ORIGINAL ARTICLE

A regulatory SNP in AKAP13 is associated with blood pressure in Koreans

Kyung-Won Hong, Ji-Eun Lim and Bermseok Oh

High blood pressure contributes to more than 10 million deaths per year worldwide through stroke and ischemic heart disease. Yet, genome-wide association studies (GWASs) have identified a small fraction of its underlying genetic factors. To identify biologically important single-nucleotide polymorphisms (SNPs) that regulate variations in blood pressure, we analyzed SNPs in a genome-wide association study. Genome-wide genotype data (original study $n=7551$, SNP$=352\,228$; replication study $n=3703$, SNP$=20$) were obtained from the Korea National Institute of Health, wherein $29\,921$ of $352\,228$ SNPs lay within 5 kbp upstream of genes. Linear regression analysis was performed for systolic and diastolic blood pressure (DBP) by controlling for cohort, age, sex and body mass index. For the 20 SNPs that were associated with both blood pressure values, a replication study was performed in an independent population. A total of 20 SNPs were significantly associated with both blood pressure values in the original study, 13 of which lay in a conserved transcription factor-binding site. One SNP (rs11638762), in the GATA-3 binding site upstream of the AKAP13 gene, was significantly replicated in another cohort (*P*-value of the meta-analysis $=1.4\times10^{-5}$ for systolic blood pressure and 6.3×10^{-4} for DBP). A functional GWAS was performed using upstream SNPs, and a novel genetic factor (AKAP13), which is essential for cardiac myocyte development in mice, was identified as a regulator of blood pressure.

INTRODUCTION

Blood pressure is an important clinical parameter of health. High blood pressure contributes to more than 10 million deaths per year worldwide through stroke and ischemic heart disease.[1,2] It is believed that 30–50% of the variability in blood pressure is influenced by multiple genetic factors, prompting extensive efforts to identify its genetic underpinnings.

As the seminal genome-wide association study (GWAS) was reported by the Wellcome Trust Case-Control Consortium,[3] such studies have identified >250 genetic loci, in which common genetic variants have been reproducibly associated with polygenetic traits.[4] Although the loci that are responsible for common diseases and traits, such as type 2 diabetes, coronary artery disease and serum lipid levels, have been faithfully mapped by several GWASs, other diseases, such as hypertension and stroke, have been intractable to reliable GWAS mapping.[5]

Recently, two large meta-analyses of blood pressure GWASs were conducted in subjects of European descent (Global BPgen consortium, $34\,433$ subjects;[6] CHARGE consortium, $29\,136$ subjects[7]). Yet, the genetic variants that have been identified by the many large blood pressure genetic studies explain merely 1% of blood pressure variation.[7] Therefore, our understanding of the allelic variation that affects blood pressure in the general population is in its infancy. More diverse strategies for GWASs have been required to identify additional alleles that are associated with blood pressure.

We reported a GWAS of quantitative traits, including blood pressure, in Korean Association REsource,[8] implicating the *ATP2B1* gene as influencing blood pressure. Our conventional approach, however, failed to identify a sufficient number of genetic factors to understand the underlying mechanism of blood pressure regulation. Therefore, we employed an alternative approach, using nonsynonymous single-nucleotide polymorphisms (SNPs) in the GWAS data, and identified more SNPs located in arginine decarboxylase, phospholipase D1, brain-derived nuclear factor and an unknown gene (AC089987).[9] Although these nonsynonymous SNPs did not have strong *P*-values ($1.0\times10^{-3}<P<4.8\times10^{-2}$), they appeared to be biologically linked to blood pressure.[9]

This limited success encouraged us to examine other regulatory SNPs that were obtained in the GWAS data of Korean Association REsource, generated by Affymetrix 5.0 SNP array (Affymetrix, Inc., Santa Clara, CA, USA), to identify additional genetic factors that regulate blood pressure. Single-nucleotide changes in regulatory regions can produce new regulatory elements that are recognized by alternate transcription factors,[10] and many functional analyses of regulatory SNPs have demonstrated this effect on gene expression experimentally.[11–14] To this end, $29\,921$ SNPs in the upstream region

Department of Biomedical Engineering, School of Medicine, Kyung Hee University, Seoul, Korea
Correspondence: Dr B Oh, Department of Biomedical Engineering, School of Medicine, Kyung Hee University, 1 Hoeki-dong, Dongdaemun-gu, Seoul 130-702, Korea.
E-mail: ohbs@khu.ac.kr

of genes genome-wide were analyzed for their association with blood pressure.

MATERIALS AND METHODS

Subjects

The subjects and their genotypes in the original study have been reported.[8] In total, 7551 individuals came from KyungGi-Do province, near Seoul, Korea, and entered a GWAS of the link between blood pressure and upstream SNP genotypes. To recapitulate the association results, 3703 persons were recruited from community health care centers in Seoul and Busan, Korea. This study was approved by the International Review Board of the Korea National Institute of Health.

The basic characteristics and blood pressures of the subjects are listed in Table 1. In the original study, blood pressure was measured three times in the supine position. Before the first measurement, the participants rested for 5 min, and three measurements were taken at least 30 s apart. The average of the three measurements was used for this study. In the replication study, blood pressure was measured twice in the seated position, and the average value was used.

Genotypes

DNA samples for the original study were isolated from the peripheral blood of participants and genotyped using the Affymetrix Genomewide Human SNP array 5.0 (Affymetrix, Inc.). In the replication study, DNA samples were genotyped using the Affymetrix Genomewide Human SNP array 6.0 (Affymetrix, Inc.). The quality controls have been described in elsewhere.[8] Briefly, the accuracy of the genotyping was calculated by Bayesian robust linear modeling using the Mahalanobis distance genotyping algorithm.[15]

Samples that had accuracies below 98% and a high missing genotype call rate ($\geqslant 4\%$), high heterozygosity ($>30\%$) or inconsistency in sex were excluded from subsequent analyses. Individuals who had a tumor or were undergoing antihypertensive therapy were excluded. Related individuals whose estimated identity-by-state values were high (>0.80)[3] were also excluded. The 352 228 genotyped SNPs (excluding sex chromosome SNPs) had a missing genotype rate below 0.1, a minor allele frequency higher than 0.01 and no deviation from Hardy–Weinberg Equilibrium ($P > 1 \times 10^{-6}$).

Upstream SNP analysis

Using BIOMART version 5.0 (http://www.ensembl.org/biomart/), 29 921 upstream SNPs were selected from the QC-filtered 352 228 SNPs, based on the data in dbSNP v.130. BIOMART, a web-based tool, allowed us to use information from the latest release of a database limiting on specific upstream category.

In our quantitative analysis of systolic blood pressure (SBP) and diastolic blood pressure (DBP), linear regression was used by controlling for a subject's cohort, age, sex and body mass index.

Statistical analyses were performed using PLINK (version 1.07).[16] All tests were based on an additive model and P-values were not adjusted for multiple tests. Significant SBP- and DBP-associated SNPs were examined in the replica-tion study. The inverse-variance meta-analysis method, assuming fixed effects, was used to generate the meta-analysis statistics. Cochran's Q test was used to assess between-study heterogeneity. All meta-analysis calculations were per-formed using R statistical language (version 2.7.1). Transcription factor binding sites (TFBSs) were identified using TFsearch (http://molsun1.cbrc.aist.go.jp/research/db/TFSEARCH.html), and allelic differences in TFBSs were surveyed. Transcription factor (TF) score is calculated by TF search program. It indicated the possibility of transcription factor binding on the site. The score is calculated by the web-based program. Study power was based on Korean Association REsource parameters, including minor allele frequency, effect size and mean value of the markers.

RESULTS

A total of 29 921 of the 352 228 genotyped SNPs lay in the upstream region (-5 kbp) of genes genome-wide; this value is one-quarter of all upstream SNPs (131 593), excluding the sex chromosomes[10] (Table 2). Using these 29 921 SNPs, we conducted a genome-wide association study of blood pressure (SBP and DBP). Quantile–quantile plots and genomic inflation statistics are described in Figure 1. The phenotypes had statistics that approximated 1.00 ($1.027 \leqslant \lambda \leqslant 1.039$), indicating that there was no genomic inflation from population stratification in these subjects.

A total of 20 SNPs were significantly associated with both SBP and DBP (P-value <0.01) (Table 3). The study-power analyses indicated that 10 of the 20 SNPs had more than 80% study power at alpha $=0.05$, and the remaining SNPs had more than 60% study power.

Of the 20 SNPs, 12 were linked to the known functional genes (*PUS7, SLC6A20, ASCL4, RNF8, BCHE, ALG2, TAAR3, FUCA2, ILDR1 and SPATS2L*), and the remaining correlated with unknown genes (See Table 3). The minor alleles of 14 SNPs (rs295142, rs1900706, rs842825, rs3806650, rs11929235, rs17279465, rs195386,

Table 1 Basic characteristics of study subjects

Variables	KARE	Replication samples
Number of individuals (%)	7551	3703
Gender (men (%)/women (%))	3747 (50)/3804 (50)	1651 (45)/2052 (55)
Age (mean (*M*) year ± s.d.)	51.44 ± 8.79	53.21 ± 8.33
Body mass index (*M* kg m^{-2} ± s.d.)	24.42 ± 3.08	23.96 ± 2.90
Systolic blood pressure (*M* mm Hg ± s.d.)	115.65 ± 17.25	121.95 ± 14.80
Diastolic blood pressure (*M* mm Hg ± s.d.)	74.21 ± 11.27	77.21 ± 10.23

Abbreviation: s.d., standard deviation.

Table 2 Upstream SNP distribution in each chromosome, excluding sex chromosome

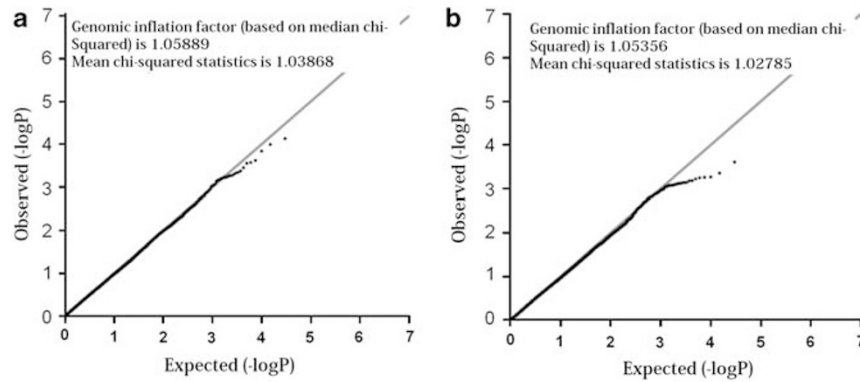|	Number of upstream SNPs			
Chromosome	In whole genome (WU et al., 2009)	%	In KARE dataset	%
1	14221	10.8	3671	12.3
2	8031	6.1	3105	10.4
3	5586	4.2	2438	8.1
4	5829	4.4	829	2.8
5	5195	3.9	1016	3.4
6	8409	6.4	2362	7.9
7	6999	5.3	2059	6.9
8	4345	3.3	829	2.8
9	6699	5.1	1599	5.3
10	5402	4.1	1992	6.7
11	8082	6.1	1438	4.8
12	5735	4.4	1092	3.6
13	2129	1.6	943	3.2
14	3902	3.0	721	2.4
15	3580	2.7	595	2.0
16	5921	4.5	596	2.0
17	10227	7.8	902	3.0
18	1789	1.4	434	1.5
19	8842	6.7	792	2.6
20	4564	3.5	1047	3.5
21	1683	1.3	586	2.0
22	4423	3.4	875	2.9
Total	131593	100	29921	100

Figure 1 Quantile–quantile plot of observed and expected *P*-values for SBP (**a**) and DBP (**b**). Genomic inflation statistics are shown in the plots.

Table 3 Associated SNPs of the original genome-wide association study of blood pressure and study power for each marker

CHR	SNPs	Linked genes	M	MAF	Tendency	Systolic blood pressure		Diastolic blood pressure		Study power[a]	Ensemble transcript ID	SNP position from transcription start site	Expected TFBS
						BETA	SBP	BETA	DBP				
2	rs295142	SPATS2L	C	0.176	↓	−1.01 ± 0.33	$2.2×10^{-3}$	−0.73 ± 0.22	$1.2×10^{-3}$	78.3	ENST00000409151	−2355	Nkx-2
2	rs1900706	SPATS2L	C	0.172	↓	−1.00 ± 0.33	$2.5×10^{-3}$	−0.73 ± 0.22	$1.1×10^{-3}$	76.7	ENST00000409151	−1481	SRY
2	rs842825	SPATS2L	T	0.179	↓	−0.93 ± 0.33	$5.1×10^{-3}$	−0.75 ± 0.22	$7.7×10^{-4}$	71.9	ENST00000438761	−2666	
3	rs3806650	BCHE	G	0.235	↓	−0.91 ± 0.30	$2.2×10^{-3}$	−0.65 ± 0.20	$1.3×10^{-3}$	78.5	ENSG00000114200	−551	AML1a
3	rs11929235	ILDR1	A	0.246	↓	−0.79 ± 0.29	$6.6×10^{-3}$	−0.57 ± 0.20	$4.0×10^{-3}$	67.9	ENST00000462014	−3901	pbx-1
3	rs17279465	SLC6A20	C	0.047	↓	−2.00 ± 0.60	$9.7×10^{-4}$	−1.39 ± 0.41	$6.8×10^{-4}$	85.5	ENST00000473146	−917	MZF1
6	rs9356478	AL022069	C	0.081	↑	1.24 ± 0.47	$7.8×10^{-3}$	0.89 ± 0.32	$4.9×10^{-3}$	67.4	ENSG00000232468	−4716	CDP
6	rs3789790	FUCA2	G	0.213	↑	0.91 ± 0.31	$3.3×10^{-3}$	0.62 ± 0.21	$3.5×10^{-3}$	75.7	ENST00000451668	−652	S8
6	rs195386	RNF8	T	0.113	↓	−1.52 ± 0.41	$1.9×10^{-4}$	−0.81 ± 0.27	$3.3×10^{-3}$	92.9	ENST00000498460	−3334	CRE-BP
6	rs6569814	TAAR3	T	0.327	↑	0.75 ± 0.27	$5.9×10^{-3}$	0.51 ± 0.18	$5.3×10^{-3}$	70.8	ENSG00000179073	−1606	Oct-1
7	rs4731112	RP11−390E23	C	0.199	↑	1.05 ± 0.32	$9.7×10^{-4}$	0.62 ± 0.22	$3.8×10^{-3}$	84.8	ENST00000422401	−4482	
7	rs7357092	AC006465	T	0.164	↑	1.41 ± 0.35	$4.6×10^{-5}$	0.79 ± 0.23	$7.9×10^{-4}$	96.9	ENST00000406829	−1870	GATA-1
7	rs3735369	PUS7	T	0.150	↓	−1.21 ± 0.35	$6.4×10^{-4}$	−0.82 ± 0.24	$6.2×10^{-4}$	86.8	ENST00000487277	−3153	
8	rs1786330	AP001205	T	0.237	↓	−0.92 ± 0.30	$2.1×10^{-3}$	−0.58 ± 0.20	$4.2×10^{-3}$	79.6	ENST00000485490	−3586	
9	rs3780616	ALG2	A	0.016	↓	−2.92 ± 1.00	$3.5×10^{-3}$	−1.80 ± 0.68	$7.8×10^{-3}$	74.2	ENSG00000119523	−3522	CREB
11	rs10768970	AC068205	C	0.240	↓	−1.04 ± 0.30	$4.8×10^{-4}$	−0.69 ± 0.20	$5.5×10^{-4}$	88.6	ENST00000500886	−1272	
12	rs11113470	ASCL4	G	0.451	↑	0.79 ± 0.26	$2.0×10^{-3}$	0.59 ± 0.17	$6.6×10^{-4}$	80.0	ENST00000342331	−3612	
15	rs11638762	AKAP13	T	0.451	↓	−1.00 ± 0.25	$9.3×10^{-5}$	−0.47 ± 0.17	$7.0×10^{-3}$	94.4	ENST00000452008	−2682	GATA-3
20	rs4811719	AL137162	T	0.430	↓	−0.95 ± 0.26	$2.4×10^{-4}$	−0.55 ± 0.17	$1.7×10^{-3}$	91.8	ENST00000496745	−4653	
22	rs11089887	RP3−434P1	G	0.186	↓	−1.09 ± 0.33	$8.7×10^{-4}$	−0.70 ± 0.22	$1.6×10^{-3}$	85.6	ENST00000433230	−1617	

Abbreviations: CHR, chromosome; DBP, diastolic blood pressure; M, minor allele; MAF, minor allele frequency; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism; TFBS, transcription factor binding site.
[a]Study power is based on KARE parameters, including minor allele frequency, effect size and mean value of the markers.

rs3735369, rs1786330, rs3780616, rs10768970, rs11638762, rs4811719 and 11089887) decreased the SBP and DBP of individuals who carried them, and the minor alleles of six SNPs (rs9356478, rs3789790, rs6569814, rs4731112, rs7357092 and rs11113470) had the opposite effect on SBP and DBP.

The 20 SNPs were examined in a replication analysis using an independent population of 3703 subjects, recruited from community health care centers in Seoul, Korea (Table 4). Their genotypes were determined using the Affymetrix Genomewide Human SNP array 6.0; 2 of the 20 SNPs were excluded during quality control. The linked gene functions and disease relations are shown in Table 5.

Three SNPs had a *P*-value <0.05 in the replication study. The rs3806650 revealed a significant association to DBP, but the direction of effect (beta=0.55 ± (s.e.) 0.27) was opposite to the original GWAS result (beta=−0.65 ± 0.20). The rs3780616 revealed a significant

association to SBP, and the direction of effect was same to the original GWAS. Meta-analysis improved the *P*-value from $3.5×10^{-3}$ in GWAS to $2.0×10^{-5}$. The rs11638762 had a consistent association with both SBP and DBP in the original and replication studies. In our meta-analysis, the size of the effect of rs11638762 was beta=−0.89 ± 0.20 ($P=1.3×10^{-5}$) for SBP and beta=−0.48 ± 0.14 ($P=6.3×10^{-4}$) for DBP.

DISCUSSION

In this study, we identified 20 upstream SNPs that significantly associated with both SBP and DBP, and three SNPs were replicated in the other cohort. As the replication samples were recruited from community healthcare centers of Seoul and Busan and had smaller sample size than original GWAS samples, we could not rule out the remaining 17 SNPs for the possible association. The 20 SNPs of GWAS

Table 4 Effect sizes and p-values of 18 SNPs in replication samples

| | | | | | Replication study (n=3703) | | | | Meta analysis | | | | | | | |
| | | | | | Systolic blood pressure | | Diastolic blood pressure | | Systolic blood pressure | | | | Diastolic blood pressure | | | |
CHR	SNPs	Linked genes	M	MAF	beta± se	P-value	beta± se	P-value	BETA	SBP	Q	P	BETA	DBP	Q	P
2	rs295142	SPATS2L	C													
2	rs1900706	SPATS2L	C	0.186	0.32±0.41	$4.4×10^{-1}$	0.27±0.29	$3.5×10^{-1}$	−0.57±0.26	2.9E−02	6.18	1.3E−02	−0.40±0.18	2.5E−02	7.47	6.3E−03
2	rs842825	SPATS2L	T	0.184	0.74±0.42	$8.0×10^{-2}$	0.17±0.29	$5.7×10^{-1}$	−0.38±0.26	1.5E−01	9.64	1.9E−03	−0.45±0.18	1.2E−02	6.16	1.3E−02
3	rs3806650	BCHE	G	0.238	0.54±0.38	$1.6×10^{-1}$	0.55±0.27	$4.0×10^{-1}$	−0.43±0.24	6.6E−02	8.87	2.9E−03	−0.25±0.16	1.2E−01	12.80	3.5E−04
3	rs11929235	ILDR1	A	0.246	−0.30±0.38	$4.4×10^{-1}$	0.06±0.27	$8.3×10^{-1}$	−0.63±0.23	6.9E−03	1.06	3.0E−01	−0.36±0.16	2.3E−02	3.53	6.0E−02
3	rs17279465	SLC6A20	C	0.045	−0.04±0.79	$9.6×10^{-1}$	−0.87±0.55	$1.2×10^{-1}$	−1.35±0.48	5.0E−03	3.85	5.0E−02	−1.22±0.33	2.2E−04	0.58	4.5E−01
6	rs9356478	AL022069	C	—	—	—	—	—	—	—	—	—	—	—	—	—
6	rs3789790	FUCA2	G	0.210	0.04±0.40	$9.2×10^{-1}$	−0.03±0.28	$9.2×10^{-1}$	0.63±0.25	1.1E−02	2.96	8.5E−02	0.40±0.17	1.7E−02	3.34	6.7E−02
6	rs195386	RNF8	T	0.112	−0.37±0.52	$4.7×10^{-1}$	−0.38±0.36	$3.0×10^{-1}$	−1.14±0.32	3.9E−04	3.03	8.2E−02	−0.67±0.22	2.5E−03	0.90	3.4E−01
6	rs6569814	TAAR3	T	0.327	0.44±0.35	$2.1×10^{-1}$	−0.11±0.25	$6.6×10^{-1}$	0.65±0.22	2.7E−03	0.49	4.8E−01	0.31±0.15	3.6E−02	4.10	4.3E−02
7	rs4731112	RP11−390E23	C	0.197	−0.17±0.41	$6.9×10^{-1}$	−0.29±0.29	$3.2×10^{-1}$	0.65±0.25	1.0E−02	5.43	2.0E−02	0.32±0.17	6.2E−02	6.39	1.1E−02
7	rs7357092	AC006465	T	0.171	−0.21±0.44	$6.4×10^{-1}$	−0.04±0.31	$9.0×10^{-1}$	0.88±0.27	1.3E−03	8.34	3.9E−03	0.52±0.19	6.0E−03	4.54	3.3E−02
7	rs3735369	PUS7	T	0.154	0.10±0.45	$8.3×10^{-1}$	0.21±0.32	$5.1×10^{-1}$	−0.78±0.28	5.4E−03	5.17	2.3E−02	−0.48±0.19	1.2E−02	6.69	9.7E−03
8	rs1786330	AP001205	T	0.239	0.27±0.39	$4.9×10^{-1}$	0.14±0.27	$6.0×10^{-1}$	−0.53±0.24	2.5E−02	5.94	1.5E−02	−0.34±0.16	3.5E−02	4.59	3.2E−02
9	rs3780616	**ALG2**	A	0.014	−4.61±1.41	**$1.1×10^{-3}$**	−1.60±0.99	$1.0×10^{-1}$	−3.47±0.81	**2.0E−05**	0.96	3.3E−01	−1.73±0.56	1.9E−03	0.03	8.7E−01
11	rs10768970	AC068205	C	0.232	−0.07±0.39	$8.5×10^{-1}$	−0.01±0.27	$9.6×10^{-1}$	−0.72±0.24	2.3E−03	3.87	4.9E−02	−0.47±0.16	3.6E−03	4.03	4.5E−02
12	rs11113470	ASCL4	G	0.451	0.01±0.32	$9.8×10^{-1}$	0.00±0.23	$9.9×10^{-1}$	0.54±0.20	8.2E−03	3.59	5.8E−02	0.40±0.14	4.1E−03	4.22	4.0E−02
15	rs11638762	**AKAP13**	T	0.457	−0.68±0.34	**$4.7×10^{-2}$**	−0.51±0.24	**$3.4×10^{-2}$**	−0.89±0.20	**1.3E−05**	0.55	4.6E−01	−0.48±0.14	**6.3E−04**	0.02	8.9E−01
20	rs4811719	AL137162	T	0.437	−0.08±0.33	$8.1×10^{-1}$	0.02±0.23	$9.4×10^{-1}$	−0.66±0.20	1.2E−03	4.29	3.8E−02	−0.36±0.14	9.6E−03	3.82	5.1E−02
22	rs11089887	RP3−434P1	G	0.185	0.11±0.42	$7.9×10^{-1}$	0.06±0.30	$8.4×10^{-1}$	−0.69±0.26	7.5E−03	5.04	2.5E−02	−0.45±0.18	1.2E−02	4.22	4.0E−02

Abbreviations: CHR, chromosome; DBP, diastolic blood pressure; M, minor allele; MAF, minor allele frequency; P, P-value for Cochran's Q test; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism; TFBS, transcription factor binding site; Q, Cochran's Q test heterogeneity between studies.
Bold characters highlight the replicated results ($P<0.05$) in the replication study samples.

Table 5 Gene function and related diseases

CHR	SNPs	Linked genes	Description	Function	Disease	Reference
2	rs295142 rs1900706 rs842825	SPATS2L	Spermatogenesis-associated, serine-rich 2-like	—	—	
3	rs3806650	BCHE	Butyrylcholinesterase	Nonspecific cholinesterase found in the blood plasma	Suxamethonium apnea	24
3	rs11929235	ILDR1	Immunoglobulin-like domain-containing receptor 1	—	—	
3	rs17279465	SLC6A20	Solute carrier family 6 (proline IMINO transporter), member 20	Na+ and Cl- coupled transporter	Iminoglycinuria	25
6	rs3789790	FUCA2	Fucosidase, alpha-L-2, plasma	Plasma alpha-L-fucosidase	Fucosidosis	26
6	rs195386	RNF8	Ring finger protein 8	A ubiquitin ligase (E3) in the ubiquitination of certain nuclear proteins.	—	27
6	rs6569814	TAAR3	Trace amine-associated receptor 3 (gene/pseudogene)	Pseudogene	—	28
7	rs3735369	PUS7	Pseudouridylate synthase 7 homolog	—	—	
9	rs3780616	ALG2	Asparagine-linked glycosylation 2, alpha-1,3-mannosyltransferase homolog (Saccharomyces cerevisiae)	Mannosylating Man(2)GlcNAc(2)-dolichol diphosphate	Glycosylation type Ih	29
12	rs11113470	ASCL4	Achaete-scute complex homolog 4	Skin development	—	30
15	rs11638762	AKAP13	A kinase (PRKA) anchor protein 13	Regulating the Rho/Rac GTPase cycle (brx in mouse), cardiac development	Leukemia, cancer	21

had the moderate P-values ($7.8×10^{-3}$ to $9.3×10^{-5}$), and thus they were not considered the candidate region of blood pressure in the previous GWAS study by using the whole SNPs of the Affymetrix SNP chip.[8] Nevertheless, three SNPs revealed the significant P-value and two of them revealed the same effect direction. Therefore, our approach that is the function-oriented association suggests a new solution of high-throughput GWAS.

The rs11638762 is the most significant result and located in a kinase anchor protein 13 (AKAP13). AKAP13 binds to the regulatory subunit of protein kinase A, anchoring it to discrete areas within cells. AKAP13 also binds RhoA to activate the Rho family GTPase;[17–19] the GTPase is a mediator of cardiac hypertrophy, and AKAP13 is upregulated in hypertrophic cardiomyocytes.[20] Moreover, cardiomyocytes in AKAP13-null mice have impaired sarcomere formation, causing
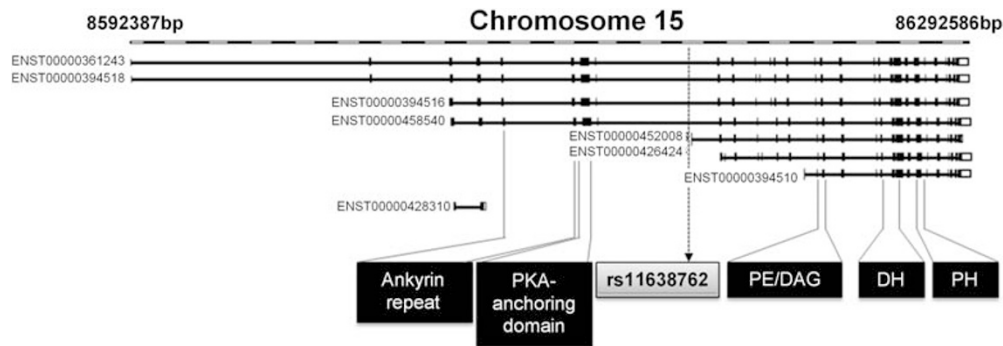
Figure 2 Genomic location, transcript variants and functional domain positions of the AKAP13 gene obtained from Ensembl (http://www.ensembl.org/index.html). PE/DAG: protein kinase C-like diacyglycerol binding site (PE/DAG), DH: dbl oncogene homology, PH: pleckstrin homology. rs11638762 is a significantly associated SNP −2682 bp of ENST00000452008 (bold highlighted).

embryos to develop thin-walled hearts and die at E10.5-11.0.[21] Altered AKAP13 expression influences genes that are essential for normal cardiac development, particularly myocyte enhancer factor 2C.[21] Therefore, we speculate that rs11638762, an SNP of AKAP13, alters AKAP13 expression levels, leading to variations in wall thickness in the heart, which might influence blood pressure, secondary to the pumping capacity of the heart.

AKAP13 lies on chromosome 15q24-25 (ref. 22) and has eight transcriptional variants, generated by alternative splicing, as observed in Ensembl (http://www.ensembl.org/index.html) (Figure 2). The longest transcript contains at least five domains, such as an ankyrin-repeat domain, a PKA-anchoring domain, a protein kinase C-like diacyglycerol consensus binding site, a dbl oncogene homology domain, and a pleckstrin homology domain; a dbl oncogene homology–pleckstrin homology domain acts as a guanine nucleotide exchange factor for the Rho GTPase.[17]

rs11638762 is located upstream of three AKAP13 transcripts (ENST00000452008, ENST00000426424 and ENST00000394510), 2682 bp upstream of ENST00000452008 (Figure 2). However, the SNP located on intron of the other AKAP13 transcript. All these three transcripts contain dbl oncogene homology–pleckstrin homology domains that bind to Rho. Moreover, the minor allele of this SNP was predicted by the TFsearch program to disrupt GATA-3 binding to the AKAP13 promoter.[23] The major allele (A) is the GATA-3 binding site is NNGATARNG (TF score =94.7); the underlined nucleotide is changed in the SNP (rs11638762) to T, possibly interrupting its transcriptional regulation. However, we should mention that if three transcripts are not the major transcripts of AKAP13, the effect of rs11638762 on differential expression of AKAP13 might not be influential on the regulation of blood pressure.

The rs3780616 revealed consistent association for SBP, but not DBP. The SNP located on the upstream of asparagines-linked glycosylation 2 alpha-1,3-mannosyltransferase homolog (ALG2).[29] The ALG2 gene has three alternative transcripts, and the SNP locates at −3522 bp upstream of all three transcripts. The minor allele of this SNP was predicted by the TF search program to create a new cAMP response element-binding (CREB) to the ALG2 promoter. The minor allele (T) sequence of the SNP site can be change to CREB binding site TGACCTCA (TF score=86.6), possibly induce the CREB binding. It has been known that ALG2 is related to cell cycle,[31] but there is no report related to blood pressure regulation in ALG2 gene.

In conclusion, rs11638762, located upstream of the AKAP13 gene, has a robust association with blood pressure, and it lies in a GATA-3 binding site, rendering the SNP a potential cause of blood pressure

variations through alterations in AKAP13 expression levels. This study also demonstrates that our approach of using functional SNPs, such as regulatory SNPs (TFBS–SNPs) in addition to a previous GWAS on nonsynonymous SNPs,[9] identifies the genetic variations that biologically linked to blood pressure regulation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Fields, L. E., Burt, V. L., Cutler, J. A., Hughes, J., Roccella, E. J. & Sorlie, P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. *Hypertension* **44**, 398–404 (2004).
2 Lawes, C. M., Vander Hoorn, S. & Rodgers, A. Global burden of blood-pressure-related disease, 2001. *Lancet* **371**, 1513–1518 (2008).
3 WTCCC. Genome-wide association study of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* **447**, 661–678 (2007).
4 Frazer, K. A., Murray, S. S., Schork, N. J. & Topol, E. J. Human genetic variation and its contribution to complex traits. *Nat. Rev. Genet.* **10**, 241–251 (2009).
5 Mohlke, K. L., Boehnke, M. & Abecasis, G. R. Metabolic and cardiovascular traits: an abundance of recently identified common genetic variants. *Hum. Mol. Genet.* **17**, R102–108 (2008).
6 Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M. D., Bochud, M., Coin, L. *et al.* Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.* **41**, 666–676 (2009).
7 Levy, D., Ehret, G. B., Rice, K., Verwoert, G. C., Launer, L. J., Dehghan, A. *et al.* Genome-wide association study of blood pressure and hypertension. *Nat. Genet.* **41**, 677–687 (2009).
8 Cho, Y. S., Go, M. J., Kim, Y. J., Heo, J. Y., Oh, J. H., Ban, H. J. *et al.* A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat. Genet.* **41**, 527–534 (2009).
9 Hong, K. W., Jin, H. S., Lim, J. E., Cho, Y. S., Go, M. J. *et al.* Non-synonymous single-nucleotide polymorphisms associated with blood pressure and hypertension. *J. Hum Hypertens.* **24** (11), 763–774 (2010).
10 Wu, L. C., Horng, J. T. & Chen, Y. A. A computation to integrate the analysis of genetic variations occurring within regulatory elements and their possible effects. *J. Comput. Biol.* **16**, 1731–1747 (2009).
11 Kim, H. A., Chun, H. Y., Kim, S. H., Park, H. S. & Suh, C. H. C-reactive protein gene polymorphisms in disease susceptibility and clinical manifestations of Korean systemic lupus erythematosus. *J. Rheumatol.* **36**, 2238–2243 (2009).
12 Kawai, T., Takeshita, S., Imoto, Y., Matsumoto, Y., Sakashita, M., Suzuki, D. *et al.* Associations between decay-accelerating factor polymorphisms and allergic respiratory diseases. *Clin. Exp. Allergy* **39**, 1508–1514 (2009).
13 Egli, R. J., Southam, L., Wilkins, J. M., Lorenzen, I., Pombo-Suarez, M., Gonzalez, A. *et al.* Functional analysis of the osteoarthritis susceptibility-associated GDF5 regulatory polymorphism. *Arthritis Rheum.* **60**, 2055–2064 (2009).

210

14 Harendza, S., Stahl, R. A. & Schneider, A. The transcriptional regulation of podocin (NPHS2) by Lmx1b and a promoter single nucleotide polymorphism. *Cell Mol. Biol. Lett.* **14,** 679–691 (2009).

15 Rabbee, N. & Speed, T. P. A genotype calling algorithm for affymetrix SNP arrays. *Bioinformatics* **22,** 7–12 (2006).

16 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559–575 (2007).

17 Diviani, D., Soderling, J. & Scott, J. D. AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation. *J. Biol. Chem.* **276,** 44247–44257 (2001).

18 Schmidt, A. & Hall, A. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev.* **16,** 1587–1609 (2002).

19 Sterpetti, P., Hack, A. A., Bashar, M. P., Park, B., Cheng, S. D., Knoll, J. H. *et al.* Activation of the Lbc Rho exchange factor proto-oncogene by truncation of an extended C terminus that regulates transformation and targeting. *Mol. Cell Biol.* **19,** 1334–1345 (1999).

20 Carnegie, G. K., Soughayer, J., Smith, F. D., Pedroja, B. S., Zhang, F., Diviani, D. *et al.* AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway. *Mol. Cell* **32,** 169–179 (2008).

21 Mayers, C. M., Wadell, J., McLean, K., Venere, M., Malik, M., Shibata, T. *et al.* The RHO-guanine nucleotide exchange factor AKAP13 (BRX) is essential for cardiac development in mice. *J. Biol. Chem.* **285,** 12344–12354 (2010).

22 Miller, B. T., Rubino, D. M., Driggers, P. H., Haddad, B., Cisar, M., Gray, K. *et al.* Expression of brx proto-oncogene in normal ovary and in epithelial ovarian neoplasms. *Am. J. Obstet. Gynecol.* **182,** 286–295 (2000).

23 Ohneda, K. & Yamamoto, M. Roles of hematopoietic transcription factors GATA-1 and GATA-2 in the development of red blood cell lineage. *Acta. Haematol.* **108,** 237–425 (2002).

24 Barta, C., Sasvari-Szekely, M., Devai, A., Kovacs, E., Staub, M. & Enyedi, P. Analysis of mutations in the plasma cholinesterase gene of patients with a history of prolonged neuromuscular block during anesthesia. *Mol. Genet. Metab.* **74,** 484–488 (2001).

25 Broer, A., Balkrishna, S., Kottra, G., Davis, S., Oakley, A. & Broer, S. Sodium translocation by the iminoglycinuria associated imino transporter (SLC6A20). *Mol. Membr. Biol.* **26,** 333–346 (2009).

26 O'Brien, J. S., Willems, P. J., Fukushima, H., de Wet, J. R., Darby, J. K., Di Cioccio, R. *et al.* Molecular biology of the alpha-L-fucosidase gene and fucosidosis. *Enzyme* **38,** 45–53 (1987).

27 Ishikawa, K., Nagase, T., Suyama, M., Miyajima, N., Tanaka, A., Kotani, H. *et al.* Prediction of the coding sequences of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. *DNA Res.* **5,** 169–176 (1998).

28 Lindemann, L., Ebeling, M., Kratochwil, N. A., Bunzow, J. R., Grandy, D. K. & Hoener, M. C. Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics* **85,** 372–385 (2005).

29 Thiel, C., Schwarz, M., Peng, J., Grzmil, M., Hasilik, M., Braulke, T. *et al.* A new type of congenital disorders of glycosylation (CDG-Ii) provides new insights into the early steps of dolichol-linked oligosaccharide biosynthesis. *J. Biol. Chem.* **278,** 22498–22505 (2003).

30 Jonsson, M., Bjorntorp Mark, E., Brantsing, C., Brandner, J. M., Lindahl, A. & Asp, J. Hash4, a novel human achaete-scute homologue found in fetal skin. *Genomics* **84,** 859–866 (2004).

31 Hoj, B. R., la Cour, J. M., Mollerup, J. & Berchtold, M. W. ALG-2 knockdown in HeLa cells results in G2/M cell cycle phase accumulation and cell death. *Biochem. Biophys. Res. Commun.* **378,** 145–148 (2009).