

# Five Blood Pressure Loci Identified by an Updated Genome-Wide Linkage Scan: Meta-Analysis of the Family Blood Pressure Program

Jeannette Simino<sup>1</sup>, Gang Shi<sup>1</sup>, Rezart Kume<sup>1</sup>, Karen Schwander<sup>1</sup>, Michael A. Province<sup>2</sup>, C. Charles Gu<sup>1</sup>, Sharon Kardia<sup>3</sup>, Aravinda Chakravarti<sup>4</sup>, Georg Ehret<sup>4,5</sup>, Richard A. Olshen<sup>6</sup>, Stephen T. Turner<sup>7</sup>, Low-Tone Ho<sup>8</sup>, Xiaofeng Zhu<sup>9</sup>, Cashell Jaquish<sup>10</sup>, Dina Paltoo<sup>10</sup>, Richard S. Cooper<sup>11</sup>, Alan Weder<sup>12</sup>, J. David Curb<sup>13</sup>, Eric Boerwinkle<sup>14</sup>, Steven C. Hunt<sup>15</sup> and Dabeeru C. Rao<sup>1</sup>

## BACKGROUND

A preliminary genome-wide linkage analysis of blood pressure in the Family Blood Pressure Program (FBPP) was reported previously. We harnessed the power and ethnic diversity of the final pooled FBPP dataset to identify novel loci for blood pressure thereby enhancing localization of genes containing less common variants with large effects on blood pressure levels and hypertension.

## METHODS

We performed one overall and 4 race-specific meta-analyses of genome-wide blood pressure linkage scans using data on 4,226 African-American, 2,154 Asian, 4,229 Caucasian, and 2,435 Mexican-American participants (total  $N = 13,044$ ). Variance components models were fit to measured (raw) blood pressure levels and two types of antihypertensive medication adjusted blood pressure phenotypes within each of 10 subgroups defined by race and network. A modified Fisher's method was used to combine the  $P$  values for each linkage marker across the 10 subgroups.

## RESULTS

Five quantitative trait loci (QTLs) were detected on chromosomes 6p22.3, 8q23.1, 20q13.12, 21q21.1, and 21q21.3 based on significant linkage evidence (defined by logarithm of odds (lod) score  $\geq 3$ ) in at least one meta-analysis and lod scores  $\geq 1$  in at least 2 subgroups defined by network and race. The chromosome 8q23.1 locus was supported by Asian-, Caucasian-, and Mexican-American-specific meta-analyses.

## CONCLUSIONS

The new QTLs reported justify new candidate gene studies. They may help support results from genome-wide association studies (GWAS) that fall in these QTL regions but fail to achieve the genome-wide significance.

**Keywords:** blood pressure; genetics; hypertension; linkage; meta-analysis; QTL

*American Journal of Hypertension*, advance online publication 9 December 2010; doi:10.1038/ajh.2010.238

<sup>1</sup>Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri, USA; <sup>2</sup>Division of Statistical Genomics, Washington University School of Medicine, St. Louis, Missouri, USA; <sup>3</sup>Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA; <sup>4</sup>McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, Maryland, USA; <sup>5</sup>Institute of Social and Preventive Medicine, University Hospital Lausanne and Cardiology Center, Geneva University Hospital, Geneva, Switzerland; <sup>6</sup>Division of Biostatistics, Stanford University, Stanford, California, USA; <sup>7</sup>Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA; <sup>8</sup>Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; <sup>9</sup>Department of Biostatistics and Epidemiology, Case Western Reserve University, Cleveland, Ohio, USA; <sup>10</sup>Division of Cardiovascular Sciences, National Heart, Lung, Blood Institute, Bethesda, Maryland, USA; <sup>11</sup>Department of Preventive Medicine and Epidemiology, Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois, USA; <sup>12</sup>Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, Michigan, USA; <sup>13</sup>Department of Geriatric Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA; <sup>14</sup>Human Genetics Center, University of Texas Health Sciences Center, Houston, Texas, USA; <sup>15</sup>Department of Internal Medicine, University of Utah, Salt Lake City, Utah, USA. Correspondence: D.C. Rao ([rao@wubios.wustl.edu](mailto:rao@wubios.wustl.edu))

Received 23 July 2010; first decision 13 September 2010; accepted 17 October 2010.

© 2011 American Journal of Hypertension, Ltd.

According to the 2005–2006 National Health and Nutrition Examination Survey, 29% of all US adults are hypertensive with respective prevalences of 41%, 28%, and 22% among African, Caucasian, and Hispanic American adults.<sup>1</sup> Elevated blood pressure (BP) is the most common modifiable risk factor for cardiovascular disease. Thus, there is great interest in identifying the underlying genetic and environmental determinants. In 1995 the National Heart, Lung, and Blood Institute established a collaboration of four multicenter networks, known as the Family Blood Pressure Program (FBPP), to investigate the genetic determinants of interindividual BP variation in Asians, African Americans, Mexican Americans, and Caucasians.<sup>2</sup> The FBPP collaboration yielded statistical power and diversity unmatched in any individual study.<sup>3</sup>

The complete FBPP resource includes 13,516 individuals with microsatellite genotype data from two temporally distinct study phases (phases 1 and 2 were conducted from 1995–2000 to 2000–2005, respectively). We report here a linkage analysis of the complete pooled FBPP data, which has considerably

more power for detecting quantitative trait loci (QTLs) for BP. Indeed, this investigation reports multiple QTLs in comparison with previous reports based on various subsets of the interim data.<sup>4–6</sup>

The FBPP database permits an overall meta-analysis of all 10 subgroups defined by network and race and 4 race-specific meta-analyses (three cohorts each of African Americans and Caucasians, 2 cohorts of Mexican Americans, and 2 cohorts of Asians—Japanese and Chinese). We conducted all analyses using the observed BP as well as two types of antihypertensive medication adjusted systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) phenotypes to search for novel BP QTLs. Recent genome-wide association studies (GWAS) have identified a number of common variants associated with BP and/or hypertension. However, they mostly include individuals of European descent and account for only a small fraction of the phenotypic variance.<sup>7</sup> Therefore, there is a need to identify genomic regions that harbor rare variants with relatively large

effect sizes. Detecting such regions could lead to novel findings that complement the current GWAS results.

## METHODS

**Subjects.** The FBPP pooled data derive from the four separate multicenter networks: GenNet, Genetic Epidemiology Network of Atherosclerosis (GENOA), Hypertension Genetic Epidemiology Network (HyperGEN), and Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRE)<sup>2</sup> (Table 1). A detailed description of each cohort can be found elsewhere.<sup>2</sup> All FBPP studies were approved by their respective institutional review committees and all participants gave informed consent. All networks recruited families ascertained through individuals with high or low BP. GenNet recruited African-American, Caucasian, and Mexican-American nuclear families ascertained through young-/middle-aged probands with untreated high-normal BP. GENOA recruited African-American and Caucasian sibships containing at least two hypertensive members, Mexican-American

**Table 1 | Characteristics of subjects in linkage analysis**

Network	Race	$N_{fam}$	Sex	$N^a$	Mean age (years)	HT%	Meds%	Mean BMI (kg/m <sup>2</sup> )	Mean SBP (mm Hg)	Mean DBP (mm Hg)	Mean MAP (mm Hg)	Mean PP (mm Hg)
GenNet	Black	266	F	475	42	38	27	32.7	126.1	76.8	93.2	49.3
			M	320	39	30	11	27.1	129.2	79.0	95.7	50.2
	Hispanic	184	F	492	36	11	9	29.0	109.4	60.5	76.8	48.9
			M	301	33	13	8	28.1	116.2	63.3	80.9	52.9
	Caucasian	226	F	537	46	33	26	29.2	119.8	67.7	85.1	52.2
			M	434	44	34	23	28.9	127.1	72.7	90.8	54.4
Total	676	—	2,559	41	27	18	29.4	121.0	69.7	86.8	51.2	
GENOA	Black	518	F	1,155	57	73	64	32.0	130.3	69.1	89.5	61.2
			M	521	59	68	54	28.3	131.1	73.9	92.9	57.2
	Hispanic	392	F	969	55	50	39	31.5	126.9	68.3	87.9	58.6
			M	673	55	48	29	29.6	129.6	74.4	92.8	55.2
	Caucasian	478	F	768	55	73	67	30.3	130.8	72.8	92.2	58.0
			M	640	56	78	64	30.3	135.6	79.9	98.4	55.7
Total	1,388	—	4,726	56	65	54	30.6	130.4	72.3	91.6	58.2	
HyperGEN	Black	522	F	1,159	47	71	67	33.5	128.7	73.1	91.6	55.6
			M	596	46	65	56	29.7	130.8	77.1	95.0	53.7
	Caucasian	430	F	968	54	61	59	29.2	120.3	66.9	84.7	53.5
			M	882	52	61	57	29.0	122.8	72.5	89.3	50.3
Total	952	—	3,605	50	65	60	30.6	125.4	72.0	89.8	53.4	
SAPPHIRE	Chinese	406	F	871	52	60	53	24.8	127.4	73.7	91.6	53.7
			M	695	50	70	57	25.9	133.2	81.9	99.0	51.4
	Japanese	158	F	346	59	82	77	26.4	132.7	75.3	94.4	57.5
			M	242	59	84	75	27.4	136.1	83.6	101.1	52.4
Total	564	—	2,154	53	69	60	25.7	131.1	77.8	95.6	53.3	
TOTAL		3,580		13,044	51	58	49	29.7	127.1	72.4	90.6	54.7

BMI, body mass index; DBP, diastolic blood pressure; HT%, percent sample who are hypertensive; GENOA, Genetic Epidemiology Network of Atherosclerosis; HyperGEN, Hypertension Genetic Epidemiology Network; MAP, mean arterial pressure; Meds%, percent sample who are on antihypertensive/diuretic medications.  $N_{fam}$ , number of families;  $N$ , sample size; PP, pulse pressure; SAPPHIRE, Stanford Asian Pacific Program in Hypertension and Insulin Resistance; SBP, systolic blood pressure.

<sup>a</sup>The number of individuals appearing in both the phenotype and genotype datasets. 12,995 individuals have some microsatellite data, 11,932 have one or more non-outlier covariate-adjusted blood pressure values, and 13,031 have either some microsatellite or some non-outlier covariate adjusted phenotype information.

sibships containing at least two adult onset diabetic siblings and all full siblings of each index pair. HyperGEN recruited African-American and Caucasian hypertensive siblings, their parents, and one or more of a sibling's untreated offspring; sibships containing one severely hypertensive member were ascertained preferentially. SAPHIRE recruited Chinese and Japanese sib pairs that were highly concordant or discordant for hypertension.

**Genotyping.** The Mammalian Genotyping Service in Marshfield, WI, performed all genome-wide scans.<sup>8</sup> For all cohorts except GenNet Mexican Americans, we used 362 autosomal markers (with average intermarker distance 9.5 cM) after removing those with high (>70%) missing rates, that were duplicates of the same position, or were mapped to multiple locations in the genome. GenNet Mexican Americans were genotyped much later, using a subsequent (marker) screening set. After quality control, this yielded 372 Marshfield autosomal markers (with average intermarker distance 9.4 cM). Most (71%) of the GenNet Mexican-American markers overlapped or shared a genetic distance with markers used in other cohorts. Family relationships were verified using graphical representation of relationships and affected sibpair exclusion mapping, while Mendelian errors were removed via MapMaker/SIBS and Pedcheck.<sup>4</sup>

**Phenotype adjustment.** All networks used a consistent BP measurement protocol.<sup>4,6</sup> The pooled FBPP dataset includes the average of three sitting SBP or DBP measurements taken using Dinamap (for 504 GenNet African Americans and 2 GenNet Caucasians, the measurements were taken using the Omron). MAP was estimated by the sum of two-thirds the average DBP and one-third the average SBP. PP was computed as the difference between the average SBP and DBP. We excluded the BP phenotypes of participants with hypertension diagnosed after age 60 or with BMI, SBP, DBP, MAP, or PP values four or more standard deviations from the mean of the subgroup (based on network, race, and sex).

The observed blood pressures (SBP, DBP, MAP, PP) of all individuals, irrespective of antihypertensive medication use, composed the “raw” set of phenotypes. Next, we assumed the measured BP of individuals treated with antihypertensive medications was lower on average than if the individuals had not been treated. Since any attempt to adjust BP for medication use is only an approximation, we calculated two different sets of “medication adjusted” BP phenotypes. The “+10/5” medication adjusted values<sup>9</sup> were derived by adding 10 mm Hg to SBP and 5 mm Hg to DBP (only for those known to be taking medications). Similarly, the “+15/10” medication adjusted values<sup>10</sup> were obtained by adding 15 mm Hg to SBP and 10 mm Hg to DBP. The medication adjusted PP and MAP were calculated from the medication adjusted SBP and DBP values. The observed BP phenotypes were used for those untreated or with unknown medication status. Only one medication adjustment was performed for PP since both “+10/5” and “+15/10” result in the same values for all individuals (obtained by adding 5 mm Hg to PP for medication users).

We narrowed our search to primarily hypertension genes instead of genes primary for a comorbidity (such as BMI) and secondary to hypertension. Therefore, phenotypes were also adjusted for BMI in addition to age, age-squared, age-cubed, and field center within each subgroup (defined by network, race, and sex), retaining terms significant at 5%. The residual phenotypes were standardized to a mean of 0 and s.d. of 1. We excluded any covariate-adjusted phenotype value that is 4 or more s.d.s away from the mean of the subgroup (fewer than 3 values were excluded for any phenotype in any group).

**Statistical analysis.** Genome-wide linkage analyses were carried out within subgroups defined by network and race (after male and female blood pressure residuals were combined) using the multipoint variance components method as implemented in Merlin.<sup>11</sup> We used sex-averaged genetic distances from the Marshfield map and allele frequencies estimated from our samples. We tested all markers for additive genetic variance due to a QTL. For GenNet Mexican-American participants, we completed the linkage analysis at the locations of the 372 genotyped markers plus the 90 markers exclusive to the 9 other network-race subgroups. Overall and race-specific meta-analyses were conducted by combining *P* values of the 362 common markers using a modification of Fisher's method.<sup>12</sup>

**Definition of a QTL.** We define a QTL as a genomic segment yielding a significant logarithm of odds (lod) score ( $\geq 3$ ) in any meta-analysis in addition to suggestive evidence (with a lod score  $\geq 1$ ) in at least two subgroups defined by network and race for any blood pressure phenotype.

## RESULTS

After excluding singletons, our analysis sample ( $N = 13,044$ ) included 2,154 Asians, 4,226 African Americans, 2,435 Mexican Americans, and 4,229 Caucasians. **Table 1** displays the summary statistics for each network stratified by race and sex after removal of BP outliers, BMI outliers, and those with hypertension diagnosis after age 60.

Multipoint variance components lod scores are shown in **Table 2**. Loci are included if they yielded lod scores  $\geq 3$  (in bold) for any phenotype in any analysis (the overall meta-analysis, race-specific meta-analysis, or network-race specific analysis). Six of the nine distinct loci presented in **Table 2** are significantly linked to pulse pressure. Five of the nine loci met our (stringent) definition of a QTL: one QTL from each of the overall, African-American, and Hispanic meta-analyses, and two from the Caucasian meta-analysis (see **Supplementary Data** online for heritabilities at QTLs). All Asian meta- and cohort-specific linkage analyses yielded maximum lod scores  $\leq 2.57$ . Thus, this ethnic group is represented in **Table 2** only through its contribution to the overall meta-analysis.

**Table 3** shows the network-race subgroups contributing evidence to each of the five QTLs. Of the five loci with meta-analysis lod scores  $\geq 3$ , the chromosome 8q23.1 (119.22 cM) locus had two or more race-specific meta-analysis lod scores  $\geq 1$ . **Figure 1** displays the overall and race-specific meta-analysis linkage

evidence between raw DBP and chromosome 8 (positions 77–165 cM). This overall meta-analysis QTL was supported by Asians, Caucasians, and Mexican Americans. African Americans failed to show any corroborating evidence at this locus.

The African-American meta-analysis QTL on chromosome 6p22.3 (42.27 cM) was substantiated by GenNet and HyperGEN African Americans while the Caucasian meta-

analysis QTL on chromosome 21q21.1(13.05 cM) was supported by all three Caucasian studies. The fourth QTL on chromosome 21q21.3 (24.73 cM) was supported by all three Caucasians cohorts and HyperGEN African Americans. The Hispanic meta-analysis locus at 20q13.12 (62.32 cM) achieved significant linkage in GENOA Mexican Americans and had a lod score  $\geq 1$  for GenNet Caucasians. **Figure 2** displays the

**Table 2 | Genome-wide significant loci from meta-analysis and cohort-specific linkage analysis**

Race	Analysis	Chr	Pos (cM)	SBP			DBP			MAP			PP	
				Raw	+10 MA	+15 MA	Raw	+5 MA	+10 MA	Raw	+10/5 MA	+15/10 MA	Raw	MA
All	Meta	8	119.22	1.80	1.92	2.04	<b>3.12</b>	2.47	1.61	2.52	2.65	2.15	0.43	0.41
African Americans	Meta	6	42.27	0.85	0.91	0.86	0.01	0.00	0.02	0.04	0.13	0.16	<b>3.76</b>	<b>3.23</b>
	HyperGEN	1	212.44	0.88	1.39	1.55	0.00	0.05	0.17	0.17	0.41	0.61	2.82	<b>3.16</b>
Caucasians	Meta	21	13.05	<b>3.12</b>	<b>3.17</b>	2.87	0.96	0.76	0.56	1.73	1.52	1.24	<b>4.36</b>	<b>4.55</b>
	GENOA	21	13.05	2.83	2.20	1.73	1.24	0.76	0.45	2.00	1.41	0.96	<b>3.06</b>	2.69
	Meta	21	24.73	2.15	2.86	<b>3.01</b>	1.08	0.81	0.57	1.57	1.46	1.18	2.93	<b>3.60</b>
	GenNet	20	95.70	2.39	2.58	2.89	1.37	1.62	1.98	2.52	<b>3.12</b>	<b>3.09</b>	1.03	0.90
Mexican Americans	Meta	20	62.32	1.91	1.75	1.50	0.44	0.53	0.46	1.28	1.07	0.92	<b>4.40</b>	<b>3.98</b>
	GENOA	20	62.32	2.69	1.81	1.44	0.91	0.69	0.54	1.60	0.87	0.59	<b>5.43</b>	<b>4.68</b>
	GenNet	11	105.74	1.24	0.88	0.84	1.30	2.46	<b>3.09</b>	2.69	2.56	2.63	0.37	0.24
	GENOA	17	89.32	2.42	2.66	2.51	0.50	0.50	0.56	1.29	1.36	1.27	<b>3.04</b>	<b>3.15</b>

Lod scores  $\geq 3$  are indicated in bold.

+10 MA, Raw+10 for antihypertensive/diuretic takers; +10/5 MA, phenotype calculated from +10 MA SBP and +5 MA DBP; +15 MA, Raw+15 for antihypertensive/diuretic takers; +15/10 MA, phenotype calculated from +15 MA SBP and +10 MA DBP; +5 MA, Raw+5 for antihypertensive/diuretic takers; DBP, diastolic blood pressure; GENOA, Genetic Epidemiology Network of Atherosclerosis; HyperGEN, Hypertension Genetic Epidemiology Network; lod, logarithm of odds; MAP, mean arterial pressure MA, medication adjusted phenotype; PP, pulse pressure; Raw, measured phenotype; SBP, systolic blood pressure.

**Table 3 | Cohort-specific linkage results (lod scores  $\geq 1$ ) for the five blood pressure QTLs**

Meta	Chr	Position	Cohort	Phenotypes <sup>a</sup> with lod score $\geq 1$	Maximum lod score	
					Value	Phenotype
Overall	8	119.22	GenNet Caucasians	SBP, DBP, MAP	1.84	+5 DBP
			GenNet Mexican Americans	SBP	1.64	+10 SBP
			GENOA Mexican Americans	MAP	1.77	Raw MAP
			SAPPHIRE Chinese	DBP, MAP	1.52	Raw DBP
			SAPPHIRE Japanese	DBP	1.11	Raw DBP
African Americans	6	42.27	GenNet African Americans	PP	2.25	Raw PP
			HyperGEN African Americans	PP	1.64	Raw PP
Caucasians	21	13.05	GenNet Caucasians	SBP, PP	1.54	+15 SBP
			GENOA Caucasians	SBP, DBP, MAP, PP	3.06	Raw PP
			HyperGEN Caucasians	PP	1.17	MA PP
Caucasians	21	24.73	GenNet Caucasians	SBP, PP	1.71	+15 SBP
			GENOA Caucasians	SBP, PP	2.23	MA PP
			HyperGEN African Americans	SBP, DBP, MAP	1.60	+10/5 MAP
			HyperGEN Caucasians	DBP, MAP	1.26	+5 DBP
Mexican Americans	20	62.32	GenNet Caucasians	SBP	1.52	Raw SBP
			GENOA Mexican Americans	SBP, MAP, PP	5.43	Raw PP

<sup>a</sup>For the phenotype(s) listed, lod scores  $\geq 1$  for any combination of raw, +10/5 medication adjusted, and/or +15/10 medication adjusted.

DBP, diastolic blood pressure; GENOA, Genetic Epidemiology Network of Atherosclerosis; HyperGEN, Hypertension Genetic Epidemiology Network; lod, logarithm of odds; MAP, mean arterial pressure; PP, pulse pressure; QTL, quantitative trait loci; SAPPHIRE, Stanford Asian Pacific Program in Hypertension and Insulin Resistance; SBP, systolic blood pressure.

race-specific meta-analysis results for the phenotypes with the highest lod scores at their respective linkage peaks on chromosomes 6, 20, and 21.

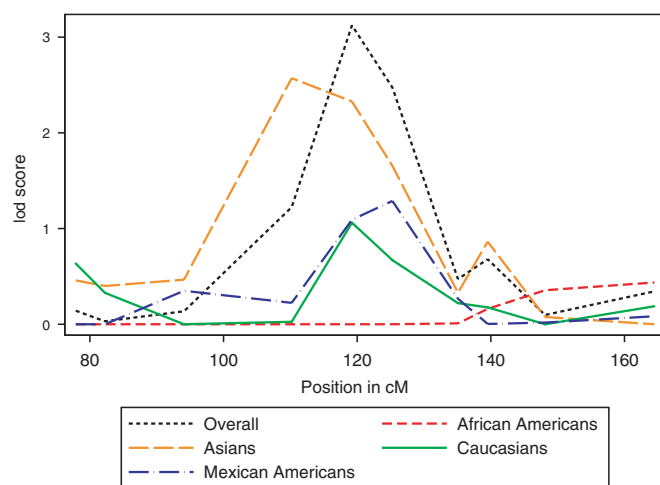
The remaining four loci on chromosome 1q31.3 (212.44 cM), 11q23.1 (105.74 cM), 17q24.2 (89.32 cM), and 20q13.32 (95.70 cM) presented in **Table 2** had significant cohort-specific lod scores but none achieved a significant meta-analysis lod score. All QTLs except 17q24.2 had lod scores  $\geq 1$  in at least one additional subgroup defined by network and race (see **Supplementary Data** online). Finally, we identified a total of 47 secondary QTLs, each one supported by a lod score  $\geq 2$  in any of the subgroups or meta-analyses (see **Supplementary Data** online). All QTLs were located on 17 of the 22 autosomes.

## DISCUSSION

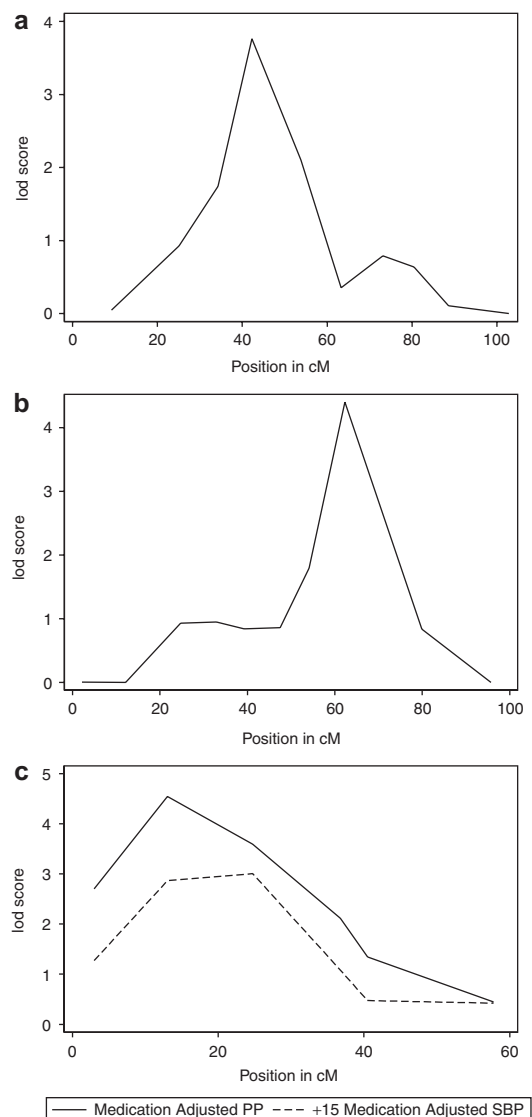
These are both exciting and challenging times to undertake genetic dissection of common complex diseases and disease related traits. They are exciting because the number of promising tools available is constantly on the increase and challenging because the current GWAS findings for BP explain a very small fraction of the total heritability. For example, recent meta-analyses from two large consortia (Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) and Global Blood Pressure Genetics Consortium (GBPGEN)) identified 13 loci through GWAS that collectively explain  $<2\%$  of SBP and DBP variances.<sup>13,14</sup> There is growing consensus that this is largely because of our reliance on “common variants” to explain the genetic heritability of common phenotypes. Finding the missing heritability is the formidable challenge we face.<sup>7</sup> While linkage analysis generally failed to advance the field of common complex traits, we believe that it has a unique role to play in the current debate. In the past, QTLs identified through linkage analysis have been followed up through common variants at positional candidate genes. It is more likely that QTLs are localizing genes with relatively low frequency variants having considerably larger effect sizes. Candidate gene-based resequencing suggests that less common genetic

variants contribute to the phenotypic variance of common diseases.<sup>15</sup> Seen in this light, we believe that the QTLs reported here for BP make an important contribution.

By harnessing the large sample size of the complete FBPP database, we were able to identify five strong QTLs for BP. The most promising QTL for DBP on chromosome 8q23.1 (119.22 cM, marker = D8S1132, lod = 3.12) was identified by the overall meta-analysis of all 10 subgroups defined by network and race. The main finding in Asians, especially given the limited sample size of the SAPPPIRe Japanese cohort ( $N = 588$ ), was their support for this QTL. Both SAPPPIRe Chinese (lod = 1.52) and SAPPPIRe Japanese (lod = 1.11) are major contributors to linkage at D8S1132. The maximum lod



**Figure 1** | Overall and race-specific meta-analysis results for diastolic blood pressure on chromosome 8. lod, logarithm of odds.



**Figure 2** | The race-specific meta-analysis linkage evidence for chromosomes 6, 20, and 21. (a) The African-American meta-analysis linkage evidence between pulse pressure (PP) and chromosome 6. (b) The Mexican-American meta-analysis linkage evidence between pulse pressure and chromosome 20. (c) The Caucasian meta-analysis linkage evidence between medication adjusted PP and chromosome 21, as well as +15 medication adjusted systolic blood pressure (SBP) and chromosome 21. lod, logarithm of odds.



score of any Asian meta-analysis across all phenotypes and markers was 2.57 for DBP on chromosome 8q22.2 at 110.2 cM (8.14 Mbps from the overall meta-analysis peak). The same genomic segment (8q22–23) was previously linked to essential hypertension in 173 affected individuals in a 2,180-member pedigree in Campora, Italy.<sup>16</sup> Significant association between SBP and marker D8S592 on chromosome 8q24.11 (125.3 cM) was reported earlier in GENOA Caucasians.<sup>17</sup> While we lacked significant evidence for this QTL in African Americans, a GWAS of 1,017 normotensive African Americans identified a significant ( $P = 1.59 \times 10^{-8}$ ) association between SBP and rs17365948 on chromosome 8q22.3 (5 Mb from our marker D8S1132).<sup>18</sup> This result was not supported in the GWAS replication sample of 980 West Africans ( $P = 0.507$ ). A disparity in minor allele frequencies (MAFs) between the African-American (MAF = 0.113) and West African (MAF = 0.005) samples could be responsible in part for the discrepancy in results.<sup>18</sup> Moderate evidence for linkage (lod = 1.74) was found between D8S1132 and the log-transformed DBP in subjects from rural Nigeria.<sup>19</sup> The significant SNP (rs17365948) is in the intron of YWHAZ, which may have a role in regulating insulin sensitivity (<http://genome.ucsc.edu>).<sup>20</sup> Other candidate genes in this region include ANGPT1, ZFPM2, and OXR1. Angiopoietin, encoded by ANGPT1, induces pulmonary hypertension in rodents, is increased in humans suffering from essential hypertension which decreases after successful antihypertensive treatment.<sup>16,21</sup> ZFPM2 codes a zinc finger protein that may modulate the activity of the GATA proteins that help regulate mammalian hematopoiesis and cardiogenesis.<sup>20</sup> Oxidation resistance 1 (OXR1), the closest gene, may help prevent oxidative damage.<sup>20</sup>

The QTL for PP on chromosome 6p22.3 (42.27 cM, marker = D6S2439, lod = 3.76) from the African-American-specific meta-analysis has also been implicated for SBP in a GWAS of normotensive African Americans.<sup>18</sup> The significant ( $P = 3.42 \times 10^{-9}$ ) association between SBP and rs16877320 on chromosome 6p22.3 (8 Mb from our marker D6S2439) failed to replicate in 980 West Africans ( $P = 0.781$ ).<sup>18</sup> Again a discrepancy in MAF could be responsible in part. The MAF of the SNP in the African-American and West African samples were 0.132 and 0.023, respectively.<sup>18</sup> Linkage studies have provided evidence for this locus with other BP phenotypes in other ethnicities. Postural changes in SBP (lod = 1.9) and DBP (lod = 1.7) were both linked, at least tentatively, to D6S2439 in 498 HyperGEN Caucasian hypertensive sibpairs.<sup>22</sup> Potential or significant linkage of SBP within 10 cM of D6S2439 has been demonstrated in western Europeans (52 cM, lod = 1.97)<sup>23</sup> and GENOA Hispanics (34.2 cM,  $P = 0.002$ ).<sup>17</sup> Early studies hinted that a genomic segment near HLA class II was linked to essential hypertension.<sup>24</sup> D6S2439 is ~5.6 Mbps from HLA-A and 8.2 Mbps from HLA-DRB1. D6S2439 is also 7.2 Mbps from the tumor necrosis factor- $\alpha$  gene which was associated with obesity-related hypertension in nonmorbidly obese French-Canadians.<sup>25</sup> Tumor necrosis factor amplifies the production of angiotensinogen and endothelin-1<sup>25</sup>; the latter is a potent vasoactive peptide.<sup>24</sup>

The two QTLs at 21q21.1 (13.05 cM, marker = D21S1437) for PP (lod = 4.55) and SBP (lod = 3.17) and 21q21.3 (24.73 cM, marker = D21S2052) for PP (lod = 3.60) and SBP (lod = 3.01) may implicate one or more BP associated genes. The confidence interval for PP subsumes that for SBP, although different genetic causes may be contributing to SBP and PP. The number of peaks encompassing these two neighboring markers is indeterminate but sufficient supporting evidence is found in the intervening genomic region. A previous analysis of log-transformed pulse pressure in the FBPP phase 1 pooled data identified a significant locus in Caucasians (lod = 4.3) at 18 cM and combined African Americans and Caucasians (lod = 3.2) at 19 cM.<sup>4</sup> The same analysis yielded suggestive linkage in all racial groups combined (lod = 2.1) at 21 cM and GENOA Caucasians (lod = 2.2) at 17 cM.<sup>4</sup> A suggestive linkage (lod = 2.44) between 21q21 and MAP was established in Mexican Americans from the San Antonio Family Heart Study.<sup>26</sup> A significant association between SBP and D21S2052 at 24.73 cM was previously reported in GENOA Mexican Americans.<sup>17</sup> A promising region for DBP in proximity to 24.73 cM was also discovered during a secondary analysis of low concordant sibpairs from Anqing, China.<sup>27</sup> Admixture mapping methods have indicated that 21q21 may contain genes influencing risk of hypertension in African Americans.<sup>28,29</sup> In the admixture mapping analysis of Dallas Heart Study sample using 1,890 ancestry informative SNPs, the 1-unit Z score drop region spans from 8 to 30 cM,<sup>29</sup> which is consistent with the region identified in this study. A GWAS of normotensive African Americans identified a suggestive ( $P = 5.73 \times 10^{-6}$ ) association between DBP and rs2823756 on chromosome 21q21.1 (4 Mb from our marker D21S1437).<sup>18</sup> Perhaps clues about potential candidate genes can be gleaned from the suggestive bivariate linkage evidence (lod = 3.03) of D21S1437 on chromosome 21q21.1 (13.05 cM) with C-reactive protein and fibrinogen in GENOA Caucasians.<sup>30</sup> Both C-reactive protein and fibrinogen are inflammation indicators and predictors of cardiovascular disease.<sup>31,32</sup> C-reactive protein inhibits endothelial nitric oxide synthase expression, while fibrinogen helps determine plasma viscosity<sup>30</sup> and is a member of the coagulation cascade.

Our fifth QTL for PP on chromosome 20q13.12 (62.32 cM, marker = D20S481, lod = 4.40) was discovered due to the inclusion of Mexican-American participants. This QTL was linked previously to pulse pressure in GENOA Hispanics (lod = 4.4)<sup>4</sup> and the slope and curvature of repeated measures of SBP over multiple visits in the Framingham Heart Study.<sup>33</sup> Suggestive linkage for SBP variation was discovered near this locus in GENOA Caucasian discordant sibpairs.<sup>34</sup> A tentative linkage (lod = 1.75) between SBP and chromosome 20q13 was observed in a sample from the isolated Croatian island Vis.<sup>35</sup> A single-nucleotide polymorphism, located 4.2 Mbps from D20S481, was associated with SBP in both the Vis population and the British 1958 Birth Cohort. Barbalic *et al.* argue that the potassium voltage gate channel gene (*KCNB1*) and the prostaglandin I<sub>2</sub> (prostacyclin) synthase gene (*PTGIS*) are candidates for the association.<sup>35</sup> *KCNB1* belongs to the voltage-gated potassium channel family that is involved in insu-

lin secretion, heart rate regulation, neurotransmitter release, and more. *PTGIS*, 4.35 Mbps from our D20S481, helps catalyze prostaglandin H2 to the vasodilator and platelet aggregation inhibitor prostacyclin.<sup>35</sup> Japanese participants of the Suita Study with particular genotypes in the promoter region of the human prostacyclin synthase gene had significantly higher SBP, PP, and odds of hypertension.<sup>36</sup>

The variable effect of antihypertensive medication adjustment on linkage evidence can be seen in **Table 2**. The +15/10 and +10/5 adjustments correspond to both increased (e.g., +15 SBP in the Caucausian meta-analysis of chromosome 21 position 24.73 cM) and decreased (e.g., +10 DBP in the overall meta-analysis of chromosome 8 position 119.22) lod scores. This fluctuation in BP linkage evidence by medication adjustment scheme has been well-documented.<sup>9,10,37</sup> The analysis of raw (measured) BP values obscures the contribution of treated individuals to the familial component of BP.<sup>9</sup> We used medication adjustment to infer the underlying pretreatment BP value.<sup>10</sup> The BP values of treated individuals were augmented by a constant value to combat negative bias and the resultant loss of power.<sup>10</sup> This adjustment method works well in a variety of settings<sup>10</sup> but is an approximation that may over- or underestimate the underlying BP and therefore the exact effect on the lod score is difficult to predict. Thus, we present the linkage evidence from the raw and medication adjusted analyses hoping that they will bracket the correct results.

Characteristics of hypertension such as prevalence, age of diagnosis, and severity differ among ethnic groups.<sup>38</sup> The failure to replicate a significant locus across racial groups within the same study does not necessarily suggest that the genetic effect is race-specific. A hypertension susceptibility gene may have the same underlying biological function across races but varying etiological variants or interacting genetic/environmental modulators may lead to different observed effect sizes and hence the power to detect the gene within each race.<sup>39</sup> Specifically, the allelic heterogeneity of a locus across populations may be a reason why association results sometimes fail to replicate across studies. The 8q23.1 locus substantiated by all non-African-American FBPP cohorts, normotensive African Americans from Washington DC, and a sample of Nigerians shows the complicated interplay between genes and environment. Integrating these results from linkage with GWAS results to generate testable hypotheses is a logical next step. The so-called missing heritability<sup>7</sup> may be found only when all hypertension susceptibility loci (both common and rare), environmental factors, and relevant gene-gene and gene-environment interactions are fully identified and modeled together. Our linkage findings may advance us toward this goal by providing supporting evidence for potential BP loci that fail to meet genome-wide significance in current GWAS studies due to small effect sizes. Results reported in this study can be used to derive weighting schemes for interpreting results from GWAS.<sup>7</sup> We conclude that low frequency variants in genes localized in the QTLs may contribute to the missing heritability of hypertension.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at <http://www.nature.com/ajh>

**Acknowledgment:** We thank all the participants in GenNet, GENOA, HyperGEN, and SAPHIRE. A complete list of FBPP investigators may be found at: <http://www.biostat.wustl.edu/fbpp/Acknowledgments.html>. This research was partly supported by grants T32 HL091823, U01 HL54473, U10 HL054512, R01 HL086694, and R21 HL095054 from the National Heart, Lung, and Blood Institute.

**Disclosure:** The authors declared no conflict of interest.

- Ostchega Y, Yoon SS, Hughes J, Louis T. Hypertension awareness, treatment, and control—continued disparities in adults: United States, 2005–2006. *NCHS Data Brief* 2008;1–8.
- Multi-center genetic study of hypertension: the Family Blood Pressure Program (FBPP). *Hypertension* 2002;39:3–9.
- Boerwinkle E; Family Blood Pressure Program. All for one and one for all: introduction to a coordinated analysis of the Gly-460-Trp  $\alpha$ -adducin polymorphism. *Am J Hypertens* 2000; 13:734–735.
- Bielinski SJ, Lynch AJ, Miller MB, Weder A, Cooper R, Oberman A, Chen YD, Turner ST, Fornage M, Province M, Arnett DK. Genome-wide linkage analysis for loci affecting pulse pressure: the Family Blood Pressure Program. *Hypertension* 2005; 46:1286–1293.
- Province MA, Kardia SL, Ranade K, Rao DC, Thiel BA, Cooper RS, Risch N, Turner ST, Cox DR, Hunt SC, Weder AB, Boerwinkle E; National Heart, Lung and Blood Institute Family Blood Pressure Program. A meta-analysis of genome-wide linkage scans for hypertension: the National Heart, Lung and Blood Institute Family Blood Pressure Program. *Am J Hypertens* 2003; 16:144–147.
- Wu X, Kan D, Province M, Quertermous T, Rao DC, Chang C, Mosley TH, Curb D, Boerwinkle E, Cooper RS. An updated meta-analysis of genome scans for hypertension and blood pressure in the NHLBI Family Blood Pressure Program (FBPP). *Am J Hypertens* 2006; 19:122–127.
- Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarrroll SA, Visscher PM. Finding the missing heritability of complex diseases. *Nature* 2009; 461:747–753.
- Marshfield Clinic mammalian genotyping service. <<http://research.marshfieldclinic.org/genetics/GeneticResearch/screeningsets.asp>>.
- Cui JS, Hopper JL, Harrap SB. Antihypertensive treatments obscure familial contributions to blood pressure variation. *Hypertension* 2003; 41:207–210.
- Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med* 2005; 24:2911–2935.
- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002; 30:97–101.
- Province MA. The significance of not finding a gene. *Am J Hum Genet* 2001; 69:660–663.
- Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JJ, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; 41:677–687.
- Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer C, Bergman RN, Morken MA, Döring A, Gieger C, Illig T, Meitinger T, Org E, Pfeuffer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvänen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dörr M, Ernst F, Felix SB, Homuth G, Lohrbein R, Reffelmann T, Rettig R, Völker U,

- Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Völzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; 41:666–676.
15. Topol EJ, Frazer KA. The resequencing imperative. *Nat Genet* 2007; 39:439–440.
  16. Ciullo M, Bellenguez C, Colonna V, Nutile T, Calabria A, Pacente R, Iovino G, Trimarco B, Bourgain C, Persico MG. New susceptibility locus for hypertension on chromosome 8q by efficient pedigree-breaking in an Italian isolate. *Hum Mol Genet* 2006; 15:1735–1743.
  17. Gu CC, Hunt SC, Kardia S, Turner ST, Chakravarti A, Schork N, Olshen R, Curb D, Jaquich C, Boerwinkle E, Rao DC. An investigation of genome-wide associations of hypertension with microsatellite markers in the Family Blood Pressure Program (FBPP). *Hum Genet* 2007; 121:577–590.
  18. Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, Zhou J, Lashley K, Chen Y, Christman M, Rotimi C. A genome-wide association study of hypertension and blood pressure in African Americans. *PLoS Genet* 2009; 5:e1000564.
  19. Adeyemo A, Luke A, Wu X, Cooper RS, Kan D, Omotade O, Zhu X. Genetic effects on blood pressure localized to chromosomes 6 and 7. *J Hypertens* 2005; 23: 1367–1373.
  20. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. The human genome browser at UCSC. *Genome Res* 2002; 12:996–1006.
  21. Nadar SK, Blann AD, Lip GY. Plasma and platelet-derived vascular endothelial growth factor and angiotensin-1 in hypertension: effects of antihypertensive therapy. *J Intern Med* 2004; 256:331–337.
  22. Pankow JS, Rose KM, Oberman A, Hunt SC, Atwood LD, Djoussé L, Province MA, Rao DC. Possible locus on chromosome 18q influencing postural systolic blood pressure changes. *Hypertension* 2000; 36:471–476.
  23. Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, Blangero J, Nürnberg P, Reis A, Riegger GA, Jacob HJ, Schunkert H. A comprehensive linkage analysis for myocardial infarction and its related risk factors. *Nat Genet* 2002; 30:210–214.
  24. Vidan-Jeras B, Gregoric A, Jurca B, Jeras M, Bohinjec M. Possible influence of genes located on chromosome 6 within or near to the major histocompatibility complex on development of essential hypertension. *Pflugers Arch* 2000; 439: R60–R62.
  25. Pausova Z, Deslauriers B, Gaudet D, Tremblay J, Kotchen TA, Larochelle P, Cowley AW, Hamet P. Role of tumor necrosis factor- $\alpha$  gene locus in obesity and obesity-associated hypertension in French Canadians. *Hypertension* 2000; 36:14–19.
  26. Rutherford S, Cai G, Lopez-Alvarenga JC, Kent JW, Voruganti VS, Proffitt JM, Curran JE, Johnson MP, Dyer TD, Jowett JB, Bastarrachea RA, Atwood LD, Goring HH, Maccluer JW, Moses EK, Blangero J, Comuzzie AG, Cole SA. A chromosome 11q quantitative-trait locus influences change of blood-pressure measurements over time in Mexican Americans of the San Antonio Family Heart Study. *Am J Hum Genet* 2007; 81:744–755.
  27. Xu X, Rogus JJ, Terwedow HA, Yang J, Wang Z, Chen C, Niu T, Wang B, Xu H, Weiss S, Schork NJ, Fang Z. An extreme-sib-pair genome scan for genes regulating blood pressure. *Am J Hum Genet* 1999; 64:1694–1701.
  28. Zhu X, Luke A, Cooper RS, Quertermous T, Hanis C, Mosley T, Gu CC, Tang H, Rao DC, Risch N, Weder A. Admixture mapping for hypertension loci with genome-scan markers. *Nat Genet* 2005; 37:177–181.
  29. Zhu X, Cooper RS. Admixture mapping provides evidence of association of the VNN1 gene with hypertension. *PLoS ONE* 2007; 2:e1244.
  30. Ding K, Feng D, de Andrade M, Mosley TH Jr, Turner ST, Boerwinkle E, Kullo IJ. Genomic regions that influence plasma levels of inflammatory markers in hypertensive sibships. *J Hum Hypertens* 2008; 22:102–110.
  31. Wilhelmssen L, Svärdsudd K, Korsan-Bengtson K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med* 1984; 311:501–505.
  32. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000; 342:836–843.
  33. Jacobs KB, Gray-McGuire C, Cartier KC, Elston RC. Genome-wide linkage scan for genes affecting longitudinal trends in systolic blood pressure. *BMC Genet* 2003; 4 Suppl 1:S82.
  34. Krushkal J, Ferrell R, Mockrin SC, Turner ST, Sing CF, Boerwinkle E. Genome-wide linkage analyses of systolic blood pressure using highly discordant siblings. *Circulation* 1999; 99:1407–1410.
  35. Barbalic M, Narancic NS, Skaric-Juric T, Salihovic MP, Klaric IM, Lauc LB, Janicijevic B, Farrall M, Rudan I, Campbell H, Wright AF, Hastie ND, Rudan P. A quantitative trait locus for SBP maps near KCNB1 and PTGIS in a population isolate. *Am J Hypertens* 2009; 22:663–668.
  36. Iwai N, Katsuya T, Ishikawa K, Mannami T, Ogata J, Higaki J, Ogihara T, Tanabe T, Baba S. Human prostacyclin synthase gene and hypertension: the Suita Study. *Circulation* 1999; 100:2231–2236.
  37. Hunt SC, Ellison RC, Atwood LD, Pankow JS, Province MA, Leppert MF. Genome scans for blood pressure and hypertension: the National Heart, Lung, and Blood Institute Family Heart Study. *Hypertension* 2002; 40:1–6.
  38. Kardia SL, Rozek LS, Krushkal J, Ferrell RE, Turner ST, Hutchinson R, Brown A, Sing CF, Boerwinkle E. Genome-wide linkage analyses for hypertension genes in two ethnically and geographically diverse populations. *Am J Hypertens* 2003; 16:154–157.
  39. Rao DC, Province MA, Leppert MF, Oberman A, Heiss G, Ellison RC, Arnett DK, Eckfeldt JH, Schwander K, Mockrin SC, Hunt SC. HyperGEN Network. A genome-wide affected sibpair linkage analysis of hypertension: the HyperGEN network. *Am J Hypertens* 2003; 16:148–150.