Data in Brief 35 (2021) 106873

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Transcriptomic data showing differentially expressed genes between Notch3 and Notch4 deleted mice

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ARTICLE INFO

Article history:
Received 6 December 2020
Revised 9 February 2021
Accepted 10 February 2021
Available online 13 February 2021

Keywords:
HIVAN
Chronic kidney disease
Inflammation
Tg26

ABSTRACT

The Notch signaling pathway is an important conserved pathway for normal homeostasis during development. However, targeted deletion of Notch4 (*Notch4*^{d1}) or Notch3 (*Notch3*^{d1}) in mice is not lethal. In fact, both *Notch4*^{d1} and *Notch3*^{d1} mice develop normally and are fertile. Here we present RNA seq analysis of differential gene expression in the kidneys of *Notch4*^{d1} mice versus the *Notch3*^{d1} mice, all on FVB background. Kidneys were collected from *Notch4*^{d1} and *Notch3*^{d1} littermates at 3 months of age. RNA sequencing was carried out. The raw data were analyzed for differential gene expression using a negative binomial generalized linear model in the DeSeq2 software package. We used *P*-value $\leq$0.05 and an absolute fold change of 1.5 or greater to identify top upregulated and downregulated genes in *Notch4*^{d1} mice compared to *Notch3*^{d1} mice. The data provided will indentify targets of Notch3 and Notch4 signaling, specifically in kidney diseases where Notch3 or Notch4 are abberantly or redundantly expressed.

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https://doi.org/10.1016/j.dib.2021.106873

Specifications Table

Subject	Biology
Specific subject area	Nephrology and Notch signaling
Type of data	RNA- sequencing data showing *Notch4*^{d1} verses *Notch3*^{d1} differential regulated genes presented as supplementary data in excel form
How data were acquired	Illumina NovaSeq 6000
Data format	Abundance estimates of raw transcripts (genes) are generated by featureCounts [1]. Normalized gene expression values are presented as median of ratio values generated by the DESeq2 package [2]. Differential renal gene expression estimates of *Notch4*^{d1}mice verses *Notch3*^{d1}mice are calculated using the DESeq2 package [2].
Parameters for data collection	The data collected represents 4 *Notch4*^{d1} mice (2 males and 2 females) and 6 *Notch3*^{d1} mice (3 males and 3 females) at 3 months of age. The kidneys from *Notch4*^{d1} or *Notch3*^{d1} mice appear normal phenotypically or histologically.
Description of data collection	RNA was isolated using trizol from kidneys of *Notch4*^{d1} and *Notch3*^{d1} mice. Genomics core constructed and sequenced libraries as described in material and methods.
Data source location	Department of Internal Medicine, The Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, USA
Data accessibility	Repository name: [Sequence Read Archive (SRA)] Data identification number: [PRJNA580295 and PRJNA680191] Direct URL to data: https://www.ncbi.nlm.nih.gov/sra/PRJNA680191 https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA580295

Value of the Data

- Homozygous *Notch4*^{d1} and *Notch3*^{d1} *mice* are phenotypically normal and have no kidney abnormality. The data herein determines differential gene expression in *Notch4*^{d1} versus *Notch3*^{d1} mice, the data will help determine whether these mice are predisposed to injuries/ repairs.
- These data will be of benefit to researchers who are interested in the role of Notch signaling in different renal diseases.
- These data can be compared to the data from wild type mice that we have recently published [3]. Together, this information can be used to compare renal genes that are altered in wildtype, *Notch4*^{d1} and *Notch3*^{d1} mice.
- The data presented herein identify the underlying genes that are abnormally regulated in *Notch4*^{d1} mice versus the *Notch3*^{d1} mice.
- The data opens avenues for further testing these differential expressed genes for therapeutic options in chronic/ acute kidney diseases.
- The data can be used for discovery of novel targets that are regulated by Notch4 or Notch3 inhibition and adds to the existing knowledge regarding the *Notch4*^{d1} and *Notch3*^{d1} mouse models in an FVB background.

1. Data Description

Notch signaling is activated when Notch receptor binds to the Notch ligand. This results in the release of Notch intracellular domain (NICD), which enters the nucleus and binds to RBPjk protein and activates the downstream targets such as Hes and Hey. Notch signaling is important during development. While Notch1 or Notch2 deletion in mice is lethal, targeted deletion of Notch4 or Notch3 is not. This gives us the opportunity to learn more about the function and targets of Notch3 and Notch4 pathways, both of which are abnormally expressed in many diseases including cancers and kidney diseases [3-9]. The homozygous *Notch4*^{d1} mice exhibit deletion of exon 21 and 22 which encode 186 amino acids of the extracellular domain of the Notch4 protein. This renders the two closest exon 20 and exon 23 out of frame with respect to each other [10]. It was reported that these mice express the extracellular domain of Notch4 [11]. The homozygous *Notch3*^{d1} mice have a deletion of 2.5 kb sequence

encoding the EGF repeats 8 through 12. These mice have been reported to express no extracellular or intracellular domain [12]. We conducted RNA seq analysis in kidney homozygous for *Notch4*^{d1} and *Notch3*^{d1}, both on FVB background. The data comprise of both male and female mice. The *Notch4*^{d1} group consists of 2 males and 2 females ($n=4$). The *Notch3*^{d1} group consists of 3 males and 3 females ($n=6$). The accession codes for these samples deposited in the SRA database for two *Notch4*^{d1} males are, (SRR10362438, SRR10362437), two *Notch4*^{d1} females (SRR10362436, SRR10362435,), three *Notch3*^{d1} males (SRR13107600, SRR13107599, SRR13530986) and three *Notch3*^{d1} females (SRR13107598, SRR13107597, SRR13530987). The differential expression is presented as top downregulated and top upregulated genes in *Notch4*^{d1} versus *Notch3*^{d1} mice and are presented as supplementary data. The list of differentially expressed genes is presented as absolute fold change $\geq$ 1.5, p value $\leq$0.05. We also calculated the false discovery rate (FDR) for each differentially expressed gene (supplementary table).

2. Experimental Design, Materials and Methods

***Notch4*^{d1} and *Notch3*^{d1} mice:** *Notch4*^{d1} and *Notch3*^{d1} mice were raised in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH). Mice were housed under pathogen free conditions in micro-isolator cages on air filtered and ventilated racks. Animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kansas Medical Center (Kansas City, KS). *Notch4*^{d1} and *Notch3*^{d1} mice were bred in pure FVB background. The study comprised 3 month-old kidneys from 2 male and 2 female *Notch4*^{d1} mice and 3 male and 3 female *Notch3*^{d1} mice. Genotyping was performed using polymerase chain reaction [5, 10, 12].

2.1. Sample collection and processing

Notch4^{d1} and *Notch3*^{d1} mice were anesthetized using isoflurane at 3 months of age. Perfusion was performed using ice cold PBS to get rid of blood cells. Kidneys were collected and snap frozen for RNA isolation. Total RNA was isolated using Trizol (Fisher Scientific) and manufacturer's protocol was followed.

RNA was submitted to the Genomics core facility at the University of Kansas Medical Center where Agilent Bioanalyzer 2100 and RNA6000 nano assay kit VII (Agilent Technologies, Santa Clara, CA) were used to determine RNA integrity. RNA-seq libraries were prepared using TruSeq Stranded mRNA kit (Illumina) following manufacturer's instructions. mRNA was prepared from total RNA (500 ng per sample) using oligo-dT magnetic beads. Random hexamer primers and reverse transcriptase were used to synthesize first strand of cDNA. Double stranded cDNA (ds cDNA) was generated by removing the RNA template and synthesizing a replacement strand, incorporating dUTP in place of dTTP. For purifying ds cDNA from second strand, AMPure XP beads were used (Beckman Coulter). cDNAs were first blunted and then poly (A) tail was added to the 3′ends for ligation. Ligation of indexing adaptors was performed and PCR amplification was performed using 15 cycles on suitable DNA fragments.

2.2. RNA-seq data analyses

RNA sequencing was performed in four *Notch4*^{d1} mice (2 females and 2 males) and six *Notch3*^{d1} mice (3 females and 3 males) in an illumina NovaSeq 6000 sequencing machine (Illumina, San Diego, CA) at a strand specific 100 cycle paired-end resolution as described recently [3]. Sequencing generated between 28.0 to 32.1 million reads per sample. The read quality was assessed using the fastQC software [13]. The quality score per sequence measured in the Phred quality scale was on average above 32 for all the samples. Sequenced reads were mapped to the mouse genome (GRCm38.rel97) using STAR software, version 2.6.1c [14]. Between 91% and

96% of the sequenced reads mapped to the reference genome, resulting in 26.5 and 29.3 million mapped reads per sample.

FeatureCounts (version 1.22.1) software was used to calculate transcript abundance estimates [1]. Expression normalization and differential gene expression were calculated using the DESeq2 [2] (version 1.26.0) software and statistically significant differentially expressed genes were identified. Relative Log Expression (RLE) normalization method was used to normalize the RNA composition in each sample in DESeq2. The significance of p-values were adjusted for multiple hypotheses testing by the Benjamini and Hochberg [15] method which established a false discovery rate (FDR) for each gene.

2.3. Accession code

We submitted the raw RNA sequencing data to Sequence Read Archive (SRA) where following identification numbers were assigned: PRJNA580295 and PRJNA680191. Links provided below can be accessed for raw data. https://www.ncbi.nlm.nih.gov/sra/PRJNA680191, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA580295.

2.4. Statistical analysis

As described above, statistically significance of differentially expressed genes between *Notch4*^{d1} and *Notch3*^{d1} mice was carried out using the DESeq2 software package. Genes with an absolute fold difference of 1.5 or greater and *p*- values less than or equal to 0.05 were considered significantly differentially expressed. False discovery rate (FDR) values were further provided.

Ethics Statement

The authors guarantee that the submitted work is original and does not contain any content that can be construed as libelous or as infringing in any way on the copyright of another party. Experiments with mice were carried out in agreement with the ARRIVE guidelines and approved by Institutional Animal Care and Use Commitee (ACUP# 2018–2480).

CRediT Author Statement

Madhulika Sharma: Conceptualization, investigation, methodology, supervision and writing; **Sireesha Yerrathota:** Isolation of kidneys and preparation of RNA for sequencing; **Mackenzie Thornton:** Organizing data and reviewing; **Sumedha Gunewardena:** Software, Validation, writing and reviewing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

The work was supported by the National Institute of Health award: R01DK108433 to MS. We thank Rajni Puri for organizing RNA samples, the genomics core staff members, Clark Bloomer and Rosanne Skinner for preparation of RNA samples, library preparation and sequencing.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.106873.

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