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INTRODUCTION

Schizophrenia (SZ) is a debilitating psychiatric illness affecting approximately 21 million people worldwide (World Health Organization, 2016). Apart from genetic predisposition, early life stress (ELS) is one risk factor associated with SZ. The present study sought to unravel differences in the peripheral small RNAome between SZ patients that did or did not experience ELS.

METHODS

Study subjects: This pilot study includes 47 DSM-IV SZ patients, 24 of which experienced ELS and 23 that did not. ELS was assessed using the German version of the Childhood Trauma Screener (Glaesmer, 2013).

RNA isolation: RNA was isolated using PAXgene blood miRNA kit (Qiagen, USA) from peripheral blood leukocytes. RNA quality was measured by bioanalyzer (Agilent, USA).

Small RNAome sequencing: NEBNext multiplex small RNA library preparation kit was used for sequencing small RNAome where 100 ng RNA was used as starting material followed by adaptor ligation and primer hybridization. First strands of cDNA were generated followed by PCR enrichment. PAGE was run for size selection. For small RNAome ~150 bp band was cut and used for cluster generation.

Small RNAome quality control and alignment: We used an in-house developed pipeline to analyze small RNAome. Quality check and demultiplexing were performed using the CASAVA 1.8.2 software (Illumina, USA). To quantify small RNAome, reads were first mapped to mature micro RNA (miRNA) sequences obtained by miRBase (<http://www.mirbase.org/>) followed by further mapping to other small non-coding RNA sequences (<http://www.ensembl.org/info/data/ftp/index.html>). Reads were then mapped to the hg19 reference genome.

Expression value normalization and adjustment of covariates: The DESeq2 (version 1.6.3) R package was used to calculate differential expression. Raw expression data for library size was normalized by estimateSizeFactors function in the DESeq. For each small non-coding RNA, a ratio is calculated by dividing the read count by the geometric mean across all samples. A scaling factor for each sample is then calculated as the median of this ratio for all small non-coding RNA in the sample. The raw read counts in each sample are then divided by the scaling factor to generate the library size-normalized data followed by the differential expression for small RNAome between ELS and non-ELS samples.

RESULTS

Distribution of small RNAome: We observed no significant difference for small RNAome between groups (p-value=0.99; Figure 1).

Distribution of miRNAs: We observed higher expression of plasma miRNA biomarkers expressed by red blood cells (e.g. miR-486-5p, miR-451a and miR-92a). Normalized read count was similar in both the groups (ELS: mean of normalized reads=13990, median=12; non-ELS: mean of normalized reads=13654, median=12; Figure 2).

Differential expression analysis: By analyzing small RNAome, we identified nine differentially expressed small non-coding RNAs (eight mature miRNA and one piRNA) that satisfied FDR-adjusted p-value ≤ 0.05 , base mean ≥ 100 and fold change ≤ -0.5 or ≥ 0.5 (Figure 3). Five small non-coding RNAs were down-regulated in SZ patients with ELS:

- hsa-miR-29a-3p
- hsa-miR-130a-3p
- hsa_piR_019675
- hsa-miR-223-3p
- hsa-miR-19b-3p

Four miRNAs were up-regulated in SZ patients with ELS:

- hsa-miR-185-3p
- hsa-miR-7706
- hsa-miR-16-2-3p
- hsa-miR-6842-3p

DISCUSSION

To our knowledge, this is the first study to investigate the effects of ELS on small RNAs in SZ patients. We observed nine differentially regulated small RNAs and used the miR2Disease database (<http://www.mir2disease.org/>) to identify known associations of these miRNAs with SZ. The following micro RNAs had previously been associated with SZ:

- hsa-miR-29a-3p: Camkurt et al. (2016); Perkins et al. (2007)
- hsa-miR-130a-3p, hsa-miR-223-3p: Beveridge et al. (2010)
- hsa-miR-185-3p: Stark et al. (2008)
- hsa-miR-16-2-3p: Gardiner et al. (2012)

ELS may thus directly affect pathways implicated in SZ etiology.

Distribution frequency of mapped small RNA class reads

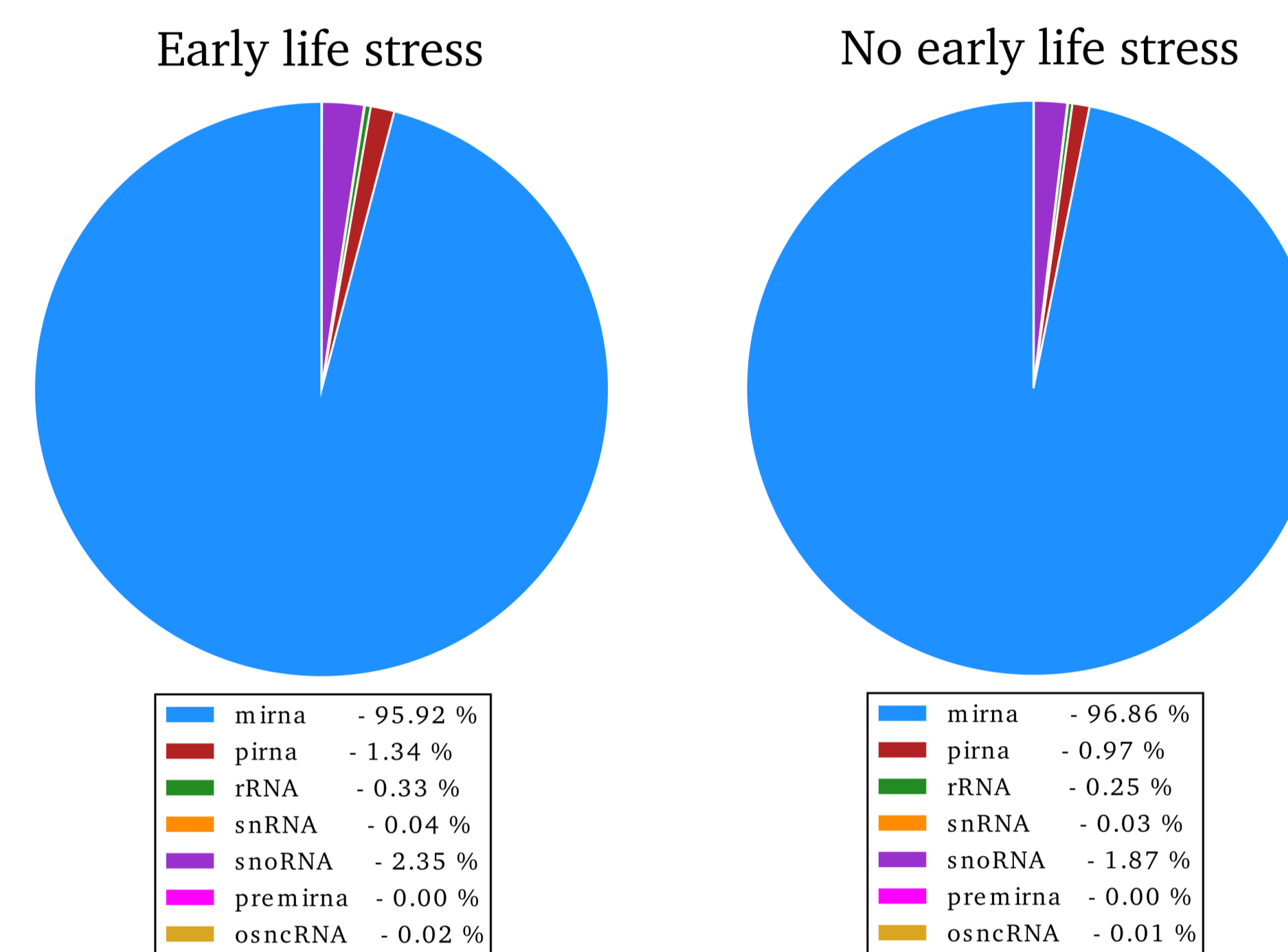


Figure 1
Small RNAome distribution in both samples. Each small non-coding RNA class is represented by different color.

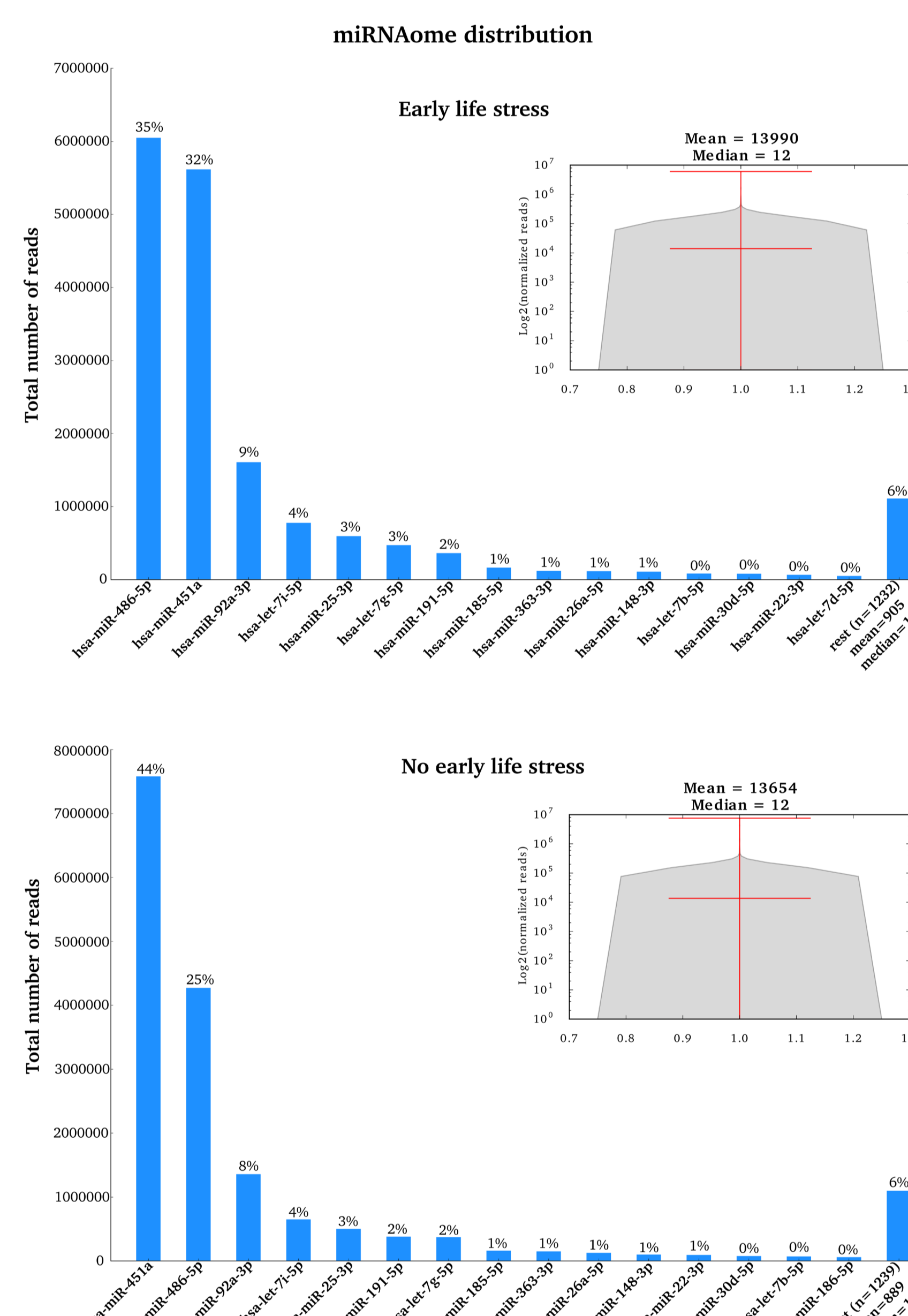


Figure 2
miRNAome distributions in ELS (top) and non-ELS (bottom) samples. Horizontal axes represent miRNAs. Vertical axes represent normalized read counts. Inset images show distributions of all normalized reads as distribution plots. Here, vertical axis represents \log_{10} of normalized reads.

Differentially expressed small non-coding RNAs associated with early life stress

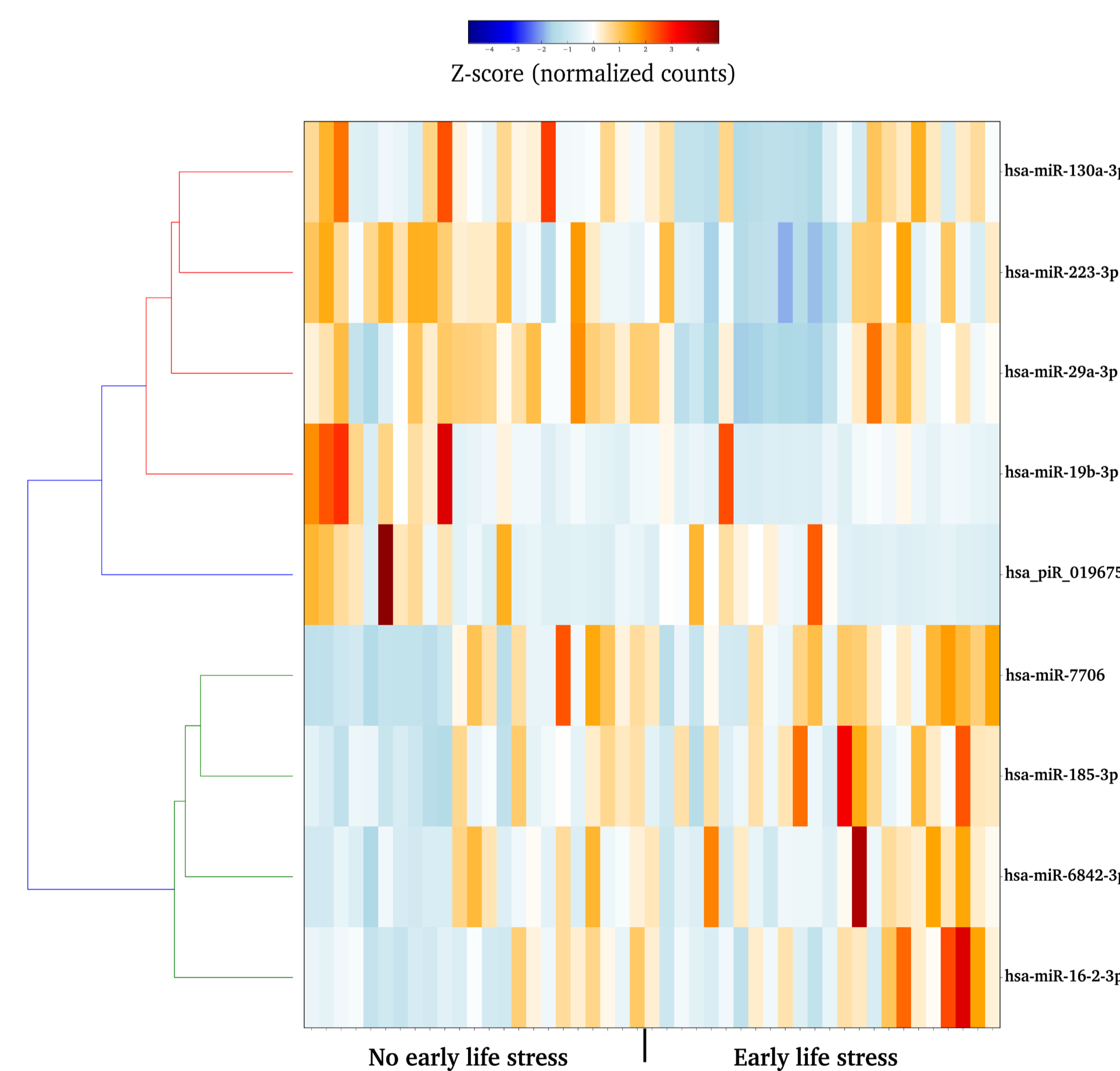


Figure 3
Differentially expressed small non-coding RNAs associated with ELS. Rows: small non-coding RNAs; columns: individual samples. Each cell in the matrix shows the expression level of a small non-coding RNA in an individual sample. Dark-red and dark-blue in cells reflect high and low expression levels, respectively (z score from normalized counts).

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