



Analyzing the effects of perinatal protein malnutrition on the miRNAome of young adult mice



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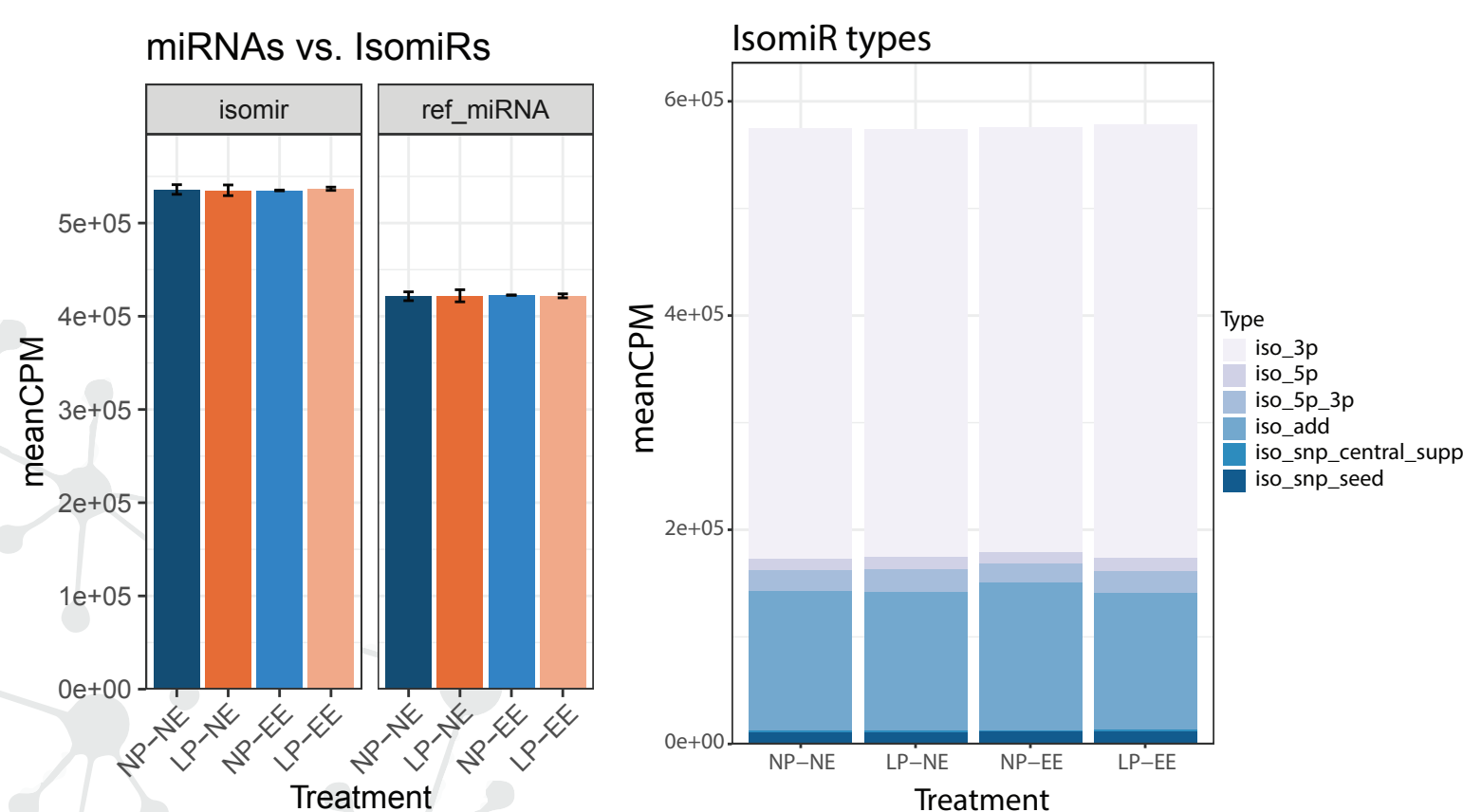
Introduction

Male mice were subjected to perinatal malnourishment and exposed to a postweaning enriched environment. Animals fed perinatally with low-protein food (LP) presented anxiety-like behaviour and increased glucose uptake in the hypothalamus. Anxiety was reversed in those animals exposed to an enriched environment (EE). In this study, we aim to analyze epigenetic mechanisms that might be responsible for the altered phenotype and its reversion.

Methods

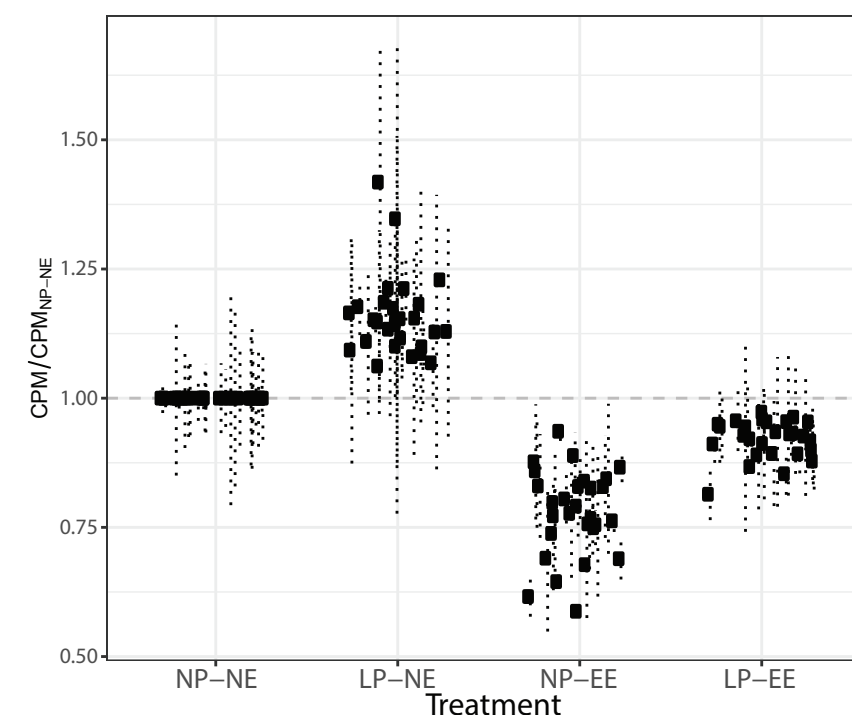
We used 2 samples of each group from P56 young-adult male mice. First, RNA from the hypothalamus was isolated using mirVana™ PARIS™ kit and assessed for quality control by Agilent Bioanalyzer obtaining an RNA Integrity Number (RIN) above 8. Next, TruSeq small RNA libraries were generated from the samples according to Illumina's protocol employing 4 to 5 µg of total RNA. Samples were then labeled and loaded in the corresponding lanes. The total passing filter (PF) reads ranged from 20M to 30 (1 × 50bp). After that, miRNA and isomiR were annotated using miraligner and converted to GFF3-srna format with mirtop.

miRNA and isomiR abundance

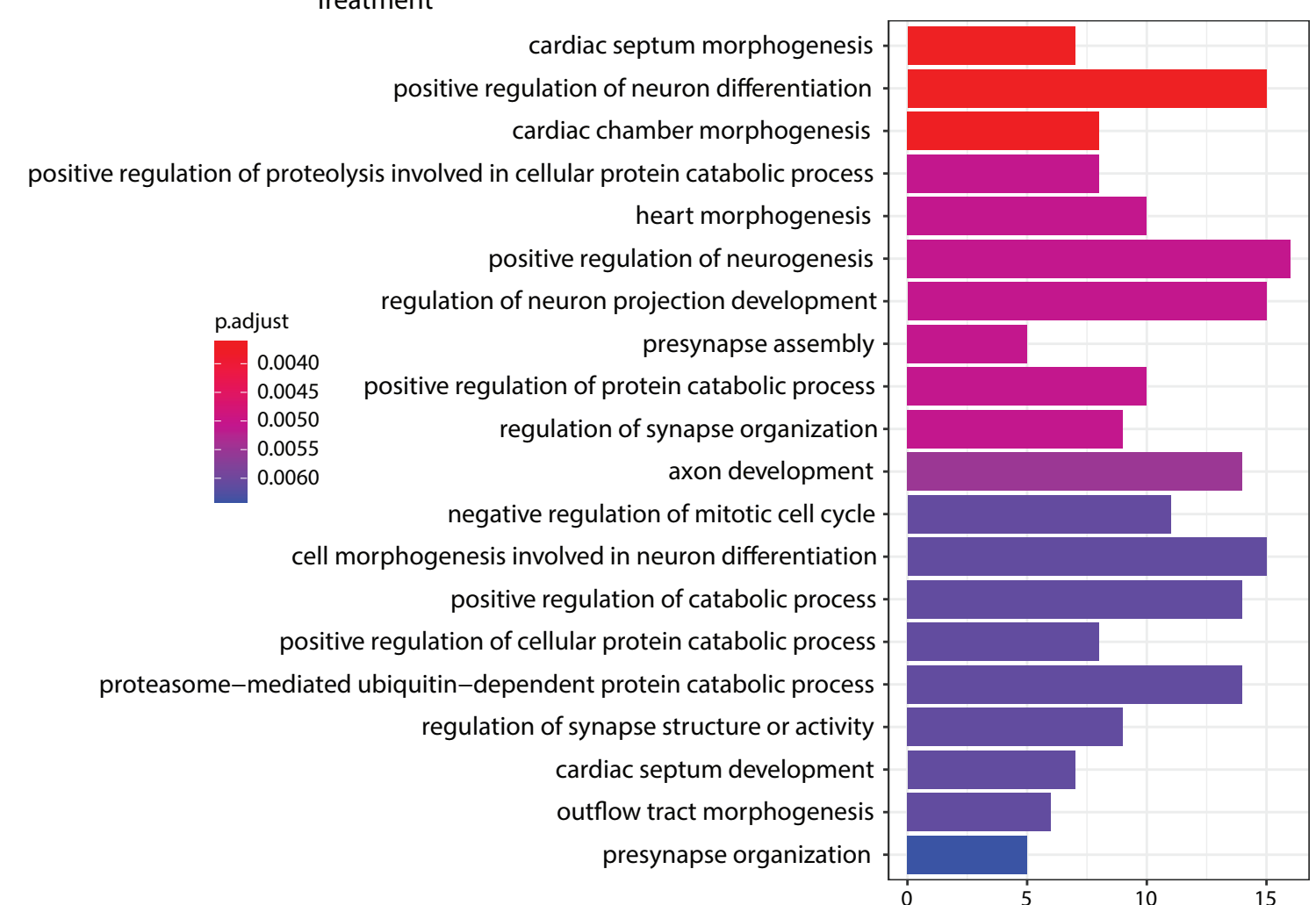


isomiRs are prominently expressed in the hypothalamus of young adult male mice. Not all variants are equally expressed but the variant pattern is similar among treatments. Interestingly, sequence variants within the seed region of the canonical miRNA are conserved between treatments, suggesting that such modifications might not be driven by chance.

small RNAs expression reflects phenotype



When clustering with R Mfuzz package, we find that there is a group of miRNAs and isomiRs whose expression pattern reflects the reversion phenotype observed regarding anxiety and could therefore explain this reversion.



mRNA targets for this set of miRNAs/isomiRs were predicted using MR-microT. Gene ontology and Kegg pathway enrichment analysis of the targets performed with R clusterProfiler package showed that they are enriched in cellular pathways that could explain behavioral differences, e.g. neurogenesis, axon development and synapse organization.

IsomiRs might play an important role in epigenetic regulation in the hypothalamus of young adult male mice accounting alongside canonical miRNAs for the differences seen in anxiety-like behavior of malnourished mice exposed to an enriched environment.