

Supplementary Table S1: Physical and functional details of studied SNPs of AGT, ACE, AGTR1 and ADD1

Gene	SNP	Region	Synonymous/ Non-synonymous	Base pair change (Ancestral allele> altered allele)	Amino acid change	Importance/Involvement in other disease
AGT	-532C/T (rs5046)	5' UTR	Synonymous	C>T	-	Part of core-promoter element 1 or lie in the upstream of promoter and play critical role in transcriptional regulation. ¹⁻⁴
	-217G/A (rsID:5049)	5' UTR	Synonymous	G>A	-	
	-152G/A (rsID:11568020)	Promoter	Synonymous	G>A	-	
	-20A/C (rsID:5050)	Promoter	Synonymous	A>C	-	
	-6G/A (rsID:5051)	Promoter	Synonymous	G>A	-	
	174T/M (rsID:4762)	Exon	Non-synonymous Missense mutation	C>T	T [Threonine]⇒ M [Methionine]	Increase the risk of high BP and also known to increase the plasma AGT concentration. ^{3, 5, 6}
	235M/T (rsID:699)	Exon	Non-synonymous Missense mutation	T>C	M [Methionine] ⇒ T [Threonine]	
ACE	I/D (rsID:4646944)	Intron	-	Insertion (I)> Deletion (D) of a 287bp marker	-	The D allele is associated with elevated ACE levels in various cardiovascular diseases and serves as risk allele for EH. ⁷⁻⁹
AGTR1	1166A/C (rsID:5186)	3' UTR	Synonymous	A>C	-	The C allele of 1166A/C polymorphism interferes with the base-pairing complementariness between AGTR1 mRNA and microRNA-155 and thereby increases AGTR1 protein expression that associates with increased risk of cardiovascular diseases. ¹⁰
ADD1	614G/T (rsID:4961)	Exon	Non-synonymous	G>T	G [Glycine]⇒ T [Tryptophan]	This SNP leads to stimulation of sodium-potassium adenosine triphosphatase activity in renal tubular cells, which increases renal sodium reabsorption and is involved in BP control. ¹¹

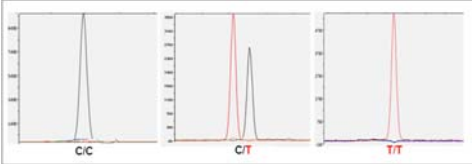
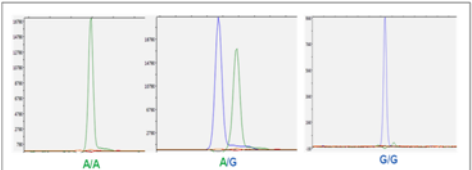
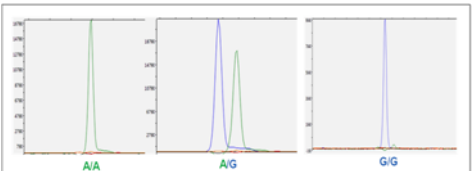
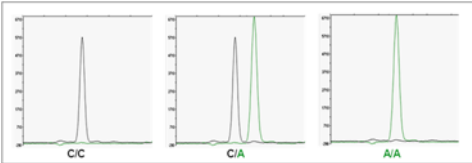
A: Adenine; G:guanine; T: thymine; C:Cytosine

Supplementary Table S2: Primers and PCR conditions of the polymorphisms of genotyped using Restriction Fragment Length Polymorphism

Gene	SNP and rsID	Primer sequence	PCR Cycling conditions	Restriction enzyme	Digestion condition	Product size (bp)
	-6G/A (rsID:5051)	F 5' TGC ACC GGC TCA CTC TGT TCA -3' R 5' ATC TCC CGG GCC TTT TCC TCC TA -3'	I 94°C 4', D 94°C 30", A 66°C 30", E 72°C 40", 38cy, FE 72°C 10'	<i>BstNI</i>	60°C 3 hours	GG=183, 107, 55 GA=183, 129, 107, 55 AA=129, 107, 55
<i>AGT</i>	174T/M (rsID:4762)	F 5'- TGG CAC CCT GGC CTC TCT CTA TCT -3' R 5'- CAG CCT GCA TGA ACC TGT CAA TCT -3'	I 95°C 4', D 95°C 15", A 67°C 45", E 72°C 45", 38cy, FE 72°C 10'	<i>NcoI</i>	37°C overnight	TT=303 TM=303, 211, 92 MM=211, 92
	235M/T (rsID:699)	F: 5'-CCG TTT GTG CAG GGC CTG GCT CTC T- 3' R: 5'-CAG GGT GCT GTC CAC ACT GGA CCC C -3'	I 95°C 4', D 95°C 15", A 65°C 45", E 72°C 45" 38 cy, FE 72°C 10'	<i>Tth III</i>	65°C 2 hours	TT=141 TM=165,141 MM=165
<i>ACE</i>	I/D (rsID:4646944)	F: 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' R: 5' GAT GTC GCC ATC ACA TTC GTCAGA T 3'	I 94°C 4'; D 94°C 1'; A 58°C 45", E 72°C 45"; 30 cy, FE 72°C 7'	-	-	DD= 190 ID=190, 490 II =490
<i>AGTRI</i>	1166A/C (rsID:5186)	F: 5' GCA GCA CTT CAC TAC CAA ATG GGC 3' R: 5' CAG GAC AAA AGC AGG CTA GGG AGA 3'	I 94°C 3'; D 94°C 30"; A 58°C 45", E 72°C 45"; 30 cy, FE 72°C 7'	<i>Hae III</i>	37°C overnight	AA= 255 AC =231, 255 CC =231
<i>ADDI</i>	614G/T (rsID:4961)	F: 5' CTC CTT TGC TAG TGA CGG TGA TTC G3' R: 5' TTG GGA CTG CTT CCA TTC GGC C C3'	I 94°C 3'; D 94°C 30"; A 61°C 45", E 72°C 45"; 30 cy, FE 72°C 7'	<i>Sau 96I</i>	65°C 2 hours	TT= AA= 147 GT =147, 122, 25 GG =122, 25

F, forward; R, reverse; I, initial denaturation; A, annealing; D, denaturation; E, extension; FE, final extension;cy, cycles.

Supplementary Table S3: Primers and PCR conditions of the polymorphisms of AGT genotyped using SNaPshot

Gene	SNP and rsID	Primer sequence	PCR Cycling conditions	SNaPshot cycling condition	Genotyping	Product size (bp)
AGT	-532C/T (rsID:5046)	F 5' GTC CCT TCA GTG CCC TAA TAC CAT G 3' R 5' GAC CCT GCT GCC CGC TCA T 3' IP 5' GCC CTA TTT ATA GCT GAG3'	I 95°C 4", D 95°C 30", A 64°C 40", E 72°C 40" 32 cy, FE 72°C 10"			484
	-217G/A (rsID:5049)	F 5' TCA GAA GGC CTG GGTGGTTGG 3' R 5' CCTCTCCCGGCCTTTTCCTC 3' IP 5' TGTAAGCTGACCCTGCACC 3'		D 96°C 10", A 58°C 5", E 60°C 30", 35cy		260
	-152G/A (rsID:1156 8020)	F 5' TCAGAAGGCCTGGGTGGTTGG 3' R 5' CCTCTCCCGGCCTTTTCCTC 3' IP 5' TCCTGGAAGAGGTCCCAGC 3'	I 95°C 4", D 95°C 30", A 64°C 40", E 72°C 40" 32 cy, FE 72°C 10"			260
	-20A/C (rsID:5050)	F 5' TCAGAAGGCCTGGGTGGTTGG 3' R 5' CCTCTCCCGGCCTTTTCCTC 3' IP 5' GCCCTATTTATAGCTGAG 3'				257

F, forward; R, reverse; IP, internal primer; A, annealing; D, denaturation; E, extension; cy, cycles.

Supplementary table S4: Goodness-of-fit test for observed and expected genotypes distribution of AGT ACE, AGTR1 and ADD1 polymorphisms in patients and controls.

Gene	SNP	Genotype	Patients		Controls	
			Observed	Expected	Observed	Expected
<i>AGT</i>	-532C/T (rs5046)	-532CC	38%	42%	51%	53%
		-532CT	53%	46%	45%	40%
		-532TT	09%	12%	04%	07%
		χ^2	2.196		1.986	
		<i>P</i>	0.334		0.37	
	-217G/A (rs5049)	-217GG	47%	48%	63%	61%
		-217GA	44%	43%	30%	34%
		-217AA	09%	09%	07%	05%
		χ^2	0.044		1.336	
		<i>P</i>	0.978		0.513	
	-152G/A (rs11568020)	-152GG	92%	90%	94%	94%
		-152GA	06%	10%	06%	06%
		-152AA	02%	00%	00%	00%
		χ^2	2.700		0.000	
		<i>P</i>	0.259		1.000	
	-20A/C (rs5050)	-20AA	28%	32%	50%	52%
-20AC		58%	49%	45%	40%	
-20CC		14%	19%	05%	08%	
χ^2		0.469		1.827		
<i>P</i>		0.177		0.401		
-6G/A (rs5051)	-6GG	14%	16%	32%	29%	
	-6GA	51%	48%	44%	50%	
	-6AA	35%	36%	24%	21%	
	χ^2	0.465		1.459		
	<i>P</i>	0.792		0.482		
174T/M (rs4762)	174TT	84%	83%	82%	81%	
	174TM	15%	16%	16%	18%	
	174MM	01%	01%	02%	01%	
	χ^2	0.074		1.234		
	<i>P</i>	0.963		0.539		
235M/T (rs699)	235MM	16%	15%	29%	32%	
	235MT	44%	47%	55%	49%	
	235TT	40%	38%	16%	19%	
	χ^2	0.363		1.489		
	<i>P</i>	0.834		0.475		

ACE	I/D (rs4646994)	II	34%	33%	38%	37%
		ID	46%	49%	47%	48%
		DD	20%	18%	15%	15%
		χ^2	0.436		0.048	
		<i>P</i>	0.804		0.976	
AGTRI	1166A/C (rs5186)	1166AA	82%	82%	83%	82%
		1166AC	16%	17%	15%	17%
		1166CC	02%	01%	02%	01%
		χ^2	1.056		1.247	
		<i>P</i>	0.589		0.536	
ADD1	614G/T (rs4961)	GG	57%	58%	61%	61%
		GT	38%	36%	34%	34%
		TT	05%	06%	05%	05%
		χ^2	0.295		0.000	
		<i>P</i>	0.863		1.000	

Comparison between observed and expected frequencies was performed; *P*-values and χ^2 tests were calculated by EPIINFO version 6.

Supplementary table S5: Genotype and allele distribution of the AGT ACE, AGTR1 and ADD1 polymorphisms in controls and patients

Genes/ SNPs	Genotype/ Alleles	Controls n, (% distribution)	Patients	Logistic regression analysis		
				χ^2	P-value	OR (95%CI)
AGT -532C/T	-532CC	202 (51%)	209 (38%)	–	–	Reference
	-532CT	180 (45%)	288 (53%)	0.71	0.400	1.2(0.8–1.7)
	-532TT	18 (05%)	48 (09%)	2.04	0.153	1.9(0.8–4.7)
	-532C	584 (73%)	706 (65%)	–	–	Reference
	-532T	216 (27%)	384 (35%)	9.54	0.002	1.4(1.1–1.8)
-217G/A	-217GG	251 (63%)	259 (48%)	–	–	Reference
	-217GA	121 (30%)	238 (44%)	0.81	0.368	1.2(0.8–1.8)
	-217AA	28 (07%)	48 (09%)	1.41	0.235	0.6(0.3–1.3)
	-217G	623 (78%)	756 (69%)	–	–	Reference
	-217A	177 (22%)	334 (31%)	8.09	0.004	1.4(1.1–1.8)
-152G/A	-152GG	374 (94%)	500 (92%)	–	–	Reference
	-152GA	26 (7.0%)	35 (6.0%)	0.10	0.752	0.9(0.5–1.8)
	-152AA	0 (0%)	10 (02%)	–	–	–
	-152G	774 (97%)	1035 (95%)	–	–	Reference
	-152A	26 (03%)	55 (05%)	0.18	0.667	1.1(0.6–2.0)
-20A/C	-20AA	200 (50%)	152 (28%)	–	–	Reference
	-20AC	180 (45%)	315 (58%)	38.99	4.2E-10	3.4(2.3–4.9)
	-20CC	20 (5%)	78 (14%)	21.05	4.5E-06	5.8(2.7–12.3)
	-20A	580 (72%)	619 (57%)	–	–	Reference
	-20C	220 (28%)	471 (43%)	40.4	2.1E-10	2.1(1.7–2.7)
-6G/A	-6GG	127 (32%)	79 (14%)	–	–	Reference
	-6GA	175 (44%)	276 (51%)	22.40	2.2E-06	3.1(1.9–4.9)
	-6AA	98 (25%)	190 (35%)	10.18	0.001	2.3(1.4–3.7)
	-6G	429 (54%)	434 (40%)	–	–	Reference
	-6A	371 (46%)	656 (60%)	23.1	1.5E-06	1.7(1.4–2.1)
174T/M	174TT	328 (82%)	456 (84%)	–	–	Reference
	174TM	65 (16%)	83 (15%)	3.29	0.070	0.6(0.4–1.0)
	173MM	07 (02%)	06 (01%)	2.32	0.128	0.3(0.1–1.4)
	174T	721 (90%)	995 (91%)	–	–	Reference
	174M	79 (10%)	95 (09%)	0.74	0.389	0.9(0.8–1.1)
235M/T	235MM	116 (29%)	87 (16%)	–	–	Reference
	235MT	221 (55%)	241 (44%)	0.33	0.564	1.1(0.7–1.8)
	235TT	63 (16%)	217 (40%)	37.0	1.2E-09	4.9(2.9–8.1)
	235M	453 (57%)	415 (38%)	–	–	Reference
	235T	347 (43%)	675 (62%)	58.0	2.6E-14	2.4(1.9–2.9)
ACE I/D	II	150 (38%)	186 (34%)	–	–	Reference

	ID	188 (47%)	252 (46%)	1.08	0.299	0.8(0.6–1.2)
	DD	62 (16%)	107 (20%)	2.65	0.104	1.5(0.9–2.5)
	I	488 (61%)	624 (57%)	–	–	Reference
	D	312 (39%)	466 (43%)	2.38	0.123	1.2(0.9–1.5)
<i>AGTRI</i>						
1166A/C	1166AA	332 (83%)	450 (83%)	–	–	Reference
	1166AC	60 (15%)	86 (16%)	2.99	0.084	1.5(0.9–2.4)
	1166CC	08 (02%)	09 (02%)	2.60	0.107	0.4(0.1–1.2)
	1166A	724 (90%)	986 (90%)	–	–	Reference
	1166C	76 (10%)	104 (10%)	1.56	0.211	0.8(0.5–1.1)
<i>ADDI</i>						
614G/T	460GG	245 (61%)	311 (57%)	–	–	Reference
	460GT	137 (34%)	209 (38%)	2.13	0.145	1.3(0.9–1.9)
	460TT	18 (05%)	25 (05%)	0.08	0.782	1.1(0.5–2.8)
	460G	627 (78%)	831 (76%)	–	–	Reference
	460T	173 (22%)	259 (24%)	0.963	0.326	0.9(0.6–1.2)

n, number subjects. *P*-value, χ^2 and odds ratio (OR) were calculated using multivariate logistic regression analysis after adjustment for age, gender, BMI, smoking, alcohol, triglyceride and cholesterol.

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