

Transcriptional Profiling of the Ductus Arteriosus: Comparison of Rodent Microarrays and Human RNA Sequencing

Michael T. Yarboro¹, Matthew D. Durbin, M.D.², Jennifer L. Herington, Ph.D.^{3,4}, Elaine L. Shelton, Ph.D.^{3,4}, Tao Zhang, M.D., Ph.D.⁵, Cris G. Ebby⁶, Jason Z. Stoller, M.D.⁵, Ronald I. Clyman M.D.⁷, Jeff Reese, M.D.^{1,3}

Affiliations:

¹Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232

²Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, 46202

³Division of Neonatology, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232

⁴Department of Pharmacology, Vanderbilt University, Nashville, TN 37232

⁵Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, PA 19104

⁶Rutgers New Jersey Medical School, Newark, NJ 08901

⁷Department of Pediatrics, Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA 94143

Corresponding Author:

Jeff Reese, MD

1125 Light Hall / MRB IV Bldg.

2215 B Garland Ave.

Vanderbilt University Medical Center

Nashville, TN 37232-0656

jeff.reese@vanderbilt.edu

Phone: (615) 322-3476

Fax: (615) 343-6182

Acknowledgements: Supported by NIH HL128386 (J.R.), NHLBI HL109199 (R.I.C.), NHLBI HL46691 (R.I.C.), and NIH 5T32HD007502-20 (M.T.Y.). Special thanks to Xiaowen Wang (Partek Inc.) for contributions to microarray and RNA-seq analyses.

This is the author's manuscript of the article published in final edited form as:

Yarboro, M. T., Durbin, M. D., Herington, J. L., Shelton, E. L., Zhang, T., Ebby, C. G., ... Reese, J. (2018). Transcriptional profiling of the ductus arteriosus: Comparison of rodent microarrays and human RNA sequencing. *Seminars in Perinatology*. <https://doi.org/10.1053/j.semperi.2018.05.003>

Abstract:

DA closure is crucial for the transition from fetal to neonatal life. This closure is supported by changes to the DA's signaling and structural properties that distinguish it from neighboring vessels. Examining transcriptional differences between these vessels is key to identifying genes or pathways responsible for DA closure. Several microarray studies have explored the DA transcriptome in animal models but varied experimental designs have led to conflicting results. Thorough transcriptomic analysis of the human DA has yet to be performed. A clear picture of the DA transcriptome is key to guiding future research endeavors, both to allow more targeted treatments in the clinical setting, and to understand the basic biology of DA function. In this review, we use a cross-species cross-platform analysis to consider all available published rodent microarray data and novel human RNAseq data in order to provide high priority candidate genes for consideration in future DA studies.

Keywords: ductus arteriosus, PDA, microarray, RNA-seq, transcriptional profiling, aorta

Abbreviations:

Ao: Aorta

DEG: Differentially Expressed Gene

bioDBnet: biological Database network

BP: Biological Process

CC: Cellular Component

DAVID: Database for Annotation, Visualization, and Integrated Discovery

ECM: extracellular matrix

FDR: False Discovery Rate

FPKM: Fragments Per Kilobase of transcript per Million mapped reads

GO: Gene Ontology

GEO: Gene Expression Omnibus

hg38: human genome version 38

RMA: Robust Multi-Array

KEGG: Kyoto Encyclopedia of Genes and Genomes

MF: Molecular Function

UP: UniProt

Introduction

The ductus arteriosus (DA) is an essential vascular shunt connecting the pulmonary artery and aorta, allowing oxygenated blood from the placenta to bypass the developing lungs *in utero*. After birth, DA closure is required for a proper transition to neonatal life. Often, the postnatal DA fails to close, resulting in persistent patency of the ductus arteriosus (PDA). PDA accounts for nearly 10% of congenital heart defects^{1,2}, including more than 30% of preterm infants with a birth weight of <1500g^{3,4}.

Effective DA closure is dependent on a combination of signaling and structural changes which support constriction and eventual remodeling of the vessel⁵⁻⁷. Despite the proximity and common neural crest lineage of their smooth muscle cells⁸, the ascending aorta (Ao) doesn't undergo these changes, suggesting transcriptional differences between these vessels may define the DA's function. Numerous attempts to understand these differences at the transcriptome level have provided insight, but varied experimental design and statistical analyses have created contradictions and ambiguity in the literature. Further, the transcriptome of the human DA has not been explored with the advanced genomic techniques now available, such as RNA-seq. A clear picture of the DA's transcriptional profile is key to guiding future research endeavors, both to allow more targeted treatments in the clinical setting, and to understand the basic biology underlying DA function.

The goals of this study were to: 1) define differentially expressed genes (DEGs) in DA versus Ao samples that were commonly identified in previously published microarray datasets using rodent models, 2) identify human DA-enriched transcripts using RNA-seq analysis, and 3) explore transcriptional commonalities between the rodent and human DA. Although cross-species and cross-platform comparisons are fraught with limitations, identification of robust markers of DA identity or novel DA-enriched pathways promises to provide unique insights into DA development and function.

Methods

Microarray Meta-Analysis:

Microarray data were obtained from NCBI's Gene Expression Omnibus (GEO) database (Table 1). All available studies were considered, but only investigations that included a DA/Ao comparison were selected for analysis. For the array data from Bokenkamp *et al.*⁹, values for E21 laser micro-dissected endothelium and smooth muscle cells were pooled. For the array data from Hseih *et al.*¹⁰, only the F344 control samples were considered. CEL files of selected data sets were evaluated in Partek Genomics Suite version 7.17.1222 (Partek Inc.). All data were normalized using the Robust Multi-Array (RMA) method. One-way ANOVA was used to analyze contrasts of interest, namely vessel type, to generate lists of DEGs between DA and Ao. Permissive DEG lists (fold change ≥ 1.2) were then separated by increased or decreased DA/Ao expression to generate UP and DOWN lists respectively. A naïve vote counting strategy was used to evaluate consistency between studies. Shared genes between UP lists or DOWN lists from each study were determined using Partek Genomics Suite.

RNA-seq Analysis:

Previable (21 - 21 5/7 weeks gestation) tissue samples for human RNA-seq analysis were obtained as previously described¹¹. Tissues were homogenized in Trizol with the IKA T10 basic Ultra-Turrax. Total RNA was isolated using the RNeasy Mini kit (Qiagen). RNA quality was assessed using the Agilent 2100 Bioanalyzer. RNA samples with RIN score ≥ 6.5 were considered for further study. 1 microgram of total RNA was used for library construction using the ScriptSeq Complete Gold Kit (Illumina). The libraries, four biological replicates per vessel type, were sequenced on an Illumina HiSeq 2000 by 100bp pair-end sequencing. RNA-seq data was uploaded to Partek Flow (Partek Inc.). Trimming of raw reads (both ends) was based on a minimum read length of 25 and discarded bases after 85. Trimmed reads were aligned to Human Genome Version 38 (hg38) using STAR – 2.5.3a and quantified to Refseq Transcripts 83 using Partek's E/M method. Aligned counts were FPKM normalized with an offset of 1. Gene

specific analysis (GSA) was used to detect DA vs. Ao DEGs. DEGs with an FDR of ≤ 0.1 and a fold change ≥ 2 were considered significant. Volcano plot and dendrogram heat map figures were generated using Partek Flow.

Comparison of Microarray and RNA-seq DEGs:

Rodent gene symbols were converted to human orthologues using biological Database network (bioDBnet) to identify genes common between microarray and RNA-seq DEG lists. Lists were manually aligned to determine genes differentially expressed in both microarray and RNA-seq analyses. Microarray and RNA-seq gene lists were submitted independently for functional annotation using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Functional terms from Gene Ontology (GO) Biological Process (BP), GO Cellular Component (CC), GO Molecular Function (MF), Kyoto Encyclopedia of Genes and Genomes (KEGG), and UniProt (UP) Keywords databases were evaluated for similarities. Lists of functional terms and keywords from each database were then manually aligned. In order to create comparison diagrams, significant terms ($p \leq 0.05$) were chosen based on count (defined as number of genes identified in each term), and ordered by $-\text{Log}(p \text{ value})$.

Results

Comparison of Published Rodent Microarrays

Our microarray meta-analysis focused on studies containing DA to Ao comparisons in term animals, since there were too few preterm studies for comparison. Comparison of DA to Ao expression allowed DA-specific genes to be distinguished from temporally-regulated genes that are important for generalized vessel development. Differential expression was recalculated from raw data using a uniform statistical approach. Array data from three mammalian species (rat, mouse, sheep) were available, but differences in experimental design and genome annotation limited the use of data from sheep¹². Four rodent studies met pre-specified criteria (vessel type, gestational stage) and were included for analysis^{9,10,13,14} (**Table 1**). There were 444 genes

identified as differentially expressed in at least three of four arrays. Of these, 87 genes were consistently increased in DA versus Ao (DA enriched), while 189 genes were consistently decreased in DA versus Ao (Ao enriched) (**Figure 1**). Complete gene lists are provided for both DA enriched (**Table S1**) and Ao enriched (**Table S2**) gene sets. Interestingly, many of the genes that were common to at least 3 studies, such as *Abcc9*^{11,15-17}, *Cacna1c*¹⁸, *Edn1*^{11,13,19}, *Pde4b*^{12,20}, *Ptger4*^{11,21-26}, and *Tfap2b*^{11,27-33}, have previously been identified as significant for DA function (**Table S1**, blue typeface). A more thorough listing of previously identified genes significant for DA function can be found in Lewis *et al.*³⁴. Identification of these established genes suggests that this approach was sufficient to detect genes and pathways relevant for DA function.

Human RNA-seq Analysis

cDNA libraries were prepared for paired DA and Ao samples from 4 subjects. Libraries were sequenced to an average of 60 million genomic reads with an average genomic coverage rate of 67.7% (**Table 2**). Reads were aligned to human genome version 38 and quantified against Refseq Transcripts 83. GSA was then used to detect DEGs from counts normalized to Fragments Per Kilobase of transcript per Million (FPKM)-mapped reads. Hierarchical clustering analysis resulted in a heat map of RNA-seq samples (**Figure 2**). This heat map demonstrates that vessel identity was the primary determinant of clustered expression patterns. Interestingly, 77% of probes selected for hierarchical clustering were DA enriched, compared to 23% Ao enriched, suggesting the DA's phenotype is driven by expression of DA-specific genes, as opposed to suppression of Ao-specific genes. Overall, 2082 genes showed differential expression between DA and Ao with a p-value of 0.05 and fold change of ≥ 2 (**Figure 3**). 186 of these genes met a permissive FDR criteria (Benjamini-Hochberg) of 0.10 or less, with 118 showing increased expression in the DA compared to Ao and 68 showing decreased expression in DA compared to Ao. Complete gene lists from this analysis are provided for both DA enriched (**Table S4**) and Ao enriched (**Table S5**) transcripts. The 20 most highly expressed (by FPKM) DEGs in the human DA were also identified (**Table S6**).

Intersection of Human and Rodent DEGs

To compare the findings of the microarray and RNA-seq studies, rodent gene symbols were converted to human orthologues (bioDBnet). Of the 276 DEGs from the rodent microarray analysis and the 186 DEGs from the human RNA-seq, 11 genes were common to both studies (**Figure 4**). Several of these genes, including *ABCC9*^{11,15-17}, *PDE1C*^{11,20,35}, *PTGER4*^{11,21-26}, and *TFAP2B*^{11,27-33} have previously been described as significant for DA identity. *TFAP2B* had a notably high fold change (37.9) similar to findings from individual microarray studies (**Table S1**)

Genes differentially expressed between DA and Ao were categorized by functional annotation (DAVID). Overlap between rodent microarray and human RNA-seq was found for GO Biological Process (48.4%), GO Cellular Component (63.2%), GO Molecular Function (47.4%), KEGG (55.6%), and UniProt (UP) Keywords (59.5%) (**Table S7**). Of the top 30 UP Keyword terms from rodent microarray and human RNA-seq, 16 were found in common (**Figure 5**). Terms such as 'Calcium', 'Cell adhesion', 'collagen', 'extracellular matrix', and 'metalloprotease' align with pathways known to be important for DA constriction and remodeling^{5,30,36-41}.

Discussion

There are currently nine published microarray studies of the DA^{9,10,12-14,26,42-44}, each performed to answer a specific question, and consequently, each with a different experimental design. In order to extract the most meaningful information from these studies, criteria were selected that would include as many studies as possible while only considering comparable data sets. We focused on studies that included DA to Ao comparisons as they distinguish between DA-specific genes and developmental genes also expressed in neighboring vasculature, while also controlling for biases inherent to each study. In addition, studies that analyzed the term-gestation time point were chosen because this developmental time point was the most represented among available microarray data sets. Although individual array studies

have identified differences between term and preterm DAs^{9,12,13}, examination of the available data suggested that differences in study design and consideration of disparate preterm time points would make data alignment unreliable and preclude inter-study comparisons. There is one published microarray study of human DA samples, though it was not considered because it was conducted on abnormal tissue (patients requiring DA stents) and contained no Ao tissue for comparison⁴⁵. Overall, 11 genes were differentially expressed in both the preterm human RNA-seq as well as the term rodent microarrays, suggesting that a small subset of genes may define DA identity over a broad developmental time span and between species. This number would be expected to increase in an analysis comparing differential time points in the same species or comparable time points across different species.

Among the DEGs identified as common between rodent microarray data sets and human RNA-seq analysis were several genes which have been previously identified as important for the DA. Two of these, *PTGER4*, which encodes the prostanoid receptor EP₄, and *TFAP2B*, which encodes transcription factor AP-2 β , have key roles in DA biology. EP₄ is a G-protein coupled receptor and primary regulator of DA patency. EP₄ is the predominant prostanoid receptor in the mammalian DA⁴⁶⁻⁵⁴. During late gestation, circulating prostaglandin E₂ (PGE₂) stimulates EP₄ to maintain DA patency⁴⁸⁻⁵⁰. Catabolism of circulating prostaglandins by 15-hydroxy-prostaglandin dehydrogenase in the newly inflated lungs helps facilitate neonatal DA closure^{55,56}. Interestingly, EP₄ knockout mice die shortly after birth with a PDA²¹⁻²⁵. Similarly, mice lacking both of the cyclooxygenase enzymes required for PGE₂ production, also die with PDA^{57,58}. Normally the removal of a dilatory stimulus would be expected to result in constriction, but these animals paradoxically present with a widely patent DA. Taken together, these findings suggest that prostaglandins, acting via the EP₄ receptor pathway, may play a role in developmentally programming the DA in addition to their acute vasodilatory function^{21,26,57,59,60}. The prevalence of EP₄ among microarray studies and its high fold change in our human RNA-seq data support its role as an important regulator of DA development and function.

TFAP2B is thought to regulate proliferation and differentiation during development. Mutations in *TFAP2B* result in Char syndrome, a neural crest disorder typically associated with developmental abnormalities of the hands and face, as well as PDA^{27,29,32}. Single nucleotide variants in *TFAP2B* have also been linked to non-syndromic PDA^{31,33}. *TFAP2B* was highly enriched in DA vs. Ao expression in both human RNA-seq (38-fold increase) and three rodent microarrays (33-fold increase in Shelton *et al.*¹⁴). *Tfap2b* was not included in the probe set used by Jin *et al.*, and thus was found significant in all possible sources. Interestingly, the array from Bokenkamp *et al.*⁹ found *Tfap2b* to be similarly enriched in both DA SMC and DA endothelium, despite only DA SMCs originating from the neural crest⁶¹. These findings are consistent with previous speculation that *Tfap2b* may act as a critical regulator of DA gene expression. Ivey *et al.* showed that *Tfap2b* expression was specific to DA SMCs and that knockout mice lacked proper SMC differentiation⁶². They also found that *Tfap2b* expression was required for the sequential expression of hypoxia inducible factor 2 α (*Hif2a*) and endothelin-1 (*Et-1*). These data suggested the hypothesis that *Tfap2b* is a transcriptional regulator which interacts with *Hif2a* and *Et-1* during development to drive differentiation of DA SMCs. In order to determine the true role of *Tfap2b* in DA development, transcriptional comparisons must be made between the DA of *Tfap2b* null and wild-type mice.

Of the eleven DEGs common to the human RNA-seq and rodent microarrays, one gene, phosphodiesterase 1C, (*PDE1C*) showed discordant results for DA vs. Ao expression between species. *Pde1c* had consistently decreased DA/Ao expression in three rodent microarray studies (-1.3, -2.4, and -2.9 fold change), but increased DA/Ao expression in human RNA-seq data (33.2 fold change). PDE1C catalyzes hydrolysis of the second messengers, cAMP and cGMP. Given the importance of both EP₄ and nitric oxide signaling via cAMP and cGMP, respectively, in regulating DA tone^{60,63-65}, PDE1C's involvement in the DA is logical. PDE1C activity has been shown to affect DA tone⁶⁶⁻⁶⁸ and is thought to do this by attenuating the dilatory effects of EP₄ stimulation and downstream cAMP production in the DA²⁰. PDE1C is also associated with pathological vascular remodeling, driving proliferation and migration in vascular

smooth muscle cells, processes relevant for DA functionality^{69,70}. Due to this study's limitations, it is unclear whether this discrepancy arises from gestational differences, species differences, or some unknown experimental factor.

Similarly, some genes shown to be relevant for DA function in prior studies were not identified in our combined approach. For example, *KCNMA1*, which encodes a subunit of the BK_{CA} potassium channel, was identified in the microarray study by Shelton *et al.*¹⁴ as enriched in the DA (fold change 2.1). Those results were confirmed by RT-qPCR, *in situ* hybridization and functional analysis¹⁴. Despite these convincing results, *KCNMA1* was enriched in the Ao by other microarrays and our human RNA-seq data. Considering Shelton *et al.* is the only study using mice, this may represent a species-specific difference. These findings highlight the importance of multi-model comparisons in DA research.

There are several limitations in our present analysis. Previous DA microarray studies have raised concerns about the efficacy and appropriateness of directly comparing different microarray data sets^{9,12,71,72}. This is not field-specific, and there are well-known limitations to this type of comparison⁷³⁻⁷⁵. This is especially true for embryonic and pregnancy-associated tissues which have high internal variability even within tissue type^{76,77}. The primary concern is a bias towards type 2 errors, or under-detection of truly differentially expressed and biologically relevant genes.

Direct gene comparison between species also suffers from differences in naming conventions and conservation of specific orthologues. To address this, we focused on functional annotation of our gene lists, to emphasize *pathways* that were in agreement rather than individual gene identity. This strategy has been shown to be more consistent across studies and yield more reproducible, biologically informative results^{75,78}. In doing so, several aspects of vessel physiology which may be altered between the DA and neighboring vessels were identified. Genes associated with alterations in extracellular matrix (ECM) organization between DA and Ao were identified by GO biological process, GO cellular component, GO molecular function, and UP keywords in both rodent microarrays and human RNA-seq. Alterations in ECM

composition play a critical role in remodeling of the DA in both late gestation and neonatal life^{5,30,36,39,40}. Interestingly, several mouse knockout models of ECM-related genes have PDA^{79,80}. There was limited direct alignment of ECM-associated genes between the microarray and RNA-seq studies. Only periostin (POSTN), an integrin binding protein which supports cell adhesion and migration, was identified in both rodent microarrays and human RNA-seq. However, both rodent arrays and human RNA-seq studies identified several collagen family members (*Col11a1*, *Col3a1*, *Col5a2*, *Col6a3*) (*COL8A1*, *COL8A2*, *COL9A1*, *COL19A1*) and ADAMTS family genes (*Adamts1*, *Adamts9*, *Adamtsl2*, *Adam33*, *Adamtsl5*) (*ADAMTS9*, *ADAMT22*, *ADMATS8*). These data suggest that though there are species differences in orthologues, similar pathways are important for both the rodent and human DA.

This study was also hindered by the limited number of preterm datasets. Because the human RNA-seq and rodent microarray data were obtained at different gestational time points, it is difficult to draw clear, unbiased comparisons. Ambiguity still exists concerning the correlation of critical events in DA development between rodents and humans. Thus, at this point, more information on developmental milestones is needed to identify parallel features of DA regulation.

Finally, microarray studies were performed on different platforms, resulting in different sets of transcripts that could possibly be detected for each array. Comparisons between these platforms were then limited to the sets of genes that are represented on all array platforms. This is a minor shortcoming, but is further compounded when comparing microarray results to RNA-seq, which is not restricted by predetermined probe sets as a sequencing-based approach. Thus, our list of genes common to both rodent microarrays and human RNA-seq data sets is capped by the set of genes which is represented on the most limited array platform, predisposing this analysis to under-report significant genes from the RNA-seq which may have been omitted from specific microarray platforms.

In conclusion, comparison of microarray studies from animal models with new information from human RNA-seq data generated high priority candidate genes to consider in future DA studies. Identification of DA-specific or highly enriched genes in the DA is a requisite

step in finding new DA-selective drugs or providing a molecular address for homing of therapeutic agents to the target tissue. Although cross-platform and cross-species transcriptomic comparisons are fraught with limitations, they offer a unique opportunity to visualize pathways of interest to better understand DA development and function.

References:

- 1 Lloyd, T. R. & Beekman, R. H., 3rd. Clinically silent patent ductus arteriosus. *Am Heart J* **127**, 1664-1665 (1994).
- 2 Hammerman, C. Patent ductus arteriosus. Clinical relevance of prostaglandins and prostaglandin inhibitors in PDA pathophysiology and treatment. *Clin Perinatol* **22**, 457-479 (1995).
- 3 Reller, M. D., Rice, M. J. & McDonald, R. W. Review of studies evaluating ductal patency in the premature infant. *J Pediatr* **122**, S59-62 (1993).
- 4 Yu, V. Y. Patent ductus arteriosus in the preterm infant. *Early Hum Dev* **35**, 1-14 (1993).
- 5 Yokoyama, U., Minamisawa, S. & Ishikawa, Y. Regulation of vascular tone and remodeling of the ductus arteriosus. *J Smooth Muscle Res* **46**, 77-87 (2010).
- 6 Bokenkamp, R., DeRuiter, M. C., van Munsteren, C. & Gittenberger-de Groot, A. C. Insights into the pathogenesis and genetic background of patency of the ductus arteriosus. *Neonatology* **98**, 6-17, doi:10.1159/000262481 (2010).
- 7 Stoller, J. Z., Demauro, S. B., Dagle, J. M. & Reese, J. Current Perspectives on Pathobiology of the Ductus Arteriosus. *J Clin Exp Cardiol* **8**, doi:10.4172/2155-9880.S8-001 (2012).
- 8 Pfaltzgraff, E. R., Shelton, E. L., Galindo, C. L., Nelms, B. L., Hooper, C. W., Poole, S. D., Labosky, P. A., Bader, D. M. & Reese, J. Embryonic domains of the aorta derived from diverse origins exhibit distinct properties that converge into a common phenotype in the adult. *J Mol Cell Cardiol* **69**, 88-96, doi:10.1016/j.yjmcc.2014.01.016 (2014).
- 9 Bokenkamp, R., van Brempt, R., van Munsteren, J. C., van den Wijngaert, I., de Hoogt, R., Finos, L., Goeman, J., Groot, A. C., Poelmann, R. E., Blom, N. A. & DeRuiter, M. C. Dlx1 and Rgs5 in the ductus arteriosus: vessel-specific genes identified by transcriptional profiling of laser-capture microdissected endothelial and smooth muscle cells. *PLoS One* **9**, e86892, doi:10.1371/journal.pone.0086892 (2014).
- 10 Hsieh, Y. T., Liu, N. M., Ohmori, E., Yokota, T., Kajimura, I., Akaike, T., Ohshima, T., Goda, N. & Minamisawa, S. Transcription profiles of the ductus arteriosus in Brown-Norway rats with irregular elastic fiber formation. *Circ J* **78**, 1224-1233 (2014).
- 11 Waleh, N., Barrette, A. M., Dagle, J. M., Momany, A., Jin, C., Hills, N. K., Shelton, E. L., Reese, J. & Clyman, R. I. Effects of Advancing Gestation and Non-Caucasian Race on Ductus Arteriosus Gene Expression. *J Pediatr* **167**, 1033-1041 e1032, doi:10.1016/j.jpeds.2015.07.011 (2015).
- 12 Goyal, R., Goyal, D., Longo, L. D. & Clyman, R. I. Microarray gene expression analysis in ovine ductus arteriosus during fetal development and birth transition. *Pediatr Res* **80**, 610-618, doi:10.1038/pr.2016.123 (2016).
- 13 Jin, M. H., Yokoyama, U., Sato, Y., Shioda, A., Jiao, Q., Ishikawa, Y. & Minamisawa, S. DNA microarray profiling identified a new role of growth hormone in vascular remodeling of rat ductus arteriosus. *J Physiol Sci* **61**, 167-179, doi:10.1007/s12576-011-0133-3 (2011).
- 14 Shelton, E. L., Ector, G., Galindo, C. L., Hooper, C. W., Brown, N., Wilkerson, I., Pfaltzgraff, E. R., Paria, B. C., Cotton, R. B., Stoller, J. Z. & Reese, J. Transcriptional profiling reveals ductus arteriosus-specific genes that regulate vascular tone. *Physiol Genomics* **46**, 457-466, doi:10.1152/physiolgenomics.00171.2013 (2014).
- 15 Grange, D. K., Nichols, C. G. & Singh, G. K. *Cantu Syndrome and Related Disorders*. (University of Washington, 2014).

- 16 Grange, D. K., Lorch, S. M., Cole, P. L. & Singh, G. K. Cantu syndrome in a woman and her two daughters: Further confirmation of autosomal dominant inheritance and review of the cardiac manifestations. *Am J Med Genet A* **140**, 1673-1680, doi:10.1002/ajmg.a.31348 (2006).
- 17 Harakalova, M., van Harssel, J. J., Terhal, P. A., van Lieshout, S., Duran, K., Renkens, I., Amor, D. J., Wilson, L. C., Kirk, E. P., Turner, C. L., Shears, D., Garcia-Minaur, S., Lees, M. M., Ross, A., Venselaar, H., Vriend, G., Takanari, H., Rook, M. B., van der Heyden, M. A., Asselbergs, F. W., Breur, H. M., Swinkels, M. E., Scurr, I. J., Smithson, S. F., Knoers, N. V., van der Smagt, J. J., Nijman, I. J., Kloosterman, W. P., van Haelst, M. M., van Haften, G. & Cuppen, E. Dominant missense mutations in ABCC9 cause Cantu syndrome. *Nat Genet* **44**, 793-796, doi:10.1038/ng.2324 (2012).
- 18 Boczek, N. J., Miller, E. M., Ye, D., Nesterenko, V. V., Tester, D. J., Antzelevitch, C., Czocek, R. J., Ackerman, M. J. & Ware, S. M. Novel Timothy syndrome mutation leading to increase in CACNA1C window current. *Heart Rhythm* **12**, 211-219, doi:10.1016/j.hrthm.2014.09.051 (2015).
- 19 Smith, G. C. The pharmacology of the ductus arteriosus. *Pharmacol Rev* **50**, 35-58 (1998).
- 20 Liu, H., Manganiello, V., Waleh, N. & Clyman, R. I. Expression, activity, and function of phosphodiesterases in the mature and immature ductus arteriosus. *Pediatr Res* **64**, 477-481, doi:10.1203/PDR.0b013e3181827c2c (2008).
- 21 Nguyen, M., Camenisch, T., Snouwaert, J. N., Hicks, E., Coffman, T. M., Anderson, P. A., Malouf, N. N. & Koller, B. H. The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature* **390**, 78-81, doi:10.1038/36342 (1997).
- 22 Segi, E., Sugimoto, Y., Yamasaki, A., Aze, Y., Oida, H., Nishimura, T., Murata, T., Matsuoka, T., Ushikubi, F., Hirose, M., Tanaka, T., Yoshida, N., Narumiya, S. & Ichikawa, A. Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem Biophys Res Commun* **246**, 7-12, doi:10.1006/bbrc.1998.8461 (1998).
- 23 Ushikubi, F., Sugimoto, Y., Ichikawa, A. & Narumiya, S. Roles of prostanoids revealed from studies using mice lacking specific prostanoid receptors. *Jpn J Pharmacol* **83**, 279-285 (2000).
- 24 Fujino, T., Yuhki, K., Yamada, T., Hara, A., Takahata, O., Okada, Y., Xiao, C. Y., Ma, H., Karibe, H., Iwashima, Y., Fukuzawa, J., Hasebe, N., Kikuchi, K., Narumiya, S. & Ushikubi, F. Effects of the prostanoids on the proliferation or hypertrophy of cultured murine aortic smooth muscle cells. *Br J Pharmacol* **136**, 530-539, doi:10.1038/sj.bjp.0704749 (2002).
- 25 Schneider, A., Guan, Y., Zhang, Y., Magnuson, M. A., Pettepher, C., Loftin, C. D., Langenbach, R., Breyer, R. M. & Breyer, M. D. Generation of a conditional allele of the mouse prostaglandin EP4 receptor. *Genesis* **40**, 7-14, doi:10.1002/gene.20048 (2004).
- 26 Gruzdev, A., Nguyen, M., Kovarova, M. & Koller, B. H. PGE2 through the EP4 receptor controls smooth muscle gene expression patterns in the ductus arteriosus critical for remodeling at birth. *Prostaglandins Other Lipid Mediat* **97**, 109-119, doi:10.1016/j.prostaglandins.2012.02.001 (2012).
- 27 Satoda, M., Zhao, F., Diaz, G. A., Burn, J., Goodship, J., Davidson, H. R., Pierpont, M. E. & Gelb, B. D. Mutations in TFAP2B cause Char syndrome, a familial form of patent ductus arteriosus. *Nat Genet* **25**, 42-46, doi:10.1038/75578 (2000).
- 28 Zhao, F., Weismann, C. G., Satoda, M., Pierpont, M. E., Sweeney, E., Thompson, E. M. & Gelb, B. D. Novel TFAP2B mutations that cause Char syndrome provide a genotype-phenotype correlation. *Am J Hum Genet* **69**, 695-703, doi:10.1086/323410 (2001).
- 29 Mani, A., Radhakrishnan, J., Farhi, A., Carew, K. S., Warnes, C. A., Nelson-Williams, C., Day, R. W., Pober, B., State, M. W. & Lifton, R. P. Syndromic patent ductus arteriosus: evidence for haploinsufficient TFAP2B mutations and identification of a linked sleep disorder. *Proc Natl Acad Sci U S A* **102**, 2975-2979, doi:10.1073/pnas.0409852102 (2005).
- 30 Schneider, D. J. & Moore, J. W. Patent ductus arteriosus. *Circulation* **114**, 1873-1882, doi:10.1161/CIRCULATIONAHA.105.592063 (2006).

- 31 Khetyar, M., Syrris, P., Tinworth, L., Abushaban, L. & Carter, N. Novel TFAP2B mutation in nonsyndromic patent ductus arteriosus. *Genet Test* **12**, 457-459, doi:10.1089/gte.2008.0015 (2008).
- 32 Zhao, F., Bosserhoff, A. K., Buettner, R. & Moser, M. A heart-hand syndrome gene: Tfp2b plays a critical role in the development and remodeling of mouse ductus arteriosus and limb patterning. *PLoS One* **6**, e22908, doi:10.1371/journal.pone.0022908 (2011).
- 33 Chen, Y. W., Zhao, W., Zhang, Z. F., Fu, Q., Shen, J., Zhang, Z., Ji, W., Wang, J. & Li, F. Familial nonsyndromic patent ductus arteriosus caused by mutations in TFAP2B. *Pediatr Cardiol* **32**, 958-965, doi:10.1007/s00246-011-0024-7 (2011).
- 34 Lewis, T. R., Shelton, E. L., Van Driest, S. L., Kannankeril, P. J. & Reese, J. Genetics of the patent ductus arteriosus (PDA) and pharmacogenetics of PDA treatment. *Semin Fetal Neonatal Med*, doi:10.1016/j.siny.2018.02.006 (2018).
- 35 Rybalkin, S. D., Rybalkina, I., Beavo, J. A. & Bornfeldt, K. E. Cyclic nucleotide phosphodiesterase 1C promotes human arterial smooth muscle cell proliferation. *Circ Res* **90**, 151-157 (2002).
- 36 Clyman, R. I. Mechanisms regulating the ductus arteriosus. *Biol Neonate* **89**, 330-335, doi:10.1159/000092870 (2006).
- 37 Yokoyama, U., Minamisawa, S., Quan, H., Ghatak, S., Akaike, T., Segi-Nishida, E., Iwasaki, S., Iwamoto, M., Misra, S., Tamura, K., Hori, H., Yokota, S., Toole, B. P., Sugimoto, Y. & Ishikawa, Y. Chronic activation of the prostaglandin receptor EP4 promotes hyaluronan-mediated neointimal formation in the ductus arteriosus. *J Clin Invest* **116**, 3026-3034, doi:10.1172/JCI28639 (2006).
- 38 Yokoyama, U., Minamisawa, S., Shioda, A., Ishiwata, R., Jin, M. H., Masuda, M., Asou, T., Sugimoto, Y., Aoki, H., Nakamura, T. & Ishikawa, Y. Prostaglandin E2 inhibits elastogenesis in the ductus arteriosus via EP4 signaling. *Circulation* **129**, 487-496, doi:10.1161/CIRCULATIONAHA.113.004726 (2014).
- 39 de Reeder, E. G., van Munsteren, C. J., Poelmann, R. E., Patterson, D. F. & Gittenberger-de Groot, A. C. Changes in distribution of elastin and elastin receptor during intimal cushion formation in the ductus arteriosus. *Anat Embryol (Berl)* **182**, 473-480 (1990).
- 40 de Reeder, E. G., Poelmann, R. E., van Munsteren, J. C., Patterson, D. F. & Gittenberger-de Groot, A. C. Ultrastructural and immunohistochemical changes of the extracellular matrix during intimal cushion formation in the ductus arteriosus of the dog. *Atherosclerosis* **79**, 29-40 (1989).
- 41 Slomp, J., van Munsteren, J. C., Poelmann, R. E., de Reeder, E. G., Bogers, A. J. & Gittenberger-de Groot, A. C. Formation of intimal cushions in the ductus arteriosus as a model for vascular intimal thickening. An immunohistochemical study of changes in extracellular matrix components. *Atherosclerosis* **93**, 25-39 (1992).
- 42 Costa, M., Barogi, S., Socci, N. D., Angeloni, D., Maffei, M., Baragatti, B., Chiellini, C., Grasso, E. & Coceani, F. Gene expression in ductus arteriosus and aorta: comparison of birth and oxygen effects. *Physiol Genomics* **25**, 250-262, doi:10.1152/physiolgenomics.00231.2005 (2006).
- 43 Yokoyama, U., Sato, Y., Akaike, T., Ishida, S., Sawada, J., Nagao, T., Quan, H., Jin, M., Iwamoto, M., Yokota, S., Ishikawa, Y. & Minamisawa, S. Maternal vitamin A alters gene profiles and structural maturation of the rat ductus arteriosus. *Physiol Genomics* **31**, 139-157, doi:10.1152/physiolgenomics.00007.2006 (2007).
- 44 Liu, N. M., Yokota, T., Maekawa, S., Lu, P., Zheng, Y. W., Taniguchi, H., Yokoyama, U., Kato, T. & Minamisawa, S. Transcription profiles of endothelial cells in the rat ductus arteriosus during a perinatal period. *PLoS One* **8**, e73685, doi:10.1371/journal.pone.0073685 (2013).
- 45 Mueller, P. P., Drynda, A., Goltz, D., Hoehn, R., Hauser, H. & Peuster, M. Common signatures for gene expression in postnatal patients with patent arterial ducts and stented arteries. *Cardiol Young* **19**, 352-359, doi:10.1017/S1047951109004260 (2009).
- 46 Leonhardt, A., Glaser, A., Wegmann, M., Schranz, D., Seyberth, H. & Nusing, R. Expression of prostanoid receptors in human ductus arteriosus. *Br J Pharmacol* **138**, 655-659, doi:10.1038/sj.bjp.0705092 (2003).

- 47 Rheinlaender, C., Weber, S. C., Sarioglu, N., Strauss, E., Obladen, M. & Koehne, P. Changing expression of cyclooxygenases and prostaglandin receptor EP4 during development of the human ductus arteriosus. *Pediatr Res* **60**, 270-275, doi:10.1203/01.pdr.0000233066.28496.7c (2006).
- 48 Momma, K., Toyoshima, K., Takeuchi, D., Imamura, S. & Nakanishi, T. In vivo reopening of the neonatal ductus arteriosus by a prostanoid EP4-receptor agonist in the rat. *Prostaglandins Other Lipid Mediat* **78**, 117-128, doi:10.1016/j.prostaglandins.2005.04.006 (2005).
- 49 Momma, K., Toyoshima, K., Takeuchi, D., Imamura, S. & Nakanishi, T. In vivo constriction of the fetal and neonatal ductus arteriosus by a prostanoid EP4-receptor antagonist in rats. *Pediatr Res* **58**, 971-975, doi:10.1203/01.pdr.0000182182.49476.24 (2005).
- 50 Kajino, H., Taniguchi, T., Fujieda, K., Ushikubi, F. & Muramatsu, I. An EP4 receptor agonist prevents indomethacin-induced closure of rat ductus arteriosus in vivo. *Pediatr Res* **56**, 586-590, doi:10.1203/01.PDR.0000139409.25014.35 (2004).
- 51 Fan, F. L., Zhu, S., Chen, L. H., Zou, Y. L., Fan, L. H., Kang, J. H., Ma, A. Q. & Guan, Y. F. Role of prostaglandin E and its receptors in the process of ductus arteriosus maturation and functional closure in the rabbit. *Clin Exp Pharmacol Physiol* **37**, 574-580, doi:10.1111/j.1440-1681.2010.05354.x (2010).
- 52 Bouayad, A., Kajino, H., Waleh, N., Fouron, J. C., Andelfinger, G., Varma, D. R., Skoll, A., Vazquez, A., Gobeil, F., Jr., Clyman, R. I. & Chemtob, S. Characterization of PGE2 receptors in fetal and newborn lamb ductus arteriosus. *Am J Physiol Heart Circ Physiol* **280**, H2342-2349, doi:10.1152/ajpheart.2001.280.5.H2342 (2001).
- 53 Bouayad, A., Bernier, S. G., Asselin, P., Hardy, P., Bhattacharya, M., Quiniou, C., Fouron, J. C., Guerguerian, A. M., Varma, D. R., Clyman, R. I. & Chemtob, S. Characterization of PGE2 receptors in fetal and newborn ductus arteriosus in the pig. *Semin Perinatol* **25**, 70-75 (2001).
- 54 Bhattacharya, M., Asselin, P., Hardy, P., Guerguerian, A. M., Shichi, H., Hou, X., Varma, D. R., Bouayad, A., Fouron, J. C., Clyman, R. I. & Chemtob, S. Developmental changes in prostaglandin E(2) receptor subtypes in porcine ductus arteriosus. Possible contribution in altered responsiveness to prostaglandin E(2). *Circulation* **100**, 1751-1756 (1999).
- 55 de Reeder, E. G., Gittenberger-de Groot, A. C., van Munsteren, J. C., Poelmann, R. E., Patterson, D. F. & Keirse, M. J. Distribution of prostacyclin synthase, 6-keto-prostaglandin F1 alpha, and 15-hydroxy-prostaglandin dehydrogenase in the normal and persistent ductus arteriosus of the dog. *Am J Pathol* **135**, 881-887 (1989).
- 56 Coggins, K. G., Latour, A., Nguyen, M. S., Audoly, L., Coffman, T. M. & Koller, B. H. Metabolism of PGE2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. *Nat Med* **8**, 91-92, doi:10.1038/nm0202-91 (2002).
- 57 Reese, J., Paria, B. C., Brown, N., Zhao, X., Morrow, J. D. & Dey, S. K. Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. *Proc Natl Acad Sci U S A* **97**, 9759-9764 (2000).
- 58 Loftin, C. D., Trivedi, D. B., Tiano, H. F., Clark, J. A., Lee, C. A., Epstein, J. A., Morham, S. G., Breyer, M. D., Nguyen, M., Hawkins, B. M., Goulet, J. L., Smithies, O., Koller, B. H. & Langenbach, R. Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. *Proc Natl Acad Sci U S A* **98**, 1059-1064, doi:10.1073/pnas.031573498 (2001).
- 59 Reese, J., Anderson, J. D., Brown, N., Roman, C. & Clyman, R. I. Inhibition of cyclooxygenase isoforms in late- but not midgestation decreases contractility of the ductus arteriosus and prevents postnatal closure in mice. *Am J Physiol Regul Integr Comp Physiol* **291**, R1717-1723, doi:10.1152/ajpregu.00259.2006 (2006).
- 60 Reese, J., O'Mara, P. W., Poole, S. D., Brown, N., Tolentino, C., Eckman, D. M. & Aschner, J. L. Regulation of the fetal mouse ductus arteriosus is dependent on interaction of nitric oxide and COX enzymes in the ductal wall. *Prostaglandins Other Lipid Mediat* **88**, 89-96, doi:10.1016/j.prostaglandins.2008.11.001 (2009).

- 61 Wang, X., Chen, D., Chen, K., Jubran, A., Ramirez, A. & Astrof, S. Endothelium in the pharyngeal arches 3, 4 and 6 is derived from the second heart field. *Dev Biol* **421**, 108-117, doi:10.1016/j.ydbio.2016.12.010 (2017).
- 62 Ivey, K. N., Sutcliffe, D., Richardson, J., Clyman, R. I., Garcia, J. A. & Srivastava, D. Transcriptional regulation during development of the ductus arteriosus. *Circ Res* **103**, 388-395, doi:10.1161/CIRCRESAHA.108.180661 (2008).
- 63 Coceani, F. Control of the ductus arteriosus—a new function for cytochrome P450, endothelin and nitric oxide. *Biochem Pharmacol* **48**, 1315-1318 (1994).
- 64 Momma, K. & Toyono, M. The role of nitric oxide in dilating the fetal ductus arteriosus in rats. *Pediatr Res* **46**, 311-315, doi:10.1203/00006450-199909000-00010 (1999).
- 65 Richard, C., Gao, J., LaFleur, B., Christman, B. W., Anderson, J., Brown, N. & Reese, J. Patency of the preterm fetal ductus arteriosus is regulated by endothelial nitric oxide synthase and is independent of vasa vasorum in the mouse. *Am J Physiol Regul Integr Comp Physiol* **287**, R652-660, doi:10.1152/ajpregu.00049.2004 (2004).
- 66 Thebaud, B., Michelakis, E., Wu, X. C., Harry, G., Hashimoto, K. & Archer, S. L. Sildenafil reverses O₂ constriction of the rabbit ductus arteriosus by inhibiting type 5 phosphodiesterase and activating BK(Ca) channels. *Pediatr Res* **52**, 19-24, doi:10.1203/00006450-200207000-00006 (2002).
- 67 Momma, K., Toyoshima, K., Imamura, S. & Nakanishi, T. In vivo dilation of fetal and neonatal ductus arteriosus by inhibition of phosphodiesterase-5 in rats. *Pediatr Res* **58**, 42-45, doi:10.1203/01.PDR.0000156370.50874.3C (2005).
- 68 Toyoshima, K., Momma, K., Imamura, S. & Nakanishi, T. In vivo dilatation of the fetal and postnatal ductus arteriosus by inhibition of phosphodiesterase 3 in rats. *Biol Neonate* **89**, 251-256, doi:10.1159/000089954 (2006).
- 69 Chan, S. & Yan, C. PDE1 isozymes, key regulators of pathological vascular remodeling. *Curr Opin Pharmacol* **11**, 720-724, doi:10.1016/j.coph.2011.09.002 (2011).
- 70 Cai, Y., Nagel, D. J., Zhou, Q., Cygnar, K. D., Zhao, H., Li, F., Pi, X., Knight, P. A. & Yan, C. Role of cAMP-phosphodiesterase 1C signaling in regulating growth factor receptor stability, vascular smooth muscle cell growth, migration, and neointimal hyperplasia. *Circ Res* **116**, 1120-1132, doi:10.1161/CIRCRESAHA.116.304408 (2015).
- 71 Goyal, R. & Clyman, R. I. Response to Coceani et al. *Pediatr Res* **82**, 175, doi:10.1038/pr.2017.125 (2017).
- 72 Coceani, F., Scebba, F. & Angeloni, D. Ductus arteriosus: gene profile for fetal maturation versus postnatal closure. *Pediatr Res* **82**, 174, doi:10.1038/pr.2017.124 (2017).
- 73 Cahan, P., Ahmad, A. M., Burke, H., Fu, S., Lai, Y., Florea, L., Dharker, N., Kobriniski, T., Kale, P. & McCaffrey, T. A. List of lists-annotated (LOLA): a database for annotation and comparison of published microarray gene lists. *Gene* **360**, 78-82, doi:10.1016/j.gene.2005.07.008 (2005).
- 74 Cahan, P., Rovegno, F., Mooney, D., Newman, J. C., St Laurent, G., 3rd & McCaffrey, T. A. Meta-analysis of microarray results: challenges, opportunities, and recommendations for standardization. *Gene* **401**, 12-18 (2007).
- 75 Tseng, G. C., Ghosh, D. & Feingold, E. Comprehensive literature review and statistical considerations for microarray meta-analysis. *Nucleic Acids Res* **40**, 3785-3799, doi:10.1093/nar/gkr1265 (2012).
- 76 Eidem, H. R., Ackerman, W. E. t., McGary, K. L., Abbot, P. & Rokas, A. Gestational tissue transcriptomics in term and preterm human pregnancies: a systematic review and meta-analysis. *BMC Med Genomics* **8**, 27, doi:10.1186/s12920-015-0099-8 (2015).
- 77 Hughes, D. A., Kircher, M., He, Z., Guo, S., Fairbrother, G. L., Moreno, C. S., Khaitovich, P. & Stoneking, M. Evaluating intra- and inter-individual variation in the human placental transcriptome. *Genome Biol* **16**, 54, doi:10.1186/s13059-015-0627-z (2015).

- 78 Manoli, T., Gretz, N., Grone, H. J., Kenzelmann, M., Eils, R. & Brors, B. Group testing for pathway analysis improves comparability of different microarray datasets. *Bioinformatics* **22**, 2500-2506, doi:10.1093/bioinformatics/btl424 (2006).
- 79 Levet, S., Ouarne, M., Ciais, D., Coutton, C., Subileau, M., Mallet, C., Ricard, N., Bidart, M., Debillon, T., Faravelli, F., Rooryck, C., Feige, J. J., Tillet, E. & Bailly, S. BMP9 and BMP10 are necessary for proper closure of the ductus arteriosus. *Proc Natl Acad Sci U S A* **112**, E3207-3215, doi:10.1073/pnas.1508386112 (2015).
- 80 Staiculescu, M. C., Kim, J., Mecham, R. P. & Wagenseil, J. E. Mechanical behavior and matrisome gene expression in the aneurysm-prone thoracic aorta of newborn lysyl oxidase knockout mice. *Am J Physiol Heart Circ Physiol* **313**, H446-H456, doi:10.1152/ajpheart.00712.2016 (2017).

Table 1 Summary of Included and Excluded Studies using Microarray to Compare DA and Aorta

Study (ref)	Species	Strain	Description	GSE #	Platform	Probe #	Analysis	Samples	Age	Genes UP	Genes DOWN
Jin 2011 ¹³	<i>Rattus norvegicus</i>	Wistar	Term and preterm DA	3422	Affy Rat U34 A Array	8799	ANOVA	2 DA vs 2 Ao	E21	328	366
Bokenkamp 2014 ⁹	<i>Rattus norvegicus</i>	Wistar	Isolated SMC vs endothelium	51248	Affy Rat 230 2.0 Array	12088	ANOVA	6 DA vs 6 Ao	E21	2453	2347
Hsieh 2014 ¹⁰	<i>Rattus norvegicus</i>	F344	PDA in Brown-Norway rat	40534	Affy Rat 1.0 ST Array	29215	ANOVA	2 DA vs 2 Ao	E21	2027	2336
Shelton 2014 ¹⁴	<i>Mus musculus</i>	CD1 WT	Term DA expression	51664	Affy Mouse 430 2.0 Array	45101	ANOVA	4 DA vs 4 Ao	E19	1532	1958
Costa 2006 ⁴²	<i>Rattus norvegicus</i>	Long-Evans	Oxygen's effect on preterm DA	3290	Affy Rat U34 A,B,C Array	26379	Excluded (preterm only)	–	–	–	–
Yokoyama 2007 ⁴³	<i>Rattus norvegicus</i>	Wistar	Vitamin A and DA maturation	3420	Affy Rat U34 A Array	8799	Excluded (Ao data unavailable)	–	–	–	–
Gruzdev 2012 ²⁶	<i>Mus musculus</i>	129S6	DA from EP4 null mice	NA	Illumina Mouse Ref8 v1.1 Array	24613	Excluded (Ao data unavailable)	–	–	–	–
Liu 2013 ⁴⁴	<i>Rattus norvegicus</i>	Wistar	Term DA endothelium	40500	Affy Rat 1.0 ST Array	29215	Excluded (endo only)	–	–	–	–
Goyal 2016 ¹²	<i>Ovis aries</i>	–	Term and preterm DA	87840	Agilent 019921 Sheep Array	15068	Excluded (incomplete annotation)	–	–	–	–

*Abbreviations: Affy – Affymetrix, ANOVA – analysis of variance, Ao – aorta, DA – ductus arteriosus, E21 – embryonic day 21, NA – not applicable, PDA – persistent patency of the ductus arteriosus, SMC – smooth muscle cell

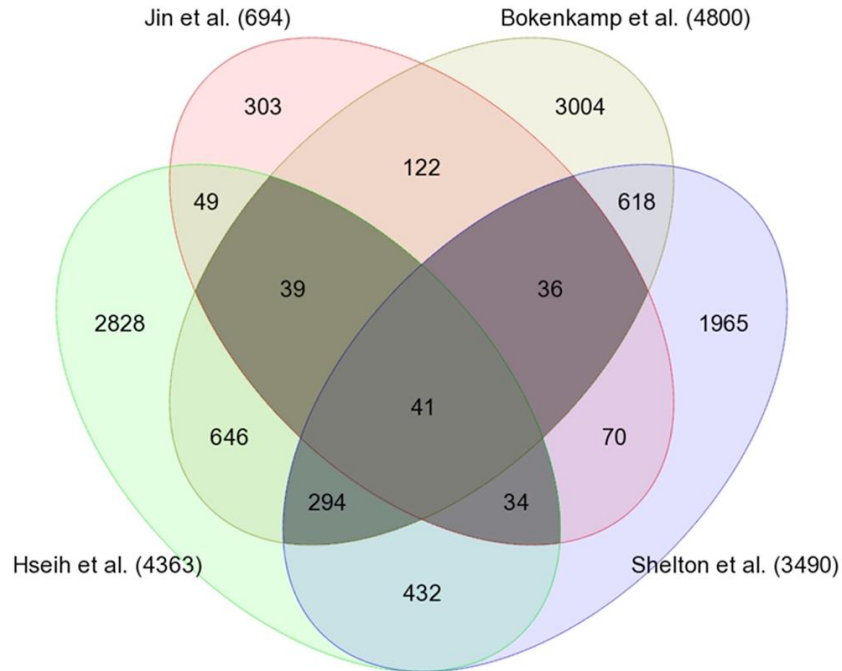


Figure 1. Venn diagram of compared rodent microarray studies. Darkly shaded areas represent 444 DEGs (p -value ≤ 0.05 , fold change ≥ 1.2) identified by at least 3 of 4 studies. Of these, 168 genes had conflicting direction of expression and were excluded from further analysis. Of the remaining 276 genes, 87 were consistently increased, while 189 were consistently decreased in DA versus aorta (genes are listed in Tables S1 and S2, respectively).

Accepted

Table 2 Summary of Genome¹ and Transcriptome² Reads and Alignment

Sample	Vessel	Genomic Reads	Genomic Alignments	Unique Alignments	Non-unique Alignments	Avg. Genomic Coverage	Transcript Reads
122	Ao	3042884	63437690 (87.7%)	24463103 (80.4%)	2215202 (7.3%)	38.6%	26761371
122	DA	6059504	97693434 (46.7%)	23044387 (38.0%)	5276853 (8.7%)	73.9%	28415074
134	Ao	5270068	121687422 (92.9%)	43540599 (82.6%)	5419786 (10.3%)	85.8%	49240999
134	DA	4855205	110777274 (91.1%)	40162140 (82.7%)	4064536 (8.4%)	75.6%	44456169
162	Ao	7558394	151451022 (85.6%)	60853248 (80.5%)	3811368 (5.0%)	72.3%	65157040
162	DA	6149504	124335202 (90.9%)	53260107 (86.6%)	2661866 (4.3%)	54.4%	56289111
208	Ao	7915546	160662212 (91.7%)	69096983 (87.3%)	3482646 (4.4%)	65.2%	73200814
208	DA	8278980	169365096 (90.9%)	71228323 (86.0%)	4003913 (4.8%)	75.9%	75899608
Mean		6037572	124051203 (87.2%)	48909215 (80.6%)	3764205 (6.6%)	67.7%	52427523

¹ Genome reads aligned to hg38² Transcriptome reads aligned to RefSeq Transcripts 83

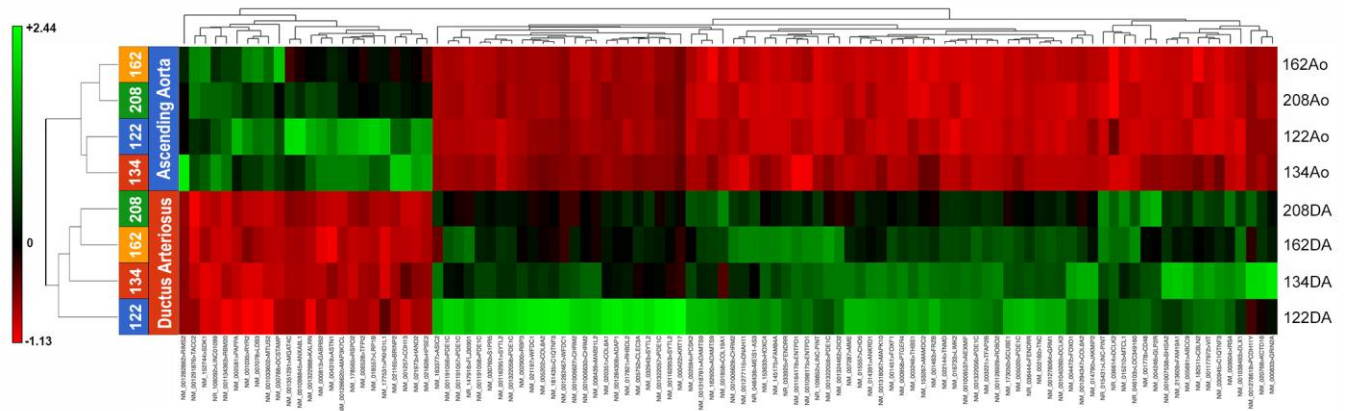


Figure 2. Dendrogram of Human RNA-seq samples. Heat map analysis of RNA-seq data showing separation of samples by vessel identity. Gene identities are specified in Tables S4, S5.

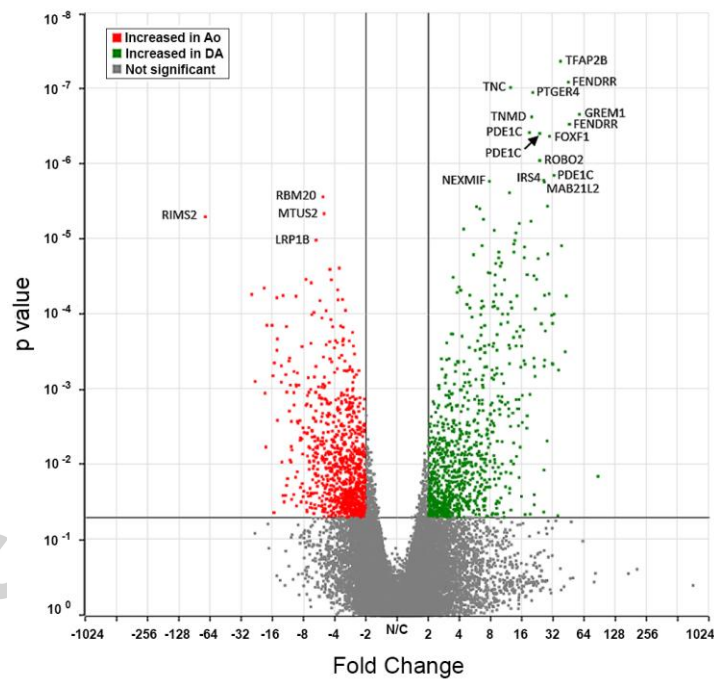


Figure 3. Volcano plot of RNA-seq differentially expressed genes. With p-value 0.05 and fold change >2, 2082 genes showed differential expression in the human DA compared to aorta: 1027 up-regulated, and 1055 down-regulated. Of these, 186 genes had an FDR (Benjamini-Hochberg) of 0.10 or less: 118 up-regulated, and 68 down-regulated (genes are listed in Tables S4 and S5, respectively).

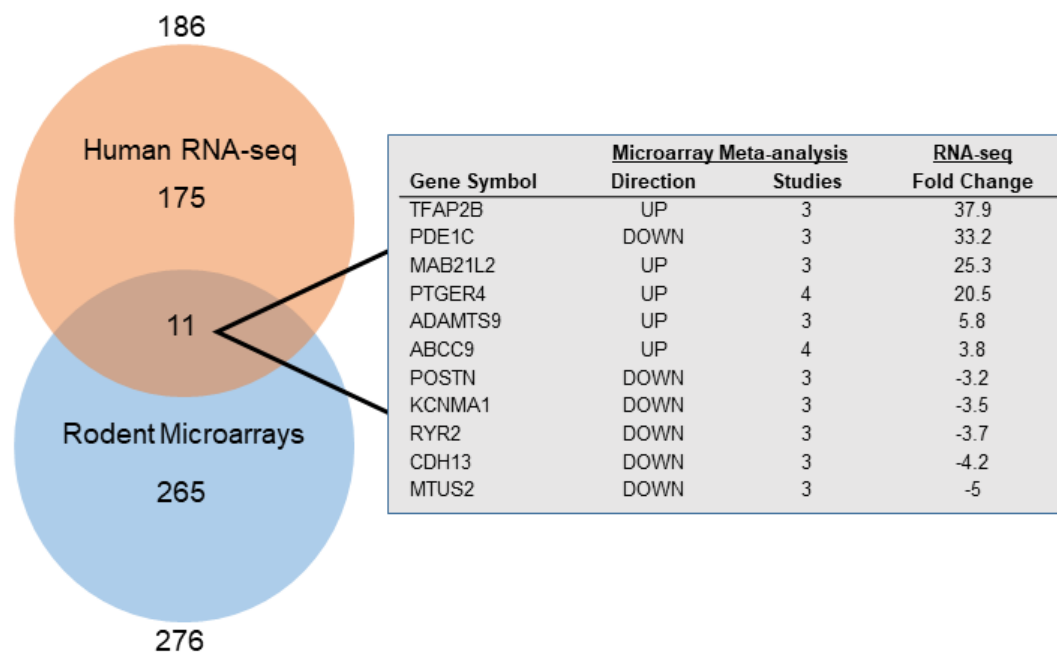


Figure 4. Venn diagram of DA vs. Ao genes common between Microarray and RNA-seq analyses. 11 genes were identified as differentially expressed between DA and aorta in both the human RNA-seq and the rodent microarray comparison. 186 genes from RNA-seq are listed in Tables S4, S5; 276 genes from microarray comparisons are listed in Tables S1, S2.

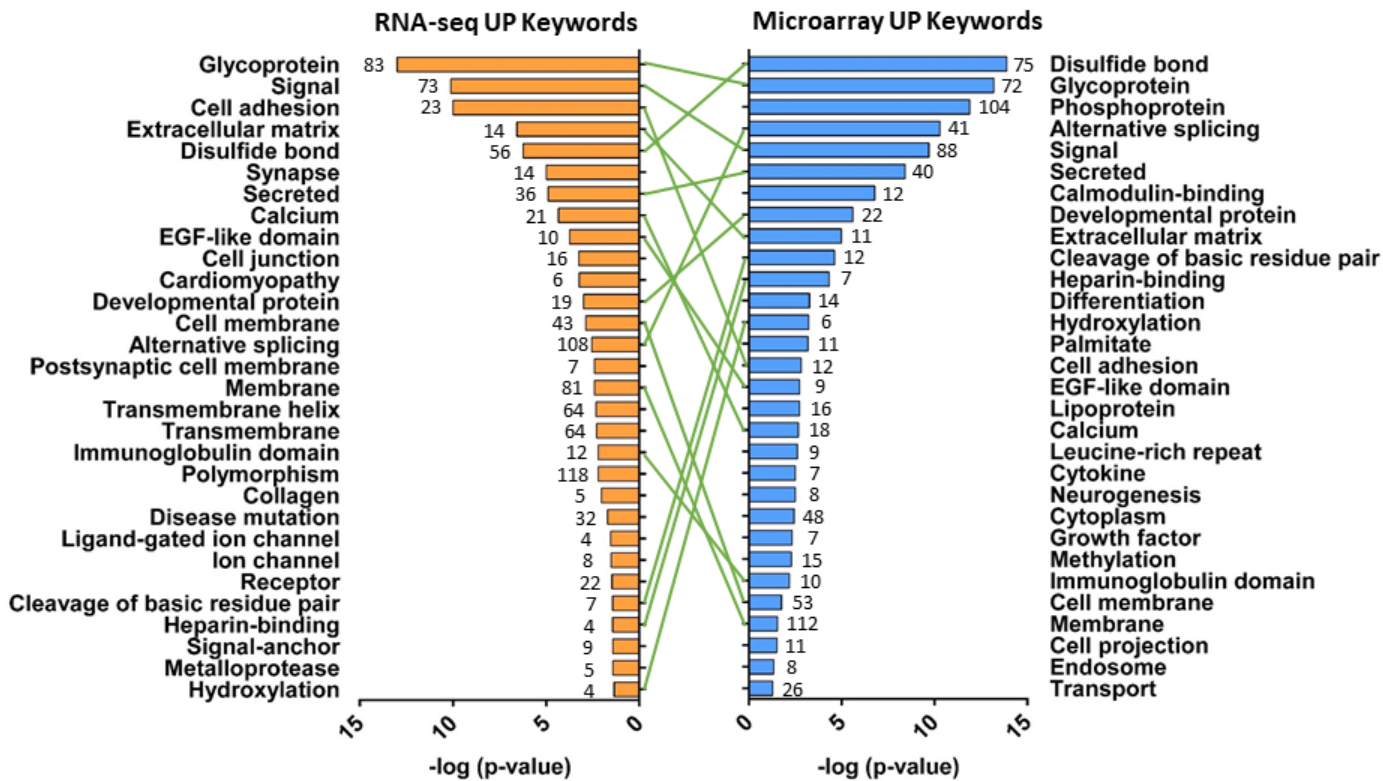


Figure 5. 'Tornadogram' showing top 30 UniProt (UP) Keywords common between Microarray and RNA-seq analyses. Genes differentially expressed in DA vs. Ao were categorized by UP Keywords (DAVID), plotted by p-value, and compared across platforms. Number of genes represented in each category shown at the end of bars.