

ONLINE DATA SUPPLEMENT

SALT-SENSITIVITY OF BLOOD PRESSURE IS ASSOCIATED WITH POLYMORPHISMS IN THE SODIUM-BICARBONATE CO-TRANSPORTER

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Rationale for Candidate Genes Selected for Study

We employed two major criteria to determine the genes to study: (1) genes from genome-wide association study (GWAS) results; and (2) genes that encode proteins known to be involved with sodium homeostasis.

We studied genes other than those reported in GWAS because as Harrap has concluded, “those genetic loci accounted for only about 2% of the genetic factors believed to influence blood pressure” (*J Am Soc Hypertens* 2009;3:231-237).

The association of 30 gene regions encoding known anti-hypertensive drug targets was recently studied. It was found that ADRB1 rs1801253, AGT rs2004776, and ACE rs4305 are associated with blood pressure or hypertension (*Hypertension* 2011;57:903-910). We also studied genes that are in chromosomal loci linked to blood pressure-related phenotypes (e.g., 11p15.4 linked to hypertension in GWAS in the CHARGE and Global BP reports (*J Am Soc Hypertens* 2009;3:231-237), i.e., cholecystokin receptor type B (CCKBR, 11p15.4). Chromosome 4p16 has been linked to hypertension in several studies (*Hypertension* 2001;38:773-778; *Hum Mol Genet* 2006;15:1735-1743); Some genes that express proteins regulating renal sodium transport are in or near this locus, including α -adducin, G protein-coupled receptor kinase type 4 [GRK4 (4p16.3)], and protein phosphatase [PP2R2C (4p16.1)]. CCKAR (4p15.1) was also studied because sodium-lithium counter transport (SLC) activity, an intermediate phenotype of primary hypertension, has been linked to a region of baboon chromosome 5, homologous to human chromosome 4p (*Am J Hypertens* 2009;22:288-293).

Deletion of dopamine receptor genes in mice (*Curr Hypertens Rep* 2011;13:55-66), e.g., DRD2 (D₂ dopamine receptor) in mice causes salt-sensitive hypertension (*Am J Hypertens* 2003;16:853-858); DRD2 gene variants have been associated with hypertension (*Clin Endocrinol* 2001;55:605-611; *Int J Cardiol* 2005;102:111-116, *J Hum Hypertens* 2001;15:553-558). GRKs are important in the desensitization of G protein-coupled receptors. Some GRK4 gene variants may be associated with salt-sensitivity (*Curr Hypertens Rep* 2011;13:55-66) and sorting nexins may be important in the resensitization of G protein-coupled receptors (*Traffic* 2010;11:508-519). We chose SNX1, SNX5, and SNX19 because they regulate dopamine receptors [*J Clin Hypertens* 2011;13(Suppl 1):A110]; *Hypertension* 2010;56:E123-E123). eNOS G894T has been reported to be associated with hypertension in some studies (*J Hypertens* 2007;25:1763-1774). Caveolin-1 is included because of a study associating caveolin-1 with low blood pressure (*Thromb Haemost* 2006;95:696-701).

SLC4A5 is located in chromosome 2p13. Fasting insulin has been linked to 2p13.2 (*Diabetes* 2007;56:137-142) and insulin resistance is associated with salt-sensitivity (*Nat Rev Cardiol* 2010;7:577-584). Because increased sodium transport is involved in genetic hypertension (*Harvey Lect* 2004-2005;100:71-101) and SLC4A5 is expressed in the nephron (*Am J Physiol Regul Integr Comp Physiol* 2007;293:R2136-2146; *Hum Mol Genet* 2012;21:1025-1036) it made an excellent candidate.

SLC4A5 has also been associated with hypertension (*Hypertension* 2006;47:532-536; *Hypertension* 2004;43:477-482.). The "C" allele of rs8179526 (allele frequency of 0.445) was associated with higher systolic blood pressure (*Biol Res Nurs* 2011 Aug 22. [Epub ahead of print]). In Caucasians, rs6731545 and rs7571842 were significantly associated with resting and sub-maximal exercise pulse pressure (PP) ($0.0004 < P < 0.0007$ and $0.002 < P < 0.003$, respectively). Additionally, rs6731545 was associated with sub-maximal-exercise systolic blood pressure and rate pressure product (both $P = 0.002$) (*Eur J Hum Genet* 2009;17:1481-1489). In these Utah pedigrees, the SLC4A5 gene (SNP hcv1137534) was significantly associated with blood pressure and the association persisted after 10 years of follow-up. These results additionally confirm the involvement of SLC4A5 with blood pressure control, although the mechanism is unclear (*Hypertension* 2006;47:532-536). In our studies, rs8179526 (SLC4A5) was significantly associated with systolic blood pressure adjusted for age, and BMI ($P = .02$). Our results relate SLC4A5 to salt-sensitivity, supporting a recent report in which its deletion in mice "induces arterial hypertension and renal metabolic acidosis" (*Hum Mol Genet* 2012;21:1025-1036).

TABLE S1. HyperPATH Demographic and Clinical Data**A. HyperPATH Demographic Data**

<u>Pre-Study Screen</u>	<u>HTN</u>
Number of subjects	211
Male	129
Female	82
Age (yrs)	49.2±8.4
BMI	26.1±2.4
Systolic BP (mmHg)	147.5±19.1
Diastolic BP (mmHg)	88.8±12.1
MAP (mmHg)	105.8±13.4

B. HyperPATH Salt-Sensitivity Data

<u>Baseline</u>	<u>Salt-Sensitive Subjects</u>	<u>Salt -Resistant Subjects</u>
Number of subjects	140	71
Male	89	40
Female	51	31
Age (yrs)	49.8 ± 8.4	48.1± 8.4
BMI	26.1 ± 2.4	26.1 ± 2.6
<u>After High Salt diet</u>		
Systolic (mmHg)	151.05 ± 17.4	140.4 ± 20.4
Diastolic (mmHg)	91.9 ± 10.3	82.6 ± 12.9
MAP (mmHg)	109.0 ± 11.2	96.7 ± 15.3
Heart Rate (Beats/min)	63.2 ± 10.0	64.1 ± 9.9
Plasma Renin Activity (ng/mL/hr)	0.55± 0.52	0.88 ± 1.6
Plasma Aldosterone (ng/dL)	5.4 ± 3.7	5.6 ± 3.7
Urinary Sodium Excretion (mmol/24 hrs)	228.6 ± 69.2	218.3 ± 69.3
Total Urine Creatinine (g/24hrs)	1.43 ± 0.4	1.44 ±0.4
<u>After Low Salt diet</u>		
Systolic (mmHg)	128.4 ± 14.9	137.8 ± 19.2
Diastolic (mmHg)	78.1 ± 10.8	82.4 ± 11.9
MAP (mmHg)	94.5 ± 11.6	95.7 ±15.6
Heart Rate (Beats/min)	64.5 ±10.1	64.8 ±8.9
Plasma Renin Activity (ng/mL/hr)	3.1 ± 8.0	2.7 ± 1.8
Plasma Aldosterone (ng/dL)	17.4 ± 10.5	18.4 ± 17.0
Urinary Sodium Excretion (mmol/24 hrs)	13.5 ±7.9	13.4 ±8.4

TABLE S2. Distribution of Genotypes in Hypertensive and Normotensive Subjects in the UVA Cohort

<u>Gene</u>	<u>SNP</u>		<u>Genotypes</u>			<u>MAF</u>	<u>HWE</u>
CYP11 β 2	rs1799998		CC	CT	TT		
		HT	6	24	15	0.4000	0.6473
		NT	26	57	29	0.4866	0.8645
ADD	rs4961		GG	GT	TT		
		HT	34	12	0	0.1304	0.2560
		NT	61	44	6	0.2523	1.000
	rs1541582		AA	AT	TT		
		HT	26	14	4	0.2500	1.000
	NT	62	41	4	0.2290	1.000	
AGT	rs699		CC	TC	TT		
		HT	8	20	19	0.3830	0.2042
		NT	20	43	49	0.8850	0.1429
AGTR1	rs5186		AA	CA	CC		
		HT	12	27	5	0.4205	1.000
		NT	49	50	12	0.3333	0.4496
ACE	rs1799752		long long	long short	short short		
		HT	5	31	10	0.4457	0.619
		NT	20	59	33	0.4420	0.06182
CAV	rs3807990		CC	CT	TT		
		HT	25	17	2	0.2386	1.000
		NT	63	37	6	0.2311	0.7967
	rs3840634		Del Del	Ins Del	Ins Ins		
		HT	2	17	24	0.2442	0.3710
	NT	2	35	70	0.1822	0.2821	
CCKAR	rs41267457		GG	GA	AA		
		HT	46	1	0	0.0213	1.000
		NT	112	2	0	0.0175	1.000
CCKBR	rs1805000		CC	CT	TT		
		HT	44	2	0	0.0217	1.000
		NT	110	0	1	0.0090	0.01099
	rs1805002		GG	GA	AA		
		HT	43	4	0	0.0851	1.000
	NT	104	9	0	0.0398	1.000	
CYP4A11	rs1126742		TT	CT	CC		
		HT	34	9	2	0.1444	1.000
		NT	76	35	2	0.1726	1.000
DRD2	rs1799732		Del Del	Ins Del	Ins Ins		

		HT	1	8	37	0.1087	0.1518
		NT	0	15	93	0.0694	1.000
	rs1800497		CC	TC	TT		
		HT	28	16	2	0.2174	1.000
		NT	62	40	8	0.2545	0.8254
	rs1079597		AA	GA	GG		
		HT	1	13	33	0.1596	1.000
		NT	6	37	69	0.2188	0.8025
	rs6276		AA	AG	GG		
		HT	25	17	4	0.2717	1.000
		NT	54	45	10	0.2982	0.5367
GRK4	rs2960306		GG	GT	TT		
		HT	26	19	10	0.3545	0.5394
		NT	46	62	22	0.227	0.2571
	rs1024323		CC	CT	TT		
		HT	24	22	9	0.3636	0.2889
		NT	45	62	23	0.4154	0.4196
	rs1801058		CC	CT	TT		
		HT	26	19	10	0.3545	0.6353
	NT	34	67	29	0.4808	0.6361	
eNOS	rs1799983		GG	TG	TT		
		HT	22	17	5	0.3068	0.6478
		NT	46	55	8	0.3257	0.2353
PPP2R2C	rs35368770		GG	GA	AA		
		HT	46	1	0	0.0213	1.000
		NT	109	1	0	0.0091	1.000
	rs35410672		CC	CT	TT		
		HT	46	0	0	0	1.000
		NT	111	2	0	0.0177	1.000
	rs3796403		AA	GA	GG		
		HT	10	29	6	0.4556	0.3556
		NT	37	56	17	0.4091	0.2939
	rs11545013		CC	AC	AA		
	HT	47	0	0	0	1.000	
	NT	110	1	0	0.0090	0.1418	
SLC4A5	rs7571842		AA	GA	GG		
		HT	14	21	12	0.4787	1.000
		NT	34	55	24	0.4558	0.4952
	rs10177833		AA	AC	CC		
		HT	12	21	9	0.5357	0.3428
	NT	34	55	19	0.4306	1.000	
SNX1	rs1130604		CC	TC	TT		
		HT	45	2	0	0.0426	1.000
		NT	112	1	0	0.0088	1.000
	rs1802376		GG	GA	AA		

		HT	45	2	0	0.0426	1.000
		NT	111	2	0	0.0177	1.000
	rs34910981		del del	ins del	ins ins		
		HT	44	2	0	0.0435	1.000
		NT	109	2	0	0.0180	1.000
SNX5	rs6045116		GG	GA	AA		
		HT	37	0	0	0	1.000
		NT	119	1	0	0.0042	1.000
SNX19	rs3751037		CC	GC	GG		
		HT	27	17	2	0.2283	0.5484
		NT	52	45	11	0.3102	0.6809
	rs3190345		AA	GA	GG		
		HT	30	13	4	0.2234	0.3497
		NT	64	37	11	0.2634	0.06756
	rs681982		AA	AC	CC		
		HT	44	2	0	0.0435	1.000
		NT	108	2	0	0.0182	1.000
	rs4414223		CC	TC	TT		
		HT	21	22	1	0.2727	1.000
		NT	36	48	16	0.4000	0.7043
	rs2298566		AA	AC	CC		
		HT	1	19	25	0.2333	0.1188
	NT	7	50	50	0.2991	0.5130	

TABLE S3. Distribution of Genotypes in Salt-Sensitive and Salt Resistant Subjects in the UVA Cohort

Gene	SNP		Genotypes			MAF	HWE
CYP11 β 2	rs1799998		CC	CT	TT		
		SS	9	17	5	0.4355	0.5207
		SR	35	64	27	0.4683	0.8225
ADD1	rs4961		GG	GT	TT		
		SS	21	10	0	0.1613	0.2909
		SR	74	52	0	0.2063	0.252
	rs1541582		AA	AT	TT		

		SS	21	8	1	0.1667	1.000
		SR	67	47	7	0.2521	0.7401
AGT	rs699		CC	TC	TT		
		SS	10	13	7	0.4500	0.495
		SR	58	50	21	0.3566	0.07772
AGTR1	rs5186		AA	CA	CC		
		SS	13	16	2	0.3226	0.4357
		SR	48	61	15	0.3669	0.5122
ACE	rs1799752		long long	long short	short short		
		SS	6	20	5	0.4839	0.1044
		SR	37	70	20	0.4331	0.1675
CAV	rs3807990		CC	CT	TT		
		SS	15	12	2	0.2759	1.000
		SR	73	42	6	0.2231	0.9896
	rs3840634		del del	ins del	ins ins		
		SS	17	12	0	0.2069	0.3043
		SR	77	44	0	0.1818	1.000
CCKAR	rs41267457		GG	GA	AA		
		SS	30	1	0	0.0161	1.000
		SR	128	2	0	0.0077	1.000
CCKBR	rs1805000		CC	CT	TT		
		SS	30	0	0	0.000	1.000
		SR	124	2	0	0.0080	0.02367
	rs1805002		GG	GA	AA		
		SS	27	4	0	0.0645	1.000
		SR	120	9	0	0.0349	1.000
CYP4A11	rs1126742		TT	CT	CC		
		SS	22	9	0	0.1452	1.000
		SR	88	39	0	0.1535	1.000
DRD2	rs1799732		del del	ins del	ins ins		
		SS	22	5	0	0.0926	1.000
		SR	108	19	0	0.0748	0.5557
	rs1800497		CC	TC	TT		
		SS	18	8	3	0.2414	0.2979
		SR	72	48	7	0.2441	0.7851
	rs1079597		AA	GA	GG		
		SS	20	10	1	0.1935	1.000
		SR	82	40	6	0.2031	0.6947
		rs6276		AA	AG	GG	
	SS	10	18	1	0.3448	0.09623	
		SR	69	44	13	0.2778	0.1455
GRK4	rs2960306		GG	GT	TT		
		SS	16	8	10	0.4118	0.002711
		SR	56	73	22	0.3874	0.8199

	rs1024323		CC	CT	TT		
		SS	14	12	8	0.4118	0.1135
		SR	55	72	24	0.3974	0.9569
	rs1801058		CC	CT	TT		
		SS	13	14	7	0.4118	0.3818
		SR	47	72	32	0.4503	0.6506
eNOS	rs1799983		GG	TG	TT		
		SS	13	12	5	0.3667	0.4485
		SR	55	60	8	0.3089	0.1142
PPP2R2C	rs35368770		GG	GA	AA		
		SS	31	0	0	0.000	1.000
		SR	124	2	0	0.0080	1.000
	rs35410672		CC	CT	TT		
		SS	31	0	0	0.000	1.000
		SR	126	2	0	0.0078	1.000
	rs3796403		AA	GA	GG		
		SS	9	16	5	0.4333	0.6377
		SR	38	69	18	0.4200	0.137
	rs11545013		CC	AC	AA		
	SS	30	0	0	0.000	1.000	
	SR	127	1	0	0.0039	1.000	
SLC4A5	rs7571842		AA	GA	GG		
		SS	18	11	2	0.2419	1.000
		SR	30	65	34	0.5155	0.9211
	rs10177833		AA	AC	CC		
		SS	17	11	0	0.1964	0.5488
	SR	29	65	28	0.4959	0.4684	
SNX1	rs1130604		CC	TC	TT		
		SS	30	0	0	0.000	1.000
		SR	127	3	0	0.0115	1.000
	rs1802376		GG	GA	AA		
		SS	31	0	0	0.000	1.000
		SR	125	4	0	0.0155	1.000
	rs34910981		del del	ins del	ins ins		
	SS	28	2	0	0.0333	1.000	
	SR	125	2	0	0.0079	1.000	
SNX5	rs6045116		GG	GA	AA		
		SS	30	0	0	0.000	1.000
		SR	126	1	0	0.0039	1.000
SNX19	rs3751037		GG	GC	GG		
		SS	28	2	0	0.0333	1.000
		SR	125	2	0	0.0079	0.779
	rs3190345		AA	GA	GG		
		SS	17	9	5	0.3065	0.0922
	SR	77	41	10	0.2383	0.1833	

	rs681982		AA	AC	CC		
		SS	28	1	0	0.0172	1.000
		SR	124	3	0	0.0118	1.000
	rs4414223		CC	TC	TT		
		SS	13	11	3	0.3148	1.000
		SR	44	59	14	0.3718	0.3897
	rs2298566		AA	AC	CC		
		SS	16	12	1	0.2414	1.000
		SR	59	57	7	0.2886	0.154