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Data Article

Transcriptomic dataset of zebrafish tissues following chronic alcohol exposure and withdrawal

Sofia Banu[a], Surabhi Srivastava[a], Arif Mohammed[a,b], Gopal Kushawah[a], Divya Tej Sowpati[a], Rakesh K Mishra[a,*]

[a] CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, Telangana, India
[b] Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia

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ABSTRACT

Alcohol is a psychoactive substance which has detrimental health effects upon consumption. Transcriptome profiling can provide insights into the dynamic changes in global gene expression profiles induced by chronic alcohol exposure and withdrawal. Male and female zebrafish were continually exposed to 0.5% ethanol for a period of 9 weeks. Upon completion of alcohol treatment, the fish were subjected to a withdrawal program for 9 weeks. Brain and liver tissues of control, alcohol exposed and withdrawal fish were isolated and the extracted RNA was sequenced on Illumina HiSeq 2000. The resultant paired end reads were mapped to the zebrafish reference genome (danRer10). The mapped transcripts were quantified for their expression and subjected to differential expression analysis across the three conditions. Gene ontology enrichment analysis of the differentially regulated genes was carried out to identify affected biological processes. The data for this project is available as a GEO dataset under Accession number GSE143416. The gene expression data discussed here accompanies the research article entitled 'Tissue-specific transcriptome recovery on withdrawal from chronic alcohol exposure in zebrafish'.

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* Corresponding author.
E-mail address: mishra@ccmb.res.in (R.K. Mishra).

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Specifications Table

Subject	Biology
Specific subject area	Transcriptomics
Type of data	Table
How data were acquired	High-throughput RNA sequencing using Illumina HiSeq 2000 platform
Data format	Raw, Processed
Parameters for data collection	Total RNA was collected from brain and liver tissues of control, alcohol exposed and withdrawal zebrafish.
Description of data collection	Wild-type (shortfin,'AB' strain) zebrafish consisting of males and females were divided into four groups of 30 fish each i.e. male control, male alcohol-exposed, female control, and female alcohol-exposed. The fish were subjected to continuous 0.5% ethanol exposure for 9 weeks followed by a 9-week withdrawal program without any ethanol in the water tank. RNA was extracted from brain and liver tissue. Samples were pooled according to their respective condition and genders and sequenced. RNAseq was performed on Illumina HiSeq 2000 to obtain paired end libraries of read length 100 bp with at least 25 million reads per sample.
Data source location	Zebrafish (Danio Rerio) maintained at CSIR-Centre for Cellular and Molecular Biology (CSIR-CCMB) zebrafish facility located at Hyderabad, Telangana, India
Data accessibility	Repository name: NCBI Gene Expression Omnibus Data identification number: GSE143416 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143416
Related research article	Sofia Banu, Surabhi Srivastava, Arif Mohammed, Gopal Kushawah, Divya Tej Sowpati, Rakesh K Mishra, Tissue-specific transcriptome recovery on withdrawal from chronic alcohol exposure in zebrafish, Alcohol. In Press. https://doi.org/10.1016/j.alcohol.2020.10.001

Value of the Data

- This data is useful for studying tissue- and gender-specific gene expression changes in transcriptomes associated with long-term alcohol exposure and withdrawal.
- This data is a good resource for researchers studying alcoholism and stress response.
- This data can be used to identify candidate genes and molecular mechanisms associated with tissue damage and recovery from alcoholism as well as in alcohol withdrawal syndrome.

1. Data Description

The dataset contains data obtained through transcriptome sequencing of control, alcohol-exposed and withdrawal zebrafish of both sexes. Brain and liver tissues of these six groups were collected for transcriptome profiling as described below:

1. Control males (CTMB1, CTMB2, CTML1, CTML2)
2. Control females (CTFB1, CTFB2, CTFB3, CTFL1, CTFL2)
3. Alcohol-exposed males (ALMB1, ALMB2, ALML1, ALML2)
4. Alcohol-exposed females (ALFB1, ALFB2, ALFL1, ALFL2)

Table 1
Details of transcriptomic data submitted to the NCBI Sequence Read Archive (SRA).

Sample	Tissue	Condition	Sex	Raw reads (In million)	Uniquely mapped (In million)	% Alignment	SRA Accession
CTMB1	Brain	Control	Male	75.26	66.46	88.31%	SRX7538216
CTMB2	Brain	Control	Male	56.37	49.47	87.77%	SRX7538217
CTFB1	Brain	Control	Female	36.34	30.27	83.31%	SRX7538218
CTFB2	Brain	Control	Female	42.33	36.35	85.88%	SRX7538219
CTFB3	Brain	Control	Female	35.59	31.00	87.12%	SRX7538220
ALMB1	Brain	Alcohol-exposed	Male	33.40	29.58	88.58%	SRX7538221
ALMB2	Brain	Alcohol-exposed	Male	28.86	25.59	88.68%	SRX7538222
ALFB1	Brain	Alcohol-exposed	Female	25.01	22.18	88.68%	SRX7538223
ALFB2	Brain	Alcohol-exposed	Female	28.61	25.11	87.78%	SRX7538224
WDMB1	Brain	Withdrawal	Male	50.33	44.28	87.98%	SRX7538225
WDMB2	Brain	Withdrawal	Male	39.55	32.95	88.32%	SRX7538226
WDMB3	Brain	Withdrawal	Male	32.64	27.00	82.72%	SRX7538227
WDFB1	Brain	Withdrawal	Female	37.35	42.49	88.73%	SRX7538228
WDFB2	Brain	Withdrawal	Female	47.31	22.98	89.81%	SRX7538229
CTML1	Liver	Control	Male	44.92	39.66	88.31%	SRX7538230
CTML2	Liver	Control	Male	48.98	40.90	83.51%	SRX7538231
CTFL1	Liver	Control	Female	35.45	31.32	88.35%	SRX7538232
CTFL2	Liver	Control	Female	41.02	36.57	89.16%	SRX7538233
ALML1	Liver	Alcohol-exposed	Male	30.02	26.95	87.25%	SRX7538234
ALML2	Liver	Alcohol-exposed	Male	29.60	26.84	90.67%	SRX7538235
ALFL1	Liver	Alcohol-exposed	Female	47.81	41.62	87.05%	SRX7538236
ALFL2	Liver	Alcohol-exposed	Female	43.39	38.76	89.33%	SRX7538237
WDML1	Liver	Withdrawal	Male	35.73	26.45	74.02%	SRX7538238
WDML2	Liver	Withdrawal	Male	35.07	28.33	80.77%	SRX7538239
WDFL1	Liver	Withdrawal	Female	44.01	39.31	89.31%	SRX7538240
WDFL2	Liver	Withdrawal	Female	29.89	26.45	88.50%	SRX7538241

5. Withdrawal males (WDMB1, WDMB2, WDMB3, WDML1,WDML2)
6. Withdrawal females (WDFB1, WDFB2, WDFL1, WDFL2)

FASTQ files and TPM value text files were deposited as a GEO dataset and are available under the accession number GSE143416. The sample details of the FASTQ files including sample statistics is described in Table 1. The TPM values corresponding to each sample for 57,918 transcripts identified for brain and liver is also available in the mentioned repository.

RNA samples isolated from brain and liver tissue were pooled according to their respective condition and genders and sequenced. The raw read information and mapping details post alignment with reference genome are shown.

2. Experimental Design, Materials and Methods

2.1. Experimental design

120 naïve wild-type (shortfin, 'AB' strain) zebrafish (*Danio rerio*), consisting of 60 males and 60 females, were split into four groups of 30 fish i.e. male control, female control, male alcohol-exposed and female alcohol-exposed. They were maintained in a 20-L water tank. To induce chronic exposure to alcohol, the fish were transferred into a new tank consisting of 0.5% ethanol every afternoon and remained in the tank for 24 h. The fish were chronically exposed to ethanol in this manner for 9 weeks. The control groups were also subjected to transfers but in ethanol-free tanks. Post completion of the alcohol program, the fish were maintained in an ethanol free holding tank for 9 weeks to induce withdrawal [1,2].

2.2. RNA isolation and sequencing

The zebrafish were anesthetized with Tricaine (Sigma, USA) and brain and liver tissues were isolated. They were dissected and immediately submerged in RNA*Later* (~200 μl in total volume; Sigma) and stored in RNase-free microcentrifuge tubes (Ambion) at -80 °C. Total RNA preparation was carried out using NucleoSpin® RNA kit (Macherey-Nagel, REF # 740955.50) as per manufacturer's protocol. Tissues were separated from RNA*Later* and were homogenized in the kit lysis buffer. Genomic DNA contamination was eliminated using on-column DNase digestion. RNA was eluted from the column using RNase and DNase free water (Sigma). After purification, the quality and quantity of RNA was checked using NanoDrop spectrophotometer and 1% agarose gel electrophoresis.

TruSeq RNA Library Prep Kit (v2 LT, non-stranded) was used for library preparation as per manufacturer's instructions. Whole genome RNAseq was performed on Illumina HiSeq 2000 to obtain paired end libraries of read length 100 $\times$ 2 with at least 25 million reads per sample. Brain and liver tissue from six groups of fish, viz control, alcohol-exposed and withdrawal from both sexes were used for analysis.

2.3. Data analysis

Quality control of the raw reads for brain and liver tissue was performed using FastQC [3]. The reads were mapped to the zebrafish reference genome (danRer10) using the aligner STAR [4]. RSEM [5] was used to obtain TPM values (normalized as Transcripts Per Million) for quantification of transcripts. Read counts were derived using Qualimap [6]. Differential expression analysis amongst the three conditions viz, control, alcohol-exposed and withdrawal was performed based on an empirical Bayesian method using the tool, EBSeq [7]. The significantly differentially expressed transcripts were obtained using a false discovery rate (FDR) cutoff of 0.05. These transcripts were further classified according to their change in regulation status if they crossed a two-fold change threshold i.e upregulated transcripts (F.C >= 2) and downregulated transcripts (F.C < 0.5). These transcripts were annotated to their corresponding genes and the enrichGO module of clusterProfiler [8] was used to carry out gene ontology enrichment analysis.

Ethics Statement

The design and implementation of this work has been approved by a local ethics committee. This study has been approved by the Institutional Animal Ethics Committee (IAEC), chaired by Dr Ghanshyam Swarup, CCMB, September 2015.

CRediT Author Statement

Sofia Banu: Formal analysis, Investigation, Visualization, Writing-Original draft; **Surabhi Srivastava:** Methodology, Project administration, Formal analysis, Writing-Original draft, Writing-Review & Editing; **Arif Mohammed:** Methodology, Investigation; **Gopal Kushawah:** Investigation; **Divya Tej Sowpati:** Conceptualization, Methodology, Supervision, Writing-Review & Editing; **Rakesh K Mishra:** Conceptualization, Supervision, Funding acquisition, Writing-Review & Editing.

Declaration of Competing Interest

None.

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