Nutrient-sensitive placental gene network dysregulation is associated with spina bifida

connorlab.ca

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BACKGROUND

- Maternal undernutrition in pregnancy is a leading global health burden, causing:

More than 1 in 10 child deaths globally

Poorer development & health outcomes in offspring

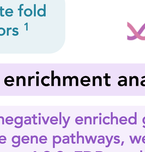
- Maternal nutrient deficiencies can lead to fetal anomalies, like spina bifida (SB)

SB affects 1 per 1000 livebirths globally

SB → increased risk of preterm birth & fetal growth restriction

- 75% of SB cases are multifactorial in origin. Known contributing factors include:

Nutrient deficiency, namely vitamins B9 (folate) & B12



Genetic variants, immune dysregulation, diabetes & medications

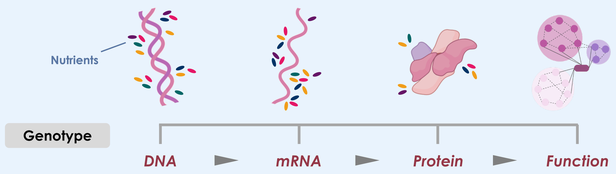
OUR FOCUS

- **Nutrient-gene interactions are poorly defined in spina bifida**

Nutrients are essential for DNA stability & repair

Host genetics influence nutrient metabolism & bioavailability

Nutrients influence gene expression & protein function



- Understanding the degree to which SB-associated genetic signatures are explained or influenced by nutrient-gene interactions could provide insights into novel, nutrient-sensitive mechanisms that underly SB disease processes

HYPOTHESIS & AIMS

- We **aimed** to identify nutrient-dependent & -interacting placental genes, pathways, and gene regulatory networks that associate with SB
- We **hypothesised** that gene signatures in placentae from fetuses with isolated SB would have multiple nutrient-gene interactions

METHODS

- Placental transcriptome for fetuses with isolated SB (cases; n=12) & fetuses with no congenital anomalies (controls; n=22) were sequenced (Clariom D™ microarray)

Differentially expressed genes (DEGs) nutrient-cofactor analysis

DEGs were identified (eBayes; FDR *q* value<0.05, absolute fold change [FC] ≥2) & screened for having nutrient-cofactors [1]

Nutrient-sensitive geneset enrichment analysis

Positively & negatively enriched gene pathways, including nutrient-sensitive gene pathways, were identified in cases (GSEA v4.2.3; FDR *q* value <0.05)

Nutrient-sensitive miRNA-DEG targetome analysis

Nutrient-sensitive miRNAs that were differentially expressed in cases were identified & used to construct miRNA-DEG targetome networks (miRWalk2.0)

Nutrient-sensitive transcription factor (TF)-DEG regulatory network analysis

TFs with nutrient cofactors that are predicted to regulate DEGs were identified (iRegulon v1.3; DNA motifs/tracks 20kb centered around transcription start site, normalised enrichment score >3) [1]

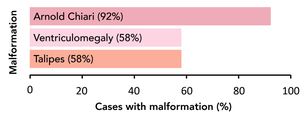
RESULTS

Maternal cohort characteristics

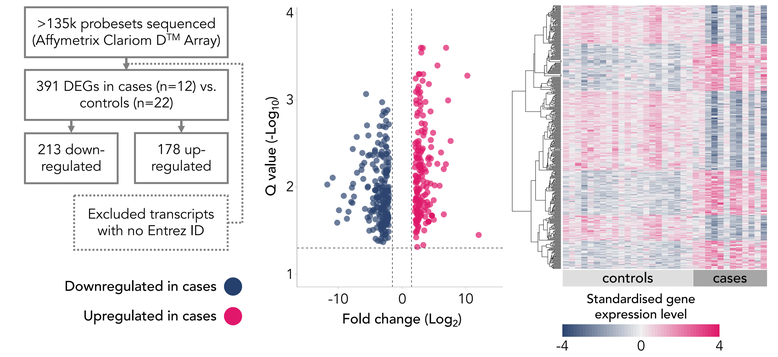
Maternal characteristics	Control (n=22)	Case (n=12)	p value
Age (years)	33 (30–35)	30 (26 – 33)	NS
Ethnicity/race (n [%])			NS
Asian	3 (13.6)	2 (16.7)	
Black	5 (22.7)	0	
Latin/Hispanic	3 (13.6)	0	
White	11 (50)	9 (75)	
Other	0	1 (8.3)	
Pre-pregnancy BMI classified (n [%])			NS
Underweight (<18.5 kg/m²)	1 (4.6)	1 (8.3)	
Normal weight (18.5–24.9 kg/m²)	14 (63.6)	7 (58.3)	
Overweight (25.0–29.9 kg/m²)	6 (27.3)	3 (25)	
Obese (≥30.0 kg/m²)	1 (4.6)	1 (8.3)	

SB starting level

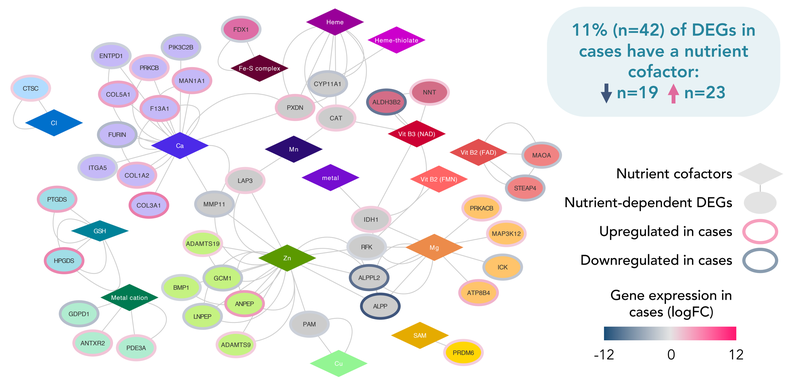
SB-associated malformations



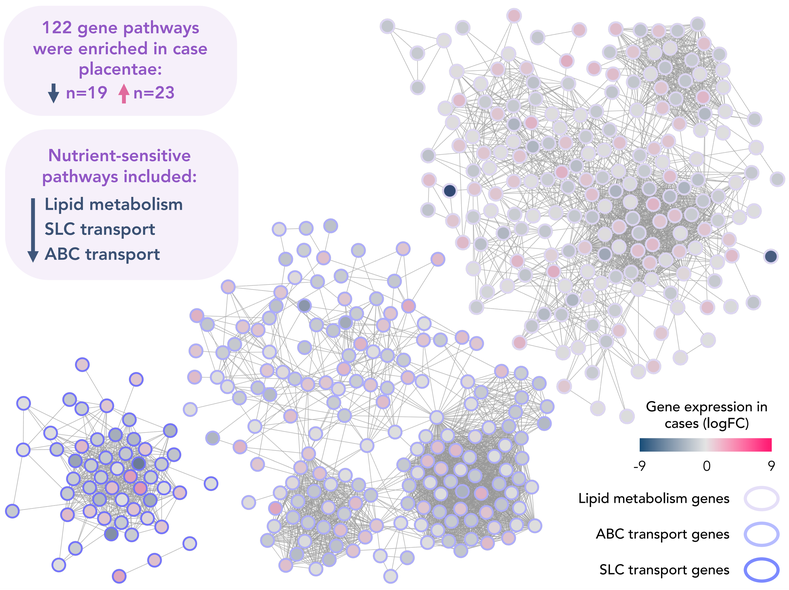
Placental gene expression was different in cases compared to controls



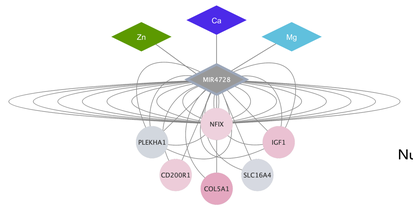
Differentially expressed genes in cases were nutrient-sensitive



Nutrient-sensitive gene pathways were downregulated in cases

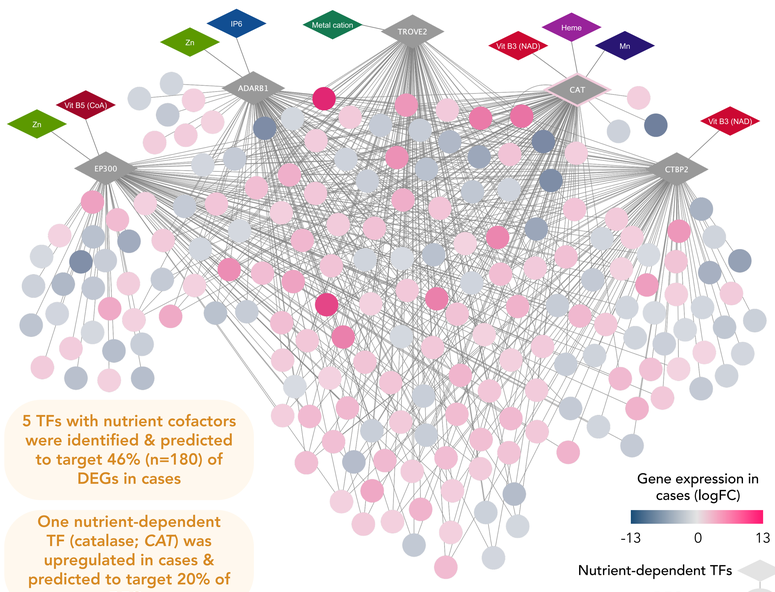


A nutrient-sensitive miRNA that was downregulated in cases targets DEGs



One nutrient-sensitive miRNA (*MIR4728*) was downregulated in cases, & was predicted to target 1.5% (n=6) of DEGs in cases

Nutrient-dependent TFs were predicted to regulate DEGs in cases



5 TFs with nutrient cofactors were identified & predicted to target 46% (n=180) of DEGs in cases

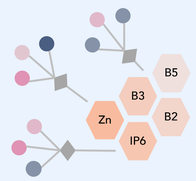
One nutrient-dependent TF (catalase; *CAT*) was upregulated in cases & predicted to target 20% of DEGs

KEY CAVEAT

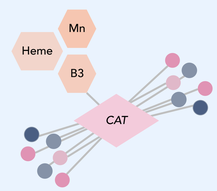
- While all study participants took a daily prenatal vitamin & folate deficiency in the Canadian population is rare, objective measures of maternal nutrient status were not available

SUMMARY & CONCLUSIONS

- We identified multiple dysregulated nutrient-dependent & -interacting genes, gene pathways, & gene regulatory networks in placentae from fetuses with SB, expanding our knowledge of placental function in SB & its comorbidities



Downregulated ABC & SLC transport in case placentae may suggest compromised barrier & nutrient transport function



Dysregulation in several nutrient-sensitive genes & regulatory networks, especially zinc- and B vitamin-sensitive genes

Dysregulation in *CAT* & known gene targets could suggest increased oxidative stress in case placentae

- Moving beyond a folic-acid centric view & integrating genetic & nutritional factors using a network-analysis approach is critical for identifying new targets for NTD prevention & improved health trajectories globally

ACKNOWLEDGEMENTS

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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