Supplemental Material

Whole Genome Expression Differences in Human Left and Right Atria Ascertained by RNA-Sequencing

Jeffrey Hsu, BA , Peter Hanna, BA, David R. Van Wagoner, PhD, John Barnard, PhD, David Serre, PhD, Mina K. Chung, MD, and Jonathan D. Smith, PhD

Supplemental Figures and Tables:

Supplemental Table 1: Taqman primer and probe design

Identifier	Sequence
Pitx2c Forward Primer	5'-GCG GTT CCT CTG GAA AGT GG-3'
Pitx2c Left Primer	5'-GCA CAC CAT CTC CGA CAC CT-3'
Probe*	5'-/56-FAM/CCC GGA GGC /ZEN/CGC AGA GAA AGATAA /3IABkFQ/-3'

*Fam fluorophore with Internal Zen and 3' Iowa Black FQ quencher modifications

Supplemental Table 2: Sample Summary

Sample ID	Age	Gender	AF status	Procedures (cardiac diagnoses)	LVEF	LA area, cm^2
1	63	M	Permanent AF*	Mitral valve repair, CABG, Maze (4+ MR, CAD)	65%	32
2	64	F	Permanent AF*	Mitral and tricuspid valve repair, Maze (4+ MR, 2-3+TR))	70%	45
3	32	F	None†	unused tissue donor	unknown	unknown
4	55	M	Permanent AF*	Mitral valve repair, CABG, Maze (flail 4+MR, CAD)	55%	52

* Permanent AF diagnosis and AF during surgery

†No history of atrial fibrillation presumed

Supplemental Table 3: Sequencing Summary of the miRNA reads.

ID	Tissue	Number of Clusters (x 10^6)	Aligned Uniquely (x 10^6)	%Aligned Uniquely	Aligned to known miRNA (x 10^6)	% Aligned to miRBase[*]
1	Right	33.2	11.3	34.1%	11.6	35.1%
1	Left	30.9	12.0	38.7%	12.7	41.0%
2	Right	33.4	11.8	35.5%	14.9	44.7%
2	Left	34.1	8.9	26.2%	14.2	41.6%
3	Right	32.3	13.5	41.8%	17.1	53.0%
3	Left	29.4	12.3	42.0%	11.3	38.4%
4	Right	28.6	9.4	33.0%	7.7	27.2%
4	Left	27.1	10.1	37.2%	7.7	28.5%

[*] In order to account for reads that aligned to multiple mature miRNAs, reads were aligned to only miRBase annotated hairpins and collapsed to their mature miRNAs.

Supplemental Table 4: Sequencing Summary of the mRNA reads.

Sample ID	Tissue	Number of Clusters (x 10^6)	% of total that are properly aligned*	Mapped to RefSeq transcripts (x 10^6)	% of total reads that mapped to RefSeq
1	Right	29.5	88%	20.8	70%
1	Left	27.2	83%	17.7	65%
2	Right	35.4	89%	28.6	81%
2	Left	24.3	86%	16.3	67%
3	Right	37.6	89%	29.9	79%
3	Left	31.1	88%	21.0	67%
4	Right	33.4	85%	21.4	64%
4	Left	41.1	70%	19.2	47%

* Paired ends were aligned at the same locus in convergent orientations.

Supplemental Table 5. Highest expressed miRNAs in the left and right atria.

Left Atria	% Total miRNAs reads	SD	Right atria	% Total miRNAs reads	SD
hsa-miR-143	32.7	1.5	hsa-miR-143	26.7	1.8
hsa-miR-378	11.0	3.3	hsa-miR-24	9.4	1
hsa-miR-24	8.7	2.4	hsa-miR-378	8.6	1.6
hsa-miR-1	7.1	2.5	hsa-miR-1	6.4	1.3
hsa-miR-30d	3.9	1.1	hsa-miR-30d	3.9	0.9
hsa-miR-21	3.0	0.4	hsa-let-7f	2.8	0.4

Supplemental Table 6: Top 10left-right atria differentially regulated GO terms.

GO Term	Right*	Left*	Hypothesis
SIGNAL TRANSDUCTION	205	94	Enriched in RA
INTRINSIC TO MEMBRANE	203	82	Enriched in RA
INTEGRAL TO MEMBRANE	198	79	Enriched in RA
CELL SURFACE RECEPTOR LINKED SIGNAL TRANSDUCTION	102	38	Enriched in RA
RECEPTOR ACTIVITY	97	30	Enriched in RA
NUCLEOBASE NUCLEOSIDE NUCLEOTIDE AND NUCLEIC ACID METABOLIC PROCESS	94	51	Enriched in RA
REGULATION OF METABOLIC PROCESS	80	35	Enriched in RA
REGULATION OF CELLULAR METABOLIC PROCESS	78	34	Enriched in RA
TRANSCRIPTION	69	28	Enriched in RA
REGULATION OF GENE EXPRESSION	67	25	Enriched in RA
CYTOPLASM	128	133	Enriched in LA
CYTOPLASMIC PART	74	93	Enriched in LA
ORGANELLE PART	46	77	Enriched in LA
INTRACELLULAR ORGANELLE PART	46	77	Enriched in LA
PROTEIN METABOLIC PROCESS	116	70	Enriched in LA
CELLULAR PROTEIN METABOLIC PROCESS	99	63	Enriched in LA
CELLULAR MACROMOLECULE METABOLIC PROCESS	99	63	Enriched in LA
MACROMOLECULAR COMPLEX	55	56	Enriched in LA
MITOCHONDRION	6	52	Enriched in LA
PROTEIN COMPLEX	54	47	Enriched in LA

Genesets were ordered with the highest number of up regulated genes in the right or left atria at the top, and by having the minimum p-value (10^{-4}) under the up or down hypothesis.
*Number of genes from the geneset that are contained within those found to be expressed higher in the left or right atria via the edgeR analysis (FDR < 0.05).

Supplemental Table7. Left-right atrial expression differences in transcripts close to SNPs associated with atrial fibrillation or PR interval identified through genome wide association studies.

Top Associated SNP	Reference	Gene	Expressed in Atria	L-R difference (FDR)	Expressed Higher
rs2200733	(1)	PITX2	Yes	5.20E-68	Left
rs1337633	(2)	KCNN3	No	NS	
		PMVK	Yes	NS	
		ADAR	Yes	NS	
		CHRNB2	No	NS	
rs1303809	(2)	SULF2	Yes	0.004	Right
		NCOA3	Yes	NS	
rs2106261	(3)	ZFHX3	Yes	NS	
		HTA	No	NS	
rs1737590	(3)	MTHFR	Yes	NS	
		CCLCN6	NA	NS	
rs11897119	(4)	MEIS1	Yes	NS	
rs11708996	(4)	SCN5A	Yes	NS	
rs6800541	(4)	SCN10A	No	NS	
rs7692808	(4)	ARHGAP24	Yes	NS	
rs251253	(4)	NKX2-5	Yes	NS	
rs3807989	(4)	CAV1	Yes	NS	
		CAV2	Yes	NS	
rs4944092	(4)	WNT11	Yes	0.04	Right
rs11047543	(4)	SOX5	No	NS	
rs1896312	(4)	TBX5	Yes	NS	
		TBX3	Yes	NS	

Supplemental Table 8. Left-right atrial miRNA expression differences after removing the non-atrial fibrillation sample #3. Of the top 26 miRNAs found to be significantly differentially expressed at the FDR 0.05 level using only the three AF sample pairs, 21 were found to be significantly differentially expressed using all four sample pairs (Table 1 in the manuscript).

miRNA	logConc	logFC	LR	P.Value	adj.P.Val	All samples (rank order)
hsa-miR-135b	-11.40	-3.16	79.03	6.13E-19	1.84E-16	3
hsa-miR-10b	-7.35	-2.21	50.59	1.14E-12	2.28E-10	1
hsa-miR-215	-10.65	-1.75	35.57	2.46E-09	3.68E-07	#N/A
hsa-miR-100	-4.35	1.58	34.21	4.95E-09	5.94E-07	2
hsa-miR-487a	-11.22	-1.25	19.27	1.13E-05	5.23E-04	4
hsa-miR-31	-9.25	-1.62	17.52	2.84E-05	1.14E-03	#N/A
hsa-miR-4792	-7.93	-1.23	16.31	5.38E-05	1.90E-03	25
hsa-miR-9	-9.01	1.02	15.58	7.92E-05	2.38E-03	10
hsa-miR-217	-9.89	-1.74	15.24	9.47E-05	2.71E-03	#N/A
hsa-miR-708	-9.66	-1.01	14.78	1.21E-04	3.30E-03	18
hsa-miR-4448	-11.11	0.97	10.64	1.11E-03	2.21E-02	5
hsa-miR-24-1*	-9.06	-0.94	10.33	1.31E-03	2.32E-02	21
hsa-miR-125b-1*	-10.64	0.87	10.32	1.32E-03	2.32E-02	12
hsa-miR-216a	-10.56	-1.59	10.00	1.57E-03	2.61E-02	#N/A
hsa-miR-585	-11.00	0.91	9.99	1.57E-03	2.61E-02	6
hsa-miR-1275	-11.01	0.87	9.95	1.61E-03	2.61E-02	7
hsa-miR-1247	-13.55	1.47	9.85	1.70E-03	2.69E-02	#N/A
hsa-miR-329	-11.97	-0.94	9.70	1.85E-03	2.84E-02	#N/A
hsa-miR-425	-6.90	-0.80	9.57	1.98E-03	2.91E-02	14
hsa-miR-708*	-12.54	-1.03	9.56	1.99E-03	2.91E-02	18
hsa-miR-1270	-13.00	1.17	9.36	2.22E-03	3.16E-02	#N/A
hsa-miR-483-3p	-11.77	-0.95	9.29	2.30E-03	3.16E-02	#N/A
hsa-miR-483-5p	-10.62	-0.92	9.28	2.31E-03	3.16E-02	8
hsa-miR-1248	-12.61	1.12	9.21	2.41E-03	3.17E-02	#N/A
hsa-miR-92b*	-12.49	1.17	9.19	2.43E-03	3.17E-02	16
hsa-miR-9*	-11.48	0.87	9.14	2.50E-03	3.19E-02	10
hsa-miR-155	-9.02	0.84	9.05	2.63E-03	3.29E-02	23
hsa-miR-4497	-10.00	0.88	8.91	2.84E-03	3.48E-02	13
hsa-miR-376c	-9.62	-0.83	8.74	3.11E-03	3.73E-02	24
hsa-miR-150	-8.93	-0.76	8.61	3.34E-03	3.93E-02	17
hsa-miR-410	-11.03	-0.84	8.55	0.003454	0.039855	#N/A
hsa-miR-125b	-4.99	0.75	8.50	0.003561	0.040311	15

Supplemental Table 9.Left-right atrial mRNA expression differences after removing the non-atrial fibrillation sample #3. The rank order of the top 10 significantly differentially expressed mRNA excluding sample #3 were compared to the rank order in which all samples were used (Table 2 in the manuscript). Although the rank ordering does change, all the significant genes within the top 10 are also significant in the previous analysis, and the top 3 genes remain the same.

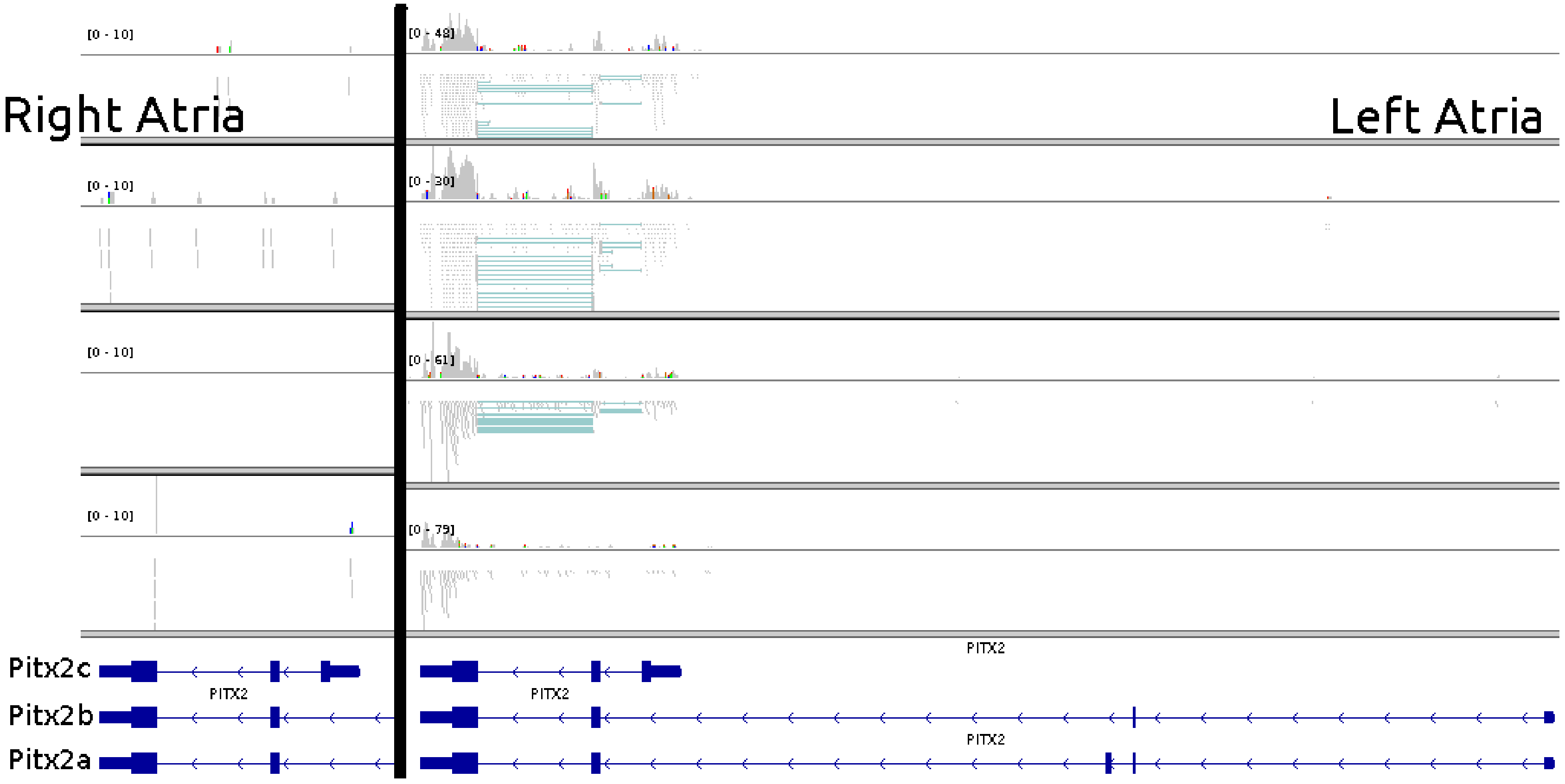
	logConc	**logFC**	**LR**	**P.Value**	**adj.P.Val**	**All samples (rank order)**	**adj.P.Val**
BMP10	-7.52	7.85	439.95	1.11E-97	1.69E-93	2	1.24E-92
HAMP	-9.84	7.08	347.37	1.58E-77	1.20E-73	1	2.77E-107
PITX2	-11.69	-6.26	226.27	3.88E-51	1.96E-47	3	5.20E-68
MSLN	-9.89	-4.84	177.85	1.43E-40	5.42E-37	37	3.14E-18
ANXA8L2	-10.34	-4.86	174.39	8.15E-40	2.19E-36	175	5.51E-09
ANXA8	-10.38	-4.85	174.27	8.63E-40	2.19E-36	135	6.15E-10
ANXA8L1	-10.38	-4.85	173.93	1.03E-39	2.23E-36	136	6.15E-10
C2orf14	-12.17	6.76	165.99	5.57E-38	1.06E-34	4	5.13E-45
SYT4	-11.54	-5.29	138.03	7.18E-32	1.21E-28	16	2.17E-23
COMP	-11.41	-3.87	137.61	8.87E-32	1.35E-28	163	2.32E-09

Supplemental Table 10.Left-right atrial non-coding RNA expression differences from Table 5 after removing the non-atrial fibrillation sample #3.

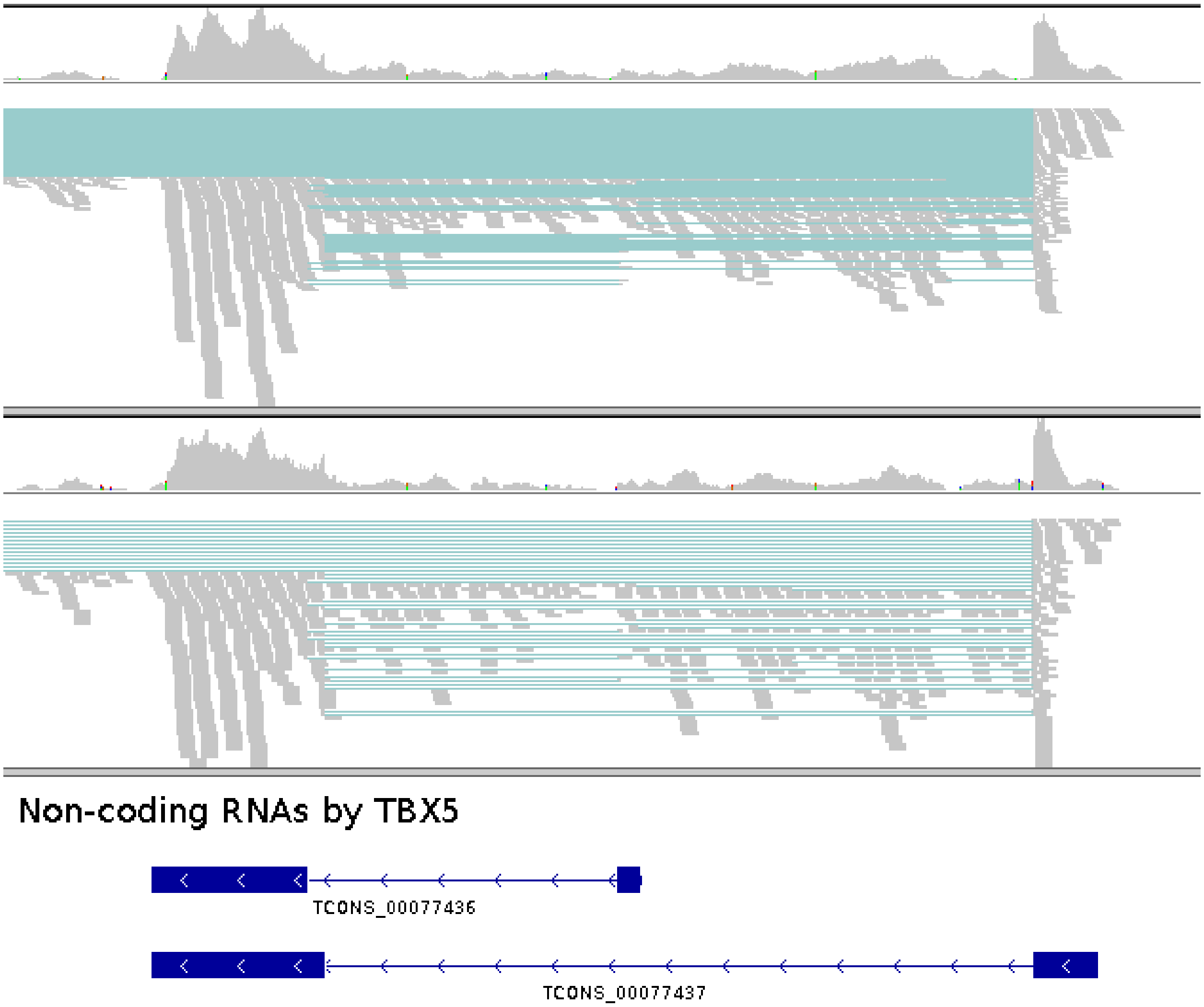
Transcript boundary	Nearest gene	Exons	Absolute Fold Change	Expressed Higher	Significant
chr12:114883593-114885094	*TBX5*	2	1.498767	Right	yes
chr6:36810354-36812333	*CPNE5*	2	2.122528	Left	yes
chr12:58325277-58329330	*XRCC6BP1*	2	No test*	No Test	No test
chr2:27939711-27961143	*SLC4A1*	2	1.93741	Left	yes
chr2:50999236-51003553	*NRXN1**	2	1.363425	Left	yes
chr19:50989460-51003531	*JOSD2*	3	1.993716	Right	yes
chr15:25243485-25247622	*SNOR108*	4	2.033991	Right	yes
chr19:50990647-50999173	*C19orf63*	2	2.823564	Right	yes
chr16:58467328-58496760	*NDRG4*	3	2.001826	Left	yes
chr13:114054026-114066562	*ADPRHL1*	2	1.221271	Left	yes
chr19:50991540-51005189	*JOSD2*	2	1.733253	Left	yes

*, After removal of sample pair #3, this transcript had ≤ 10 reads across the three remaining sample pairs and was excluded in the Cuffdiff program within Cufflinks from differential transcript isoform calling between the left and right atria.

Supplemental Figure 1: *PITX2* is expressed specifically in the left atria. There are three common PITX2 splice isoforms, and of the isoform specific splice junctions (reads highlighted in light blue), only evidence for PITX2c expression was observed.


Right Atria
Left Atria
[0 - 10]
[0 - 48]
[0 - 10]
[0 - 30]
[0 - 10]
[0 - 61]
[0 - 10]
[0 - 79]
PITX2
Pitx2c
PITX2
PITX2
Pitx2b
PITX2
Pitx2a

Supplemental Figure 2: Non-coding RNAs by TBX5. TCONS_00077436 was found to be differentially expressed between the left and right atria.



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