Data in Brief

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

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\textbf{Abstract}

The Notch signaling pathway is an important conserved pathway for normal homeostasis during development. However, targeted deletion of Notch4 (Notch4\textsuperscript{d1}) or Notch3 (Notch3\textsuperscript{d1}) in mice is not lethal. In fact, both Notch4\textsuperscript{d1} and Notch3\textsuperscript{d1} mice develop normally and are fertile. Here we present RNA seq analysis of differential gene expression in the kidneys of Notch4\textsuperscript{d1} mice versus the Notch3\textsuperscript{d1} mice, all on FVB background. Kidneys were collected from Notch4\textsuperscript{d1} and Notch3\textsuperscript{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 ≤ 0.05 and an absolute fold change of 1.5 or greater to identify top upregulated and downregulated genes in Notch4\textsuperscript{d1} mice compared to Notch3\textsuperscript{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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Value of the Data

- Homozygous *Notch4<sup>d1</sup>* and *Notch3<sup>d1</sup>* mice are phenotypically normal and have no kidney abnormality. The data herein determines differential gene expression in *Notch4<sup>d1</sup>* versus *Notch3<sup>d1</sup>* 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<sup>d1</sup>* and *Notch3<sup>d1</sup>* mice.
- The data presented herein identify the underlying genes that are abnormally regulated in *Notch4<sup>d1</sup>* mice versus the *Notch3<sup>d1</sup>* 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<sup>d1</sup>* and *Notch3<sup>d1</sup>* 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 RBPjκ 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<sup>d1</sup>* 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<sup>d1</sup>* mice have a deletion of 2.5 kb sequence
encoding the EGF repeats 8 through 12. These mice have been reported to express no extra-
cellular or intracellular domain [12]. We conducted RNA seq analysis in kidney homozygous for
Notch4\textsuperscript{d1} and Notch3\textsuperscript{d1}, both on FVB background. The data comprise of both male and female
mice. The Notch4\textsuperscript{d1} group consists of 2 males and 2 females (n = 4). The Notch3\textsuperscript{d1} group con-
sists of 3 males and 3 females (n = 6). The accession codes for these samples deposited in the
SRA database for two Notch4\textsuperscript{d1} males are, (SRR10362438, SRR10362437), two Notch4\textsuperscript{d1} females
(SRR10362436, SRR10362435), three Notch3\textsuperscript{d1} males (SRR13107600, SRR13107599, SRR13530986) and three
Notch3\textsuperscript{d1} females (SRR13107598, SRR13107597, SRR13530987). The differential expres-
sion is presented as top downregulated and top upregulated genes in Notch4\textsuperscript{d1} versus Notch3\textsuperscript{d1}
mice and are presented as supplementary data. The list of differentially expressed genes is pre-
sented as absolute fold change ≥ 1.5, p value < 0.05. We also calculated the false discovery rate
(FDR) for each differentially expressed gene (supplementary table).

2. Experimental Design, Materials and Methods

Notch4\textsuperscript{d1} and Notch3\textsuperscript{d1} mice: Notch4\textsuperscript{d1} and Notch3\textsuperscript{d1} mice were raised in accordance with
the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Insti-
tutes 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\textsuperscript{d1} and Notch3\textsuperscript{d1} mice were bred in pure FVB background. The study comprised 3 month-
old kidneys from 2 male and 2 female Notch4\textsuperscript{d1} mice and 3 male and 3 female Notch3\textsuperscript{d1} mice.
Genotyping was performed using polymerase chain reaction [5, 10, 12].

2.1. Sample collection and processing

Notch4\textsuperscript{d1} and Notch3\textsuperscript{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 manufac-
turer’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\textsuperscript{d1} mice (2 females and 2 males) and six
Notch3\textsuperscript{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 re-
cently [3]. Sequencing generated between 28.0 to 32.1 million reads per sample. The read qual-
ity 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/PRJNA580295, https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA580295.

2.4. Statistical analysis

As described above, statistically significance of differentially expressed genes between Notch4d1 and Notch3d1 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 Committee (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.

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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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