The renin–angiotensin system revisited: new genomic and clinical directions

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Experimental Data

- Constant infusion of AII over a 3-day period in rabbits
- Transient BP rise, followed by maintenance of BP in normal range
- Pathology showed multiple areas of myocardial necrosis, and, less consistently, renal tubular necrosis







Clinical Observations

- Five patients with end-stage renal disease and excessively high AII levels sustained multiple acute coronary episodes
- Three succumbed: Post-mortem showed multiple areas of myocardial necrosis
- Two survived: After bilateral nephrectomy, no more coronary events





ANGIOTENSIN I

Asp — Arg — Val — Tyr — Val — His — Pro — Phe

ANGIOTENSIN I INHIBITOR







 Following the sequencing of the Human, Mouse and Rat Genome and the development of several methods for gene expression analysis, (Microarrays, SAGE, TcDD etc) new opportunities are arising for investigation of new genes or for discovery of new gene interactions.

Serial Analysis of Gene Expression (S.A.G.E.) Velculescu et al. (1995) Science 270: 484

- 10bp unique fragment can identify a gene through Gene Bank search
- High throughput
- Quantitative
- Unbiased
- Identify known genes & ESTs & novel transcripts

EXPERIMENTAL DESIGN



Day	Blood Pressure					
3	112	124	148	153		
4	112	126	124	163		
5	93	105	143	163		
6	88	115	137	144		
7	97	115	157	155		



Functional Category	Tag Sequence	C-7dl	tag # I A-7dH	Pval	Unigene ID	Accession#	Gene
Protein	GCCCGGGAAT	9	26	0.00239	276337	XM_355500	Rpl17 (ribosomal protein L17)
Synthesis	TGGTGACAAA	2	12	0.00600	280083	NM_024212	Rpl4 (ribosomal protein L4)
	GCCTAATGTA	5	15	0.01843	212197	XM_354905	Similar to ribosomal protein L21
	CAAGGAGCTA	0	5	0.03287	195628	NM_025544	Mrps15 (mitochondrial ribosomal protein S15)
	CCTCGGAAAA	0	5	0.03287	345415	NM_023372	Rpl38 (ribosomal protein L38)
	TCTTCTCACA	1	7	0.03291	30478	BC039092	Rpl39 (ribosomal protein L39)
	CTAGTCTTTG	17	29	0.04986	354387	NM_009093	Rps29 (ribosomal protein S29)
ECM	CAAACTCTCA	5	31	0.00001	291442	NM_009242	*Sparc (secreted acidic cysteine rich glycoprotein; alias: osteonectin)
	CCAACGCTTT	0	9	0.00187	193099	XM_129845	*Fn1 (fibronectin 1)
	TGTTCATCTT	2	11	0.01100	249555	NM_009930	Col3a1 (procollagen type III α1)
	TAAGAACATA	0	5	0.03287	233547	AF011450	Col15a1 (procollagen, type XV α1)
Cytoskeletal	GCTCCCACAC	116	79	0.00657	27674	NM_009406	Tnni3 troponin I, cardiac
Organization	GAACATTGCA	0	7	0.00701	239554	NM_178440	*Myolg (myosin IG)
	AGGAAGAGCG	97	64	0.00848	290003	M76600	Myh6 (myosin, heavy polypeptide 6, cardiac)
	CTTGTTTTGA	7	0	0.00851	29733	NM_011918	"Ldb3 (LIM domain binding 3)
	CCAGGCCTTA	0	6	0.01563	272368	BC031922	*Crip1 (cysteine-rich protein 1)
	GGAGGTGGCA	13	25	0.03005	17235	NM_013808	Csrp3 (cysteine and glycine-rich protein 3)
	GGGGAAATCG	0	5	0.03287	3532	BC038926	Tmsb10 thymosin, beta 10
	GGCTGTTGAA	0	5	0.03287	196484	NM_007791	*Csrp1 (cysteine and glycine-rich protein 1)
	CCCGGGTACA	0	5	0.03287	274346	NM_011876	Ptk91 (protein tyrosine kinase 9-like; alias: twinfilin 2)
	GATTATGGCT	5	0	0.03347	295205	NM_178680	**Cmya4 (cardiomyopathy associated 4)
Calcium	TCATCTTTAA	6	0	0.01818	1971	BC003453	Calr calreticulin
Homeostasis	TTGTGGTGAT	14	5	0.03394	34145	NM_023129	Pln phospholamban
Intracellular Signaling	TAGGGAGATG	17	45	0.00017	10279	AK009959	Ankrd1 (ankyrin repeat domain 1, cardiac; alias: CARP)
	CCCCCGCAGG	21	41	0.00668	19961	XM_131840	"LOC230899, Similar to atrial natriuretic peptide precursor
	GCTCCCCCAC	5	0	0.03347	25547	NM 025331	Gng11 (quanine nucleotide binding gamma11)
Transcription	TAGTTACTTA	173	116	0.00099	347564	AK033799	**7 dbhc14 (zinc finger DHHC domain
Factors	TCGGCGCAGA	11	3	0.02990	181852	NM 175606	containing 14) Hod (homeobox only domain)
Defense	CTATCCTCTC	0	5	0.03287	200916	NM 008161	*Gpx3 (glutathione peroxidase 3)
	GAGGACTGCC	0	7	0.00701	788	BC019113	*Ly6e (lymphocyte antigen 6 locus E)
Oxvgen	CTCAGGTCTC	113	82	0.02229	201606	NM 013593	Mb (myoglobin)
Transport	CCCTTCTTCT	47	98	0.00000	196110	BC043020	*Hba-a1 (hemoglobin alpha, adult chain 1)
	TGGATCCTGA	59	78	0.04894	288567	NM 008220	Hbb-y (hemoglobin Y, beta-like embryonic)
Proteases	GTTTGCTGTG	3	11	0.02549	236553	BC006656	Ctsb (cathepsin B)
	TACTGGGAGC	0	6	0.01563	2277	BC006878	Ctsh (cathepsin H)
Protease	GGTCGTGTAT	0	6	0.01563	38888	BC002026	"Serping1 (serine proteinase inhibitor, clade G
Transport	CCAGCAAACA	8	0	0.00401	16228	XM 134169	***Slc25a4 (solute carrier family 25 member 4)
RNA	AACCCTAATA	57	29	0.00222	41867	NM 198033	**AW060766 EST
Processing	GTGTAATAAG	0	6	0.01563	155896	AF073993	Hnrpa2b1 (heterogeneous nuclear ribonucleoprotein A2/B1)
Protein	GAGCGTTTTG	1	10	0.00586	5246	NM 008907	*Ppia (peptidylprolyl isomerase A)
Folding	GGTACACCGC	6	0	0.01818	12758	NM 010219	Fkbp4 (FK 506 binding protein 4)

Differentially Expressed Genes Identified by SAGE

Functional	Tag Sequence	C-7d	tag #	Dval	Unigene	Accession #	Сепе
Energy/	CCCTGCCTTA	30	10	0.00002	2375	X03233	*Ckm (creating kingse muscle)
Metabolism	AAACCCCCAG	223	159	0.00116	MITO	101420	Cox1 (cytochrome oxidase subunit 1)
	GGGAATCACG	10	0	0.00132	41502	AK002379	Fh1 (fumarate hydratase 1)
	CAAACCTCCA	86	53	0.00455	MITO	101420	12S ribosomal RNA mitochondrial
	CAGAATGTGC	12	2	0.00499	29867	BC006815	*Ndufa2 (NADH dehydrogenase 1 alpha
	AGGAGGACTT	254	200	0.01069	MITO	J01420	subcomplex 2) NADH dehydrogenase subunit 2
	CAGGCCACAC	73	47	0.01492	238973	NM 016774	Atp5b (ATP synthese H+ transporting
							F1complex β subunit)
	CCAATGCAGC	8	1	0.02161	13445	BC003422	Oxct 3 (oxoacid CoA transferase)
	ACGCTGACTC	31	16	0.02459	12907	AF037370	Cox7a1 (cytochrome c oxidase subunit VII alpha 1)
	GGTCACACTA	25	12	0.02627	181933	NM_025710	*Uqcrfs1 (ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1)
	AGCAAAAGCC	196	156	0.02704	MITO	J01420	Mitochondrial sequence
	AACGGCTAAA	100	73	0.03029	MITO	J01420	16S ribosomal RNA
	AGGACAAATA	388	331	0.03261	MITO	J01420	Cytochrome b
	TAGCTGTAAC	5	0	0.03347	280103	NM_144900	Atp1a1 (Na+/K+ ATPase alpha 1 polypeptide
	ACATTTCTGT	5	0	0.03347	298947	NM_010027	*Ddt (D-dopachrome tautomerase)
	AAGCTCGAAA	7	1	0.03939	10406	NM_025848	Sdhd (succinate dehydrogenase, subunit D)
	CACGGGACCA	30	17	0.04438	181721	BC011388	2610041P16Rik
	AGGGCACTGG	13	5	0.05111	27396	BC048668	MGC:58837 (cytochrome c oxidase copper chaperone)
	ATAATACATA	415	537	0.00001	MITO	J01420	ATPase subunit 6
	ACCCATCGGA	1	7	0.03688	29939	BC058378	Ndufa9 (NADH dehydrogenase 1 alpha subcomplex 9)
	AAGGATGTGC	24	9	0.00833	21162	BC037149	*Grim19
	GCTTAAGTGT	0	5	0.03287	280075	NM_007874	Dp1 (deleted in polyposis 1)
	TTAAGGGGGA	17	2	0.00043	200497	BC058569	Hadha (hydroxyacyl-Coenzyme A dehydrogenase, alpha subunit)
	ACCAAGCCTA	5	5	0.00979	22220	NM_010174	*Echi (enoyi coenzyme A nydratase 1, peroxisomal) **Eabp3 (fatty acid hinding protein 3, muscle
		-		0.000-17			and heart)
	ATTGTTCACT	5	0	0.03347	255499	NM_019966	MIycd (malonyl-CoA decarboxylase)
	GGGTTCTTCC	9	2	0.03429	260164	NM_008212	Hadhsc (L-3-hydroxyacyl-Coenzyme A dehydrogenase, short chain)
Unclassified	CTAATAAAGC	5	15	0.01843	329631	NM_007990	Fau FBR-MuSV
Unknown Function	TGGGTTTAGA	7	0	0.00851	347915	NM_019814	*Hig1 (hypoxia induced gene 1)
	GGCTAAAGTT	0	8	0.00374	261043	AK009221	*2310008C07Rik
	CAGTTCTAGC	5	0	0.03347	41180	NM_021430	*2900002H16Rik
	GCCGCGAAGG	7	1	0.03939	59139	NM_134147	*D930010J01Rik
	TGTTGGTACG	23	4	0.00019	347120	NM_176841	***A430106J12Rik
	ATTTTTAAAA	6	20	0.00390	29813	NM_025856	**1700029G01Rik
	GCTGCCTTCC	26	8	0.00177	333769	AK090265	EST
	CTGAGGAAGT	0	6	0.01563	239581	BY544832	EST

Q-RT-PCR Verification of Differential Expression of a Sample of Genes Identified by SAGE



Difference in expression in mouse heart after Angll 7days infusion



The relative positions of the A530024P18 (Cmya3) gene and the 231008C07Rik EST on mouse chromosome 2



Targeting strategy of myomaxin gene and myomaxin hypomorphic allele











kDa

-200



Unstressed myomaxin hypertophic mice display cardiac hypertrophy



Fibrosis and apoptosis in unstressed myomaxin hypomorhic mice

в



WT Hypo

Blunted response in myomaxin hypomorphic mice to Angll-induced cardiac hypertrophy

Diminished pathological cardiac remodeling and hypertension in Angll-induced myomaxin hypomorphic mice

Daseline 2week

Нуро

WT

Elevated expression of hypertrophic marker genes in Angll-infused hypomorphic mice

Angll regulates myomaxin promotor through MEF2A/STAT1 cooperativity

Conclusions

- 1. Ang Il-induced cardiomyopathy is caracterized by upregulation of the newly discovered Cmya3 or myomaxin gene.
- 2. Overexpression of myomaxin is not a hemodynamic response to hypertension, as it occurs in Ang II-induced , but not salt-induced hypertension.
- 3. Transgenic mice with myomaxin hypomorphic hearts have attenuated LV hypertrophy, dilatation, fibrosis and apoptosis in response to Ang II.
- 4. Ang II downstream signaling requires activation of a MEF2A-myomaxin pathway to produce maladaptive cardiac remodeling.