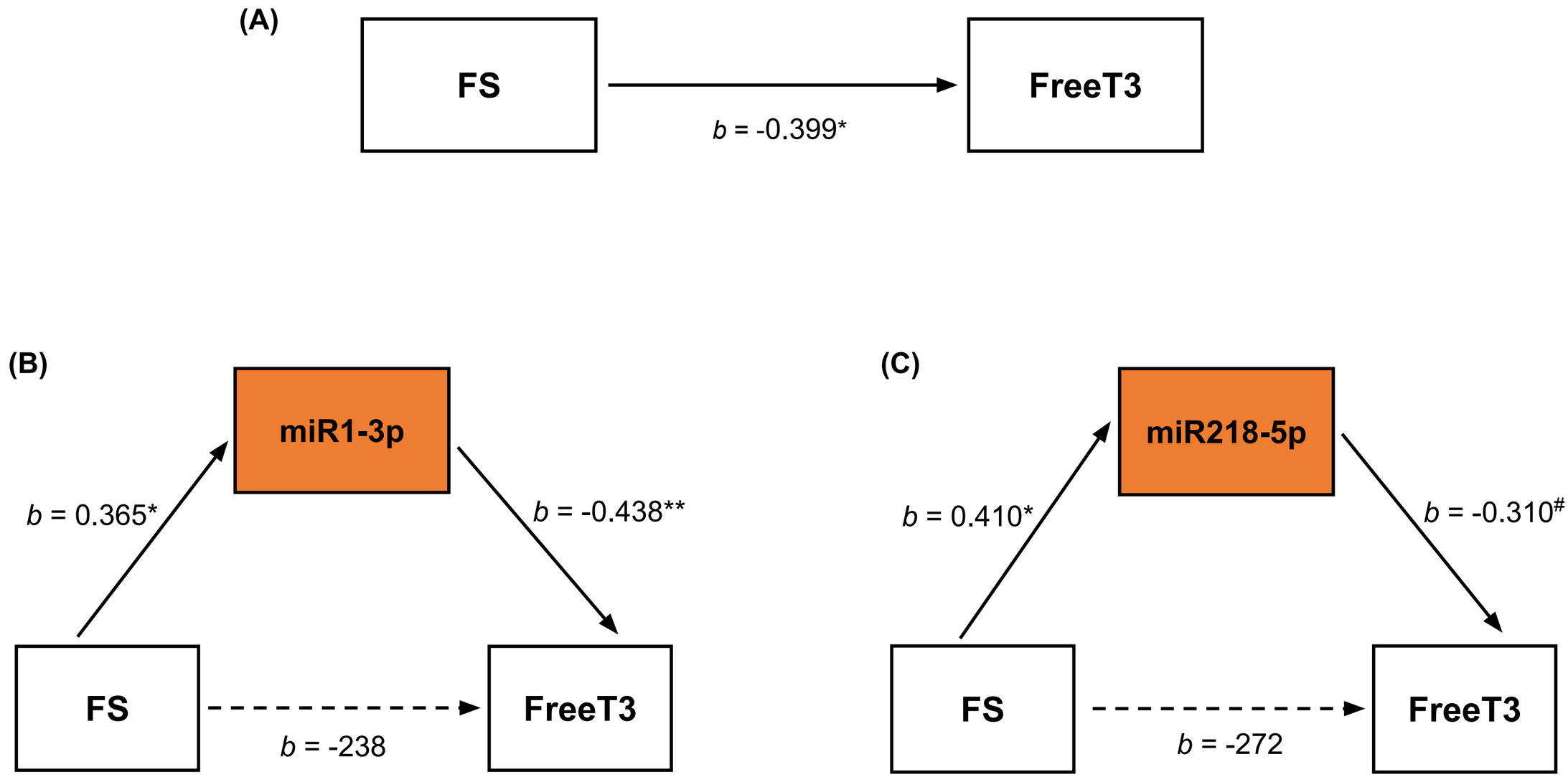
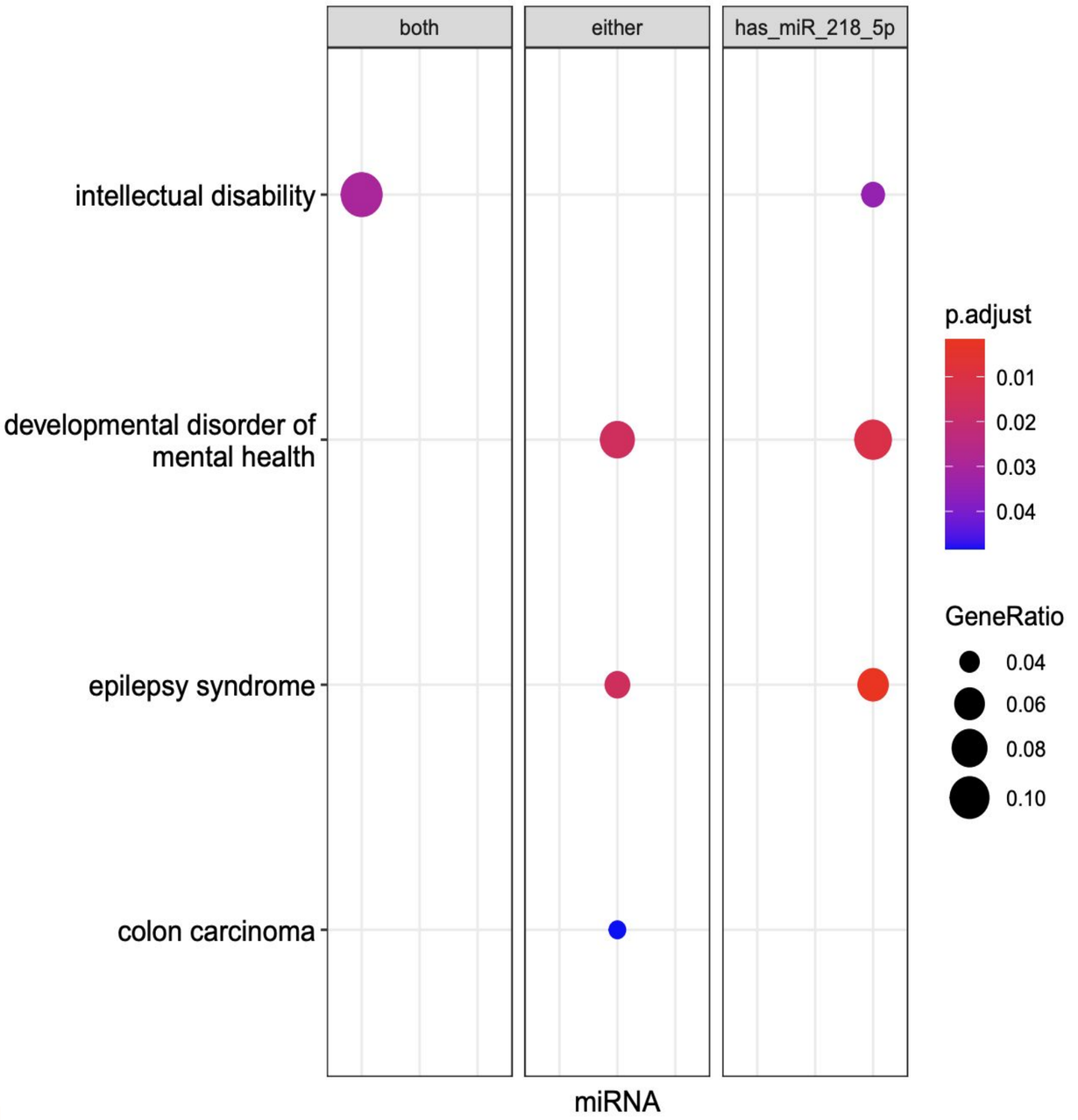


Figure 2. Causal relations from maternal fipronil sulfone to Free T3 hormone (A) and from maternal fipronil sulfone through upregulation of miR1-3p/miR218-5p to Free T3 hormone(B and C). Arrows connecting one variable to another represent unstandardized regression coefficients. *P < 0.05; **P < 0.01; #P < 0.06.

Disease Ontology Enrichment



Wiki Pathway Enrichment

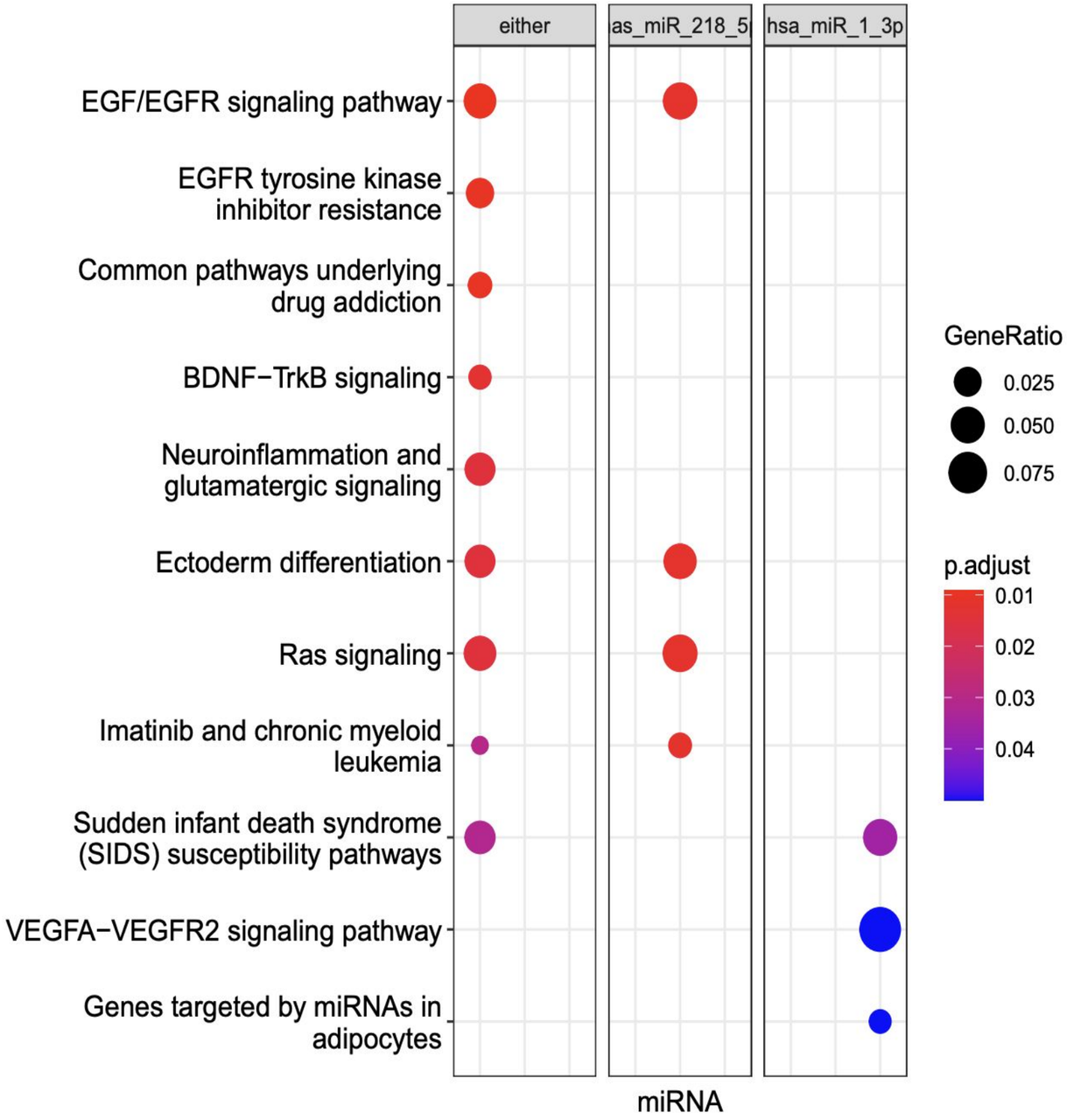
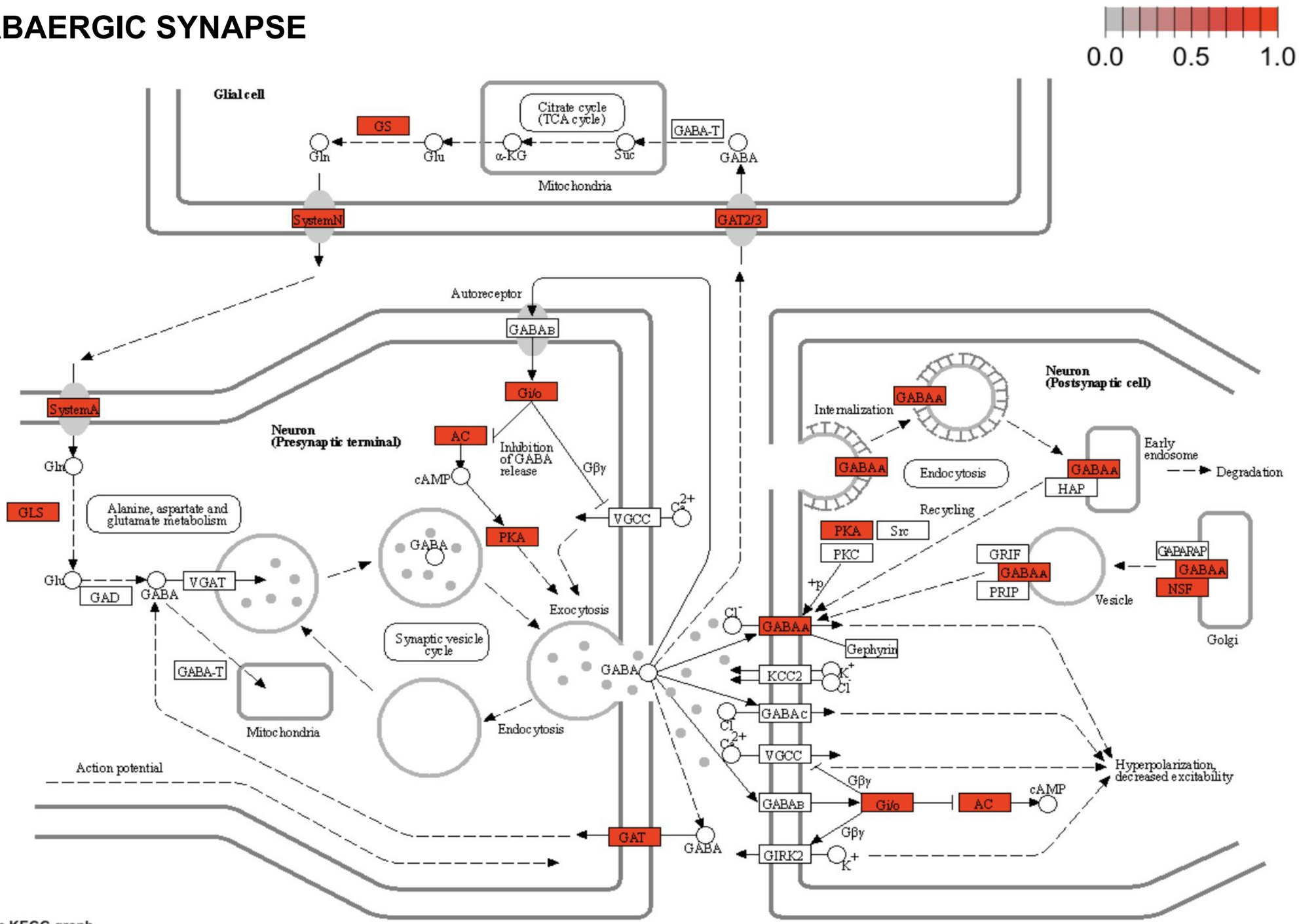


Figure 3. Pathway analyses. Disease ontology enrichment (left) and wiki pathway enrichment (right) from target genes. Enrichment analyses were done with an adjusted p-vaule cutoff of 0.05 using the Benjamini-Hochberg correction for multiple comparisons

GABAERGIC SYNAPSE



Data on KEGG graph
Rendered by Pathview

Figure 4. The functional networks showed interactions of the target mRNAs in GABAergic synapse pathway.

THYROID HORMONE SYNTHESIS

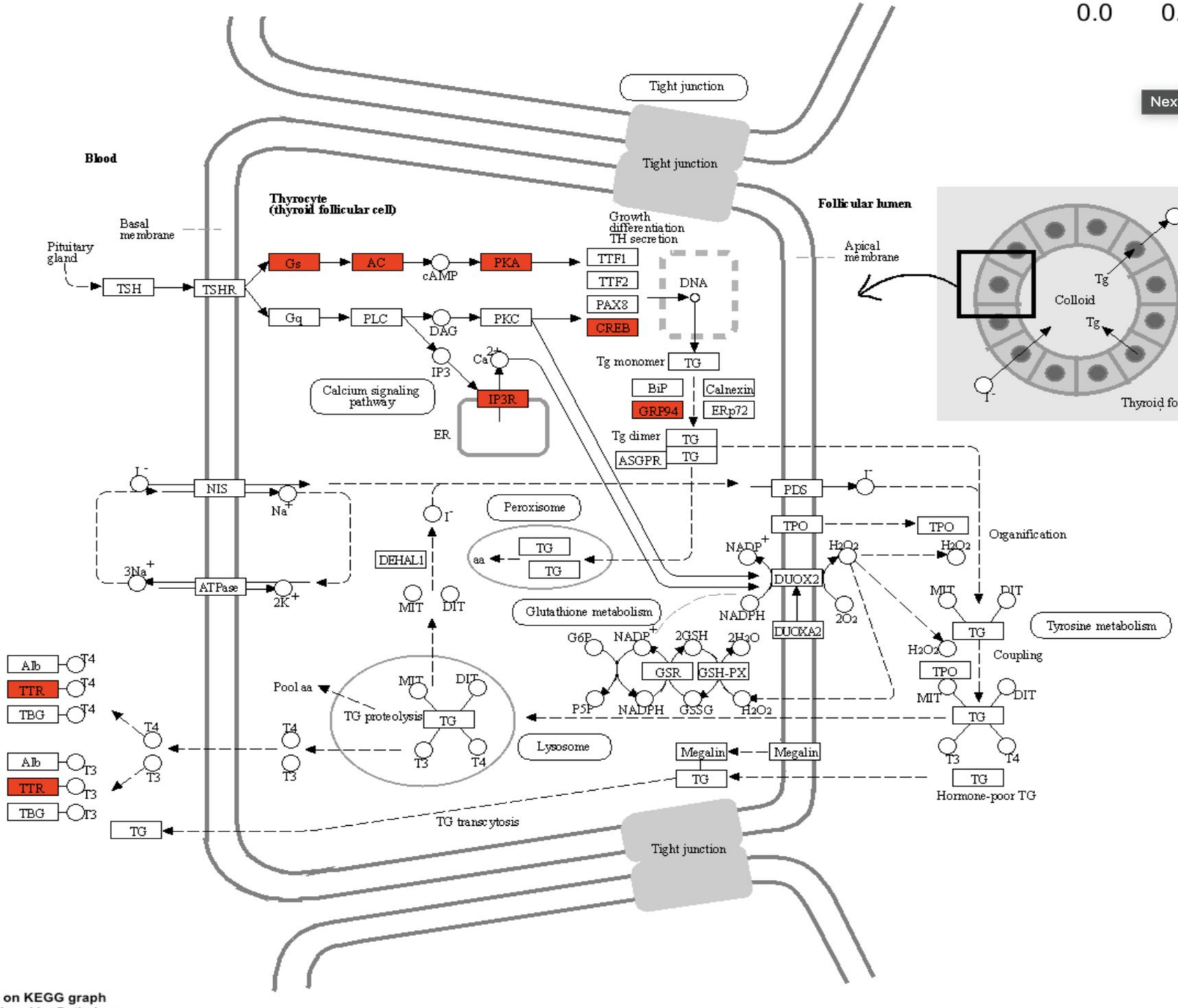


Figure 5. The functional networks showed interactions of the target mRNAs in thyroid hormone synthesis pathway

Summary

- *In utero* exposure to fipronil affects the decrement of thyroid hormones (T3 and Free T3) in the cord blood of newborns

- Upregulation of **miR1-3p** and **miR 218-5p** acts as a significant contributor to Free T3 decrease in newborns by prenatal exposure to fipronil

- Both miR1-3p and miR218-5p share 124 target genes, which interact **GABAergic synapse pathway** and **Thyroid secretion pathway**

- Our results suggest that epigenetic biomarkers for prenatal fipronil exposure and thyroid dysfunction provide mechanistic data to explain the Free T3 decrease in newborns in response to early-life fipronil exposure

  ✔ Further studies into the potential for miRNAs as informative biomarkers using a larger sample size are warranted.

QUESTIONS?