

St. Catherine University

From the Selected Works of Katherine Campbell

March, 2016

Mapping Transcriptome Profiles of In Vitro iPSC-derived Cardiac Differentiation to In Utero Heart Development

Xing Li, *Mayo Clinic*

Katherine A. Campbell, *Mayo Clinic*

Sherri M. Biendarra, *Mayo Clinic*

Andre Terzic, *Mayo Clinic*

Timothy J. Nelson, *Mayo Clinic*



This work is licensed under a [Creative Commons CC_BY-NC-ND International License](https://creativecommons.org/licenses/by-nc-nd/4.0/).



Available at: [https://works.bepress.com/katherine-](https://works.bepress.com/katherine-campbell/2/)



Mapping transcriptome profiles of *in vitro* iPSC-derived cardiac differentiation to *in utero* heart development



Xing Li ^{a,b}, Katherine A. Campbell ^{c,d,f}, Sherri M. Biendarra ^c, Andre Terzic ^{c,d,e,g}, Timothy J. Nelson ^{c,d,e,f,h,i,*}

^a Department of Health Sciences Research, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^b Division of Biomedical Statistics and Informatics, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^c Department of Molecular Pharmacology and Experimental Therapeutics, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^d Center for Regenerative Medicine, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^e Division of Cardiovascular Diseases, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^f Division of General Internal Medicine, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^g Department of Medical Genetics, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

^h Center for Transplantation and Clinical Regeneration, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

ⁱ Division of Pediatric Cardiology, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA

ARTICLE INFO

Article history:

Received 15 December 2015

Accepted 28 December 2015

Available online 30 December 2015

Keywords:

Induced pluripotent stem cell

Cardiac development

Nos3 knockout

Disease modeling

Microarray analysis

ABSTRACT

The dataset includes microarray data (Affymetrix Mouse Genome 430 2.0 Array) from WT and *Nos3*^{-/-} mouse embryonic heart ventricular tissues at 14.5 days post coitum (E14.5), induced pluripotent stem cells (iPSCs) derived from WT and *Nos3*^{-/-} mouse tail tip fibroblasts, iPSC-differentiated cardiomyocytes at Day 11, and mouse embryonic stem cells (mESCs) and differentiated cardiomyocytes as positive controls for mouse iPSC differentiation. Both *in utero* (using embryonic heart tissues) and *in vitro* (using iPSCs and differentiated cells) microarray datasets were deposited to the NCBI Gene Expression Omnibus (GEO) database. The deposited data in GEO include raw microarray data, metadata for sample source information, experimental design, sample and data processing, and gene expression matrix. The data are available under GEO Access Number GSE69317 (GSE69315 for tissue sample microarray data, GSE69316 for iPSCs microarray data, <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69317>).

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Specifications

Organism/cell line/tissue	<i>Mus musculus</i> (mouse)/ <i>Nos3</i> ^{-/-} mouse tail tip-derived iPSCs and iPSC-derived cardiomyocytes/ <i>Nos3</i> ^{-/-} (B6.129P2- <i>Nos3tm1unc/J</i> , The Jackson Laboratory, Bar Harbor, ME) and wild type (WT) CD1 (Harlan Laboratories, Indianapolis, IN) mouse heart tissues (left and right ventricular tissues, or LV and RV) at 14.5 days post coitum (E14.5)
Sex	Male mice for iPSCs
Sequencer or array type	GPL1261/[Mouse430_2] Affymetrix Mouse Genome 430 2.0 Array
Data format	Raw microarray data (.CEL files) and computed gene expression matrix using RMA
Experimental factors	WT LV vs. <i>Nos3</i> ^{-/-} LV; WT RV vs. <i>Nos3</i> ^{-/-} RV; WT iPSCs vs. <i>Nos3</i> ^{-/-} iPSCs; WT iPSC-differentiated cardiomyocytes at D11 vs. <i>Nos3</i> ^{-/-} iPSC-differentiated cardiomyocytes
Experimental features	The experiments are designed to compare the transcriptome profiles of embryonic heart tissues (LV and RV) between WT and <i>Nos3</i> ^{-/-} mice (in utero comparison). The transcriptome profiles of iPSCs derived from both WT and <i>Nos3</i> ^{-/-} mice tail tip fibroblasts and their differentiated cardiomyocytes are also compared in this study.

(continued)

Specifications	
Consent	N/A
Sample source location	<i>Nos3</i> ^{-/-} (B6.129P2- <i>Nos3tm1unc/J</i> , The Jackson Laboratory, Bar Harbor, ME) and wild type (WT) CD1 (Harlan Laboratories, Indianapolis, IN)

1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69317>. Series GSE69317.

2. Experimental design, materials and methods

The experimental design of this study is to model the *Nos3*-related cardiac defects using an induced pluripotent stem cell (iPSC) platform and investigate the underlying disease pathogenesis by mapping the transcriptome profiles using tail tip derived iPSCs of wild type (WT) CD1 (Harlan Laboratories, Indianapolis, IN) and *Nos3*^{-/-} (B6.129P2-*Nos3tm1unc/J*, The Jackson Laboratory, Bar Harbor, ME) mice back to the gene expression profiles of corresponding embryonic heart tissues.

* Corresponding author at: Department of Molecular Pharmacology and Experimental Therapeutics, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA.
E-mail address: Nelson.Timothy@mayo.edu (T.J. Nelson).

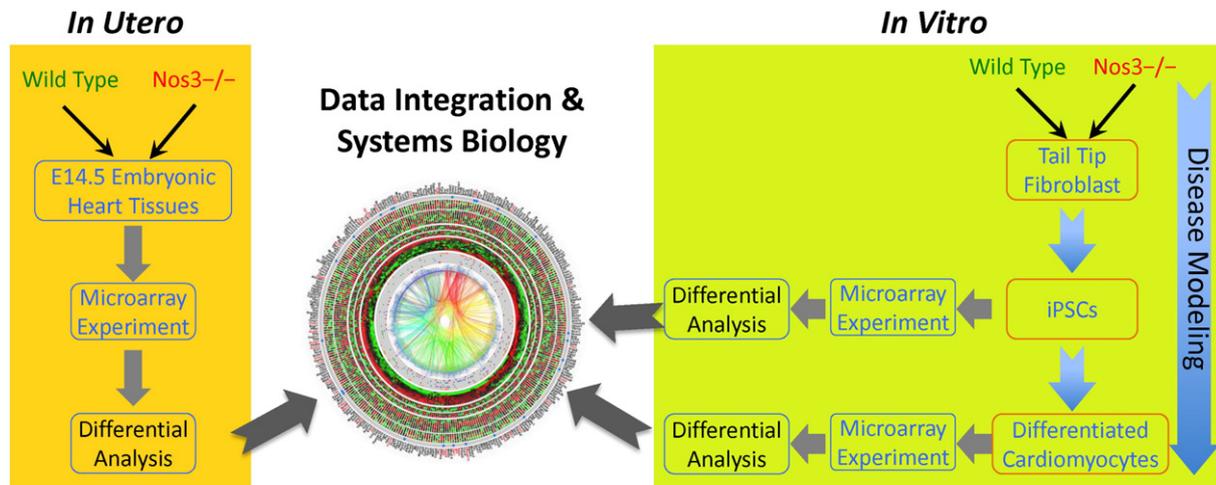


Fig. 1. Overall strategy of study design and data analysis. Transcriptome profiling using microarray was performed on embryonic heart tissues (*in utero*, left orange box). *In vitro* disease modeling for *Nos3*-related cardiac defects was conducted using induced pluripotent stem cells (iPSCs) derived from fibroblast (right green box). Microarray experiments were performed on both iPSCs and differentiated cardiomyocytes. Systems biology study integrated both *in utero* and *in vitro* transcriptome information to pinpoint the disease associated genes and networks (central circular plot).

In utero microarray experiments used WT mouse embryonic heart tissues as a gold standard. In brief, WT and *Nos3*^{-/-} embryos were harvested at 14.5 days post coitum (E14.5) and dissected for embryonic heart tissues. Biological triplicate samples were collected for microarray experiments with each sample containing tissues from 15 to 30 embryos. To accurately profile the transcriptomes in different heart chambers, left and right ventricular tissues (LV and RV) were microdissected for RNA extraction. For *in vitro* microarray experiments, tail tip fibroblasts were harvested from the distal part of the tail of WT and *Nos3*^{-/-} adult male mice. After culturing for 1–2 weeks in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Grand Island, NY), tail tip fibroblasts were infected with viral vectors from the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies, Grand Island, NY). Sendai viral vectors encoding hKOS (hKlf4, hOct4, hSox2), hcMyc, and hKlf4 were introduced at multiplicities of infection of 5:5:3, respectively. The detailed process is published in the original paper [1]. Emerging WT and *Nos3*^{-/-} iPSC colonies were harvested for RNA extraction and microarray analysis. To model the *Nos3*-related heart defects *in vitro*, WT and *Nos3*^{-/-} iPSCs were spontaneously differentiated into beating cardiomyocytes and allowed to expand until Day 11 of differentiation with medium refreshed every other day. Differentiated cardiomyocytes were identified by beating activity and expression of α -actinin. In addition, mouse embryonic stem cells (mESCs; R1, G4, CGR8) were used as additional quality controls for *in vitro* cardiac differentiation.

RNA from embryonic tissue samples, iPSCs, and differentiated cardiomyocytes was extracted with the RNeasy kit (Qiagen, Germantown, MD) for microarray experiments. GeneChip Mouse Genome 430 2.0 Arrays (Affymetrix, Santa Clara, CA) were used to profile the transcriptome in both tissue and cell line samples. Stringent quality assessment and quality control procedures were performed in this study, which are described in our previous publication [2]. According to

standard Affymetrix microarray protocol, Biotinylated complementary RNA (cRNA) was prepared from 100 ng total RNA. Following fragmentation, 10 μ g of cRNA was hybridized for 16 h at 45 °C on the microarray. GeneChips were washed and stained in the Affymetrix Fluidics Station 450 and then scanned using the GeneChip Scanner 3000 7G.

Microarray data was normalized and processed using the RMA method [3,4] to calculate the gene expression matrix. The differential analysis was performed using the LIMMA method [5]. Pairwise differential analyses were performed between the following groups: WT LV vs. *Nos3*^{-/-} LV, WT RV vs. *Nos3*^{-/-} RV, WT iPSCs vs. *Nos3*^{-/-} iPSCs, WT iPSC-derived cardiomyocytes vs. *Nos3*^{-/-} iPSC-derived cardiomyocytes (Fig. 1). Mouse ESC and ESC-derived cardiomyocytes were used as positive controls in clustering and PCA analyses. Following differential analysis, a systems biology strategy was applied to integrate differential genes from both *in utero* and *in vitro* microarray experiments along with gene interaction information to pinpoint the concordant genes and network hubs.

References

- [1] K.A. Campbell, X. Li, S.M. Biendarra, A. Terzic, T.J. Nelson, *Nos3*^(-/-) iPSCs model concordant signatures of *in utero* cardiac pathogenesis. *J. Mol. Cell. Cardiol.* 87 (Oct 2015) 228–236, <http://dx.doi.org/10.1016/j.yjmcc.2015.08.021> (Epub 2015 Sep 4).
- [2] X. Li, A. Martinez-Fernandez, K.A. Hartjes, J.P. Kocher, T.M. Olson, A. Terzic, T.J. Nelson, Transcriptional atlas of cardiogenesis maps congenital heart disease interactome. *Physiol. Genomics* 46 (13) (Jul 1 2014) 482–495, <http://dx.doi.org/10.1152/physiolgenomics.00015.2014> (Epub 2014 May 6. PMID: 24803680).
- [3] L. Gautier, L. Cope, B.M. Bolstad, R.A. Irizarry, Affy – analysis of affymetrix GeneChip data at the probe level. *Bioinformatics* 20 (2004) 307–315.
- [4] R.A. Irizarry, B. Hobbs, F. Collin, Y.D. Beazer-Barclay, K.J. Antonellis, U. Scherf, T.P. Speed, Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4 (2003) 249–264.
- [5] G.K. Smyth, Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3 (2004), 3.