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ASSOCIATION OF GENETIC VARIATION WITH BLOOD PRESSURE TRAITS AMONG EAST AFRICANS

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Conflicts of interest

The authors have no conflicts of interest to declare

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Abstract

Genetic variation may explain some of the disparity in prevalence and control of hypertension across sub-Saharan Africa. Twenty-seven blood pressure (BP) related single nucleotide polymorphisms (SNPs) were genotyped among 2881 samples from participants in the MEPI-CVD survey. Associations with known BP variants were evaluated for SBP, DBP and PP as continuous variables and for HTN as a binary variable. Eleven SNPs were associated with at least one BP trait ($P < 0.05$). Four SNPs; rs2004776, rs7726475, rs11837544 and rs2681492 whose nearest genes are *AGT*, *NPR3/SUB1*, *PLXNC1* and *ATP2B1* respectively were associated with SBP. Six SNPs: rs2004776, rs11977526, rs11191548, rs381815, rs2681492 and rs1327235 close to *AGT*, *IGFBP3*, *CYP17A1*, *PLEKHA7*, *ATP2B1* and *JAG* respectively were associated with DBP while two SNPs located within *AGT* and *IGFBP-3* genes associated with HTN. For PP, four variants rs1458038, rs11725861, rs7726475 and rs11953630 whose corresponding genes are *FGF5*, *CHIC2*, *SUB1/NPR3* and *EBF1* reached significance ($P < 0.05$). Eight SNPs were replicated in the same effect direction as the parent studies. Risk scores defined using published effect sizes were significantly associated with both SBP ($P = 0.0026$) and DBP ($P = 0.0214$). The replication of multiple BP variants among East Africans suggests that these variants may have universal effects across ethnic populations.

Graphical Abstract

Keywords

diastolic blood pressure; East Africa; genetics; SNPs; systolic blood pressure

INTRODUCTION

Cardiovascular disease is a leading contributor to disease burden in Africa accounting for nearly a half of the mortality due to non-communicable disease in the region(1). High blood pressure is the principal risk factor for cardiovascular disease (CVD). An increasing prevalence of hypertension has been documented in Africa; however detection and treatment

rates have been dismal (2). In Uganda, for example, more than a quarter of the population have documented hypertension but less than 15% of all those treated achieve target blood pressure control (3, 4). As a result high blood pressure- related CVD morbidity and mortality occurs at a much younger age in Africa compared to the rest of the world (5–7).

Blood pressure (BP) is a heritable trait with an estimated 39 –50% variation attributed to genetics (8). Further evidence is derived from several twin and family studies wherein greater correlation has been noted between monozygotic twins compared to dizygotic twins and greater resemblance of blood measurements documented within families than between families (9, 10). Hypertension is 3.8 times more frequent among persons with a positive family history of hypertension before the age of 55 years (11). In native West Africans, heritability for both systolic and diastolic blood pressure is estimated at 45% (12). Epidemiological studies in sub-Saharan Africa (SSA) have also been unanimous in their findings of a strong association between family history of hypertension and its incidence (13).

People within the sub-Saharan region are disproportionately affected by hypertension. Numerous prevalence studies have documented a higher prevalence of hypertension and poorer control in the East and Southern African regions than the North African region (2, 14). Differences in environment risks, while important, may not completely explain the disparity in prevalence of hypertension and its control across SSA (15). The other mechanisms responsible for these differences, including variation in genetic predisposition as a result of varying frequencies of common disease susceptibility patterns or the presence of population specific alleles especially in populations with a high level of genetic heterogeneity such as those of SSA (16–18) need to be further examined.

To evaluate the genetic basis of hypertension, both genome-wide association studies (GWAS) and candidate gene approaches have been employed. The strength of GWAS compared to candidate gene studies, lies in the identification of novel hypothesis-free loci and the reproducibility of the findings (19). Since 2009, GWAS has identified multiple blood pressure regulatory loci with many of these loci being replicated in independent population samples (20). Replication of GWAS findings is required to improve generalizability of study findings (21). The few BP GWAS and replications in Africans have been performed on samples derived from populations of West African origin, including African Americans (22, 23). There is a paucity of genetic studies to delineate the genetic basis of blood pressure in other African populations. It is also not clear whether previously identified blood pressure genetic variants can be generalized to sub-Saharan populations.

We therefore sought to determine whether a set of genetic loci significantly associated with blood pressure- traits could be replicated among samples from the Medical Education Partnership Initiative for Cardiovascular Disease (MEPI-CVD) survey in Uganda, East Africa.

MATERIALS AND METHODS:

The methods of the Medical Education Partnership Initiative (MEPI-CVD) Survey have been described in detail elsewhere (3, 24). The MEPI-CVD Survey was a cross-sectional study conducted between September 2012 and May, 2013 in Wakiso district of central Uganda among men and women aged 18 years and older to describe CVD risk factors in a peri-urban Ugandan population. Data on CVD risk factors was collected by trained research nurses using a World Health Organisation (WHO) modified expanded STEPs questionnaire. The study variables were age, gender, address, dietary habits, tobacco and alcohol consumption, exercise, smoke exposure, socio-economic status (housing characteristics), family history and symptoms of heart disease including angina. Self-reported history of hypertension diagnosis, diabetes, dyslipidaemia and the treatment for these conditions were also recorded. Anthropometric measurements gathered included height, weight and waist circumference. Blood pressure, fasting cholesterol and blood sugar were also measured.

All study data were collected by the study staff within the survey area and entered into a central survey database. All case report forms and laboratory reports were reviewed for data consistency before data entry. Information collected from blood analysis was electronically transferred to the central survey database.

Ethical considerations:

The survey protocol was approved by the Makerere University School of Medicine Research and Ethics Committee and the Uganda National Council of Science and Technology. Written informed consent was obtained from all study participants.

Phenotype (Blood pressure) measurement:

Participants were requested beforehand to refrain from smoking, drinking alcohol or caffeinated beverage half an hour prior to blood pressure measurement. Blood pressure and heart rate were measured with an Omron automated sphygmomanometer model HEM-907. The BP was measured on the left arm after the participant had sat for at least five minutes. The blood pressure was taken in the sitting position, legs uncrossed, the arm resting on a table and the ante-cubital fossa at the level of the lower sternum. Two arm cuffs that fitted arm circumferences 9-13 inches and 13-17 inches were used in the process. Three readings were taken three minutes apart and the mean of the closest two values were used to describe the blood pressure of the subject. Additional measurements included height which was measured to the nearest 0.1 cm as the perpendicular distance between the top of the head (the vertex) and the bottom of the feet by a SECA 214 portable stadiometer. The weight was measured to the nearest 0.1 kg using a SECA 762 weighing scale with the subjects putting on loose clothing. The waist circumference was measured to the nearest 0.1 cm at the level of the midpoint between the inferior margin of the last rib and the crest of the ilium in the mid-axillary plane using a non-stretchable tape measure.

Candidate single nucleotide polymorphism (SNP) selection:

Thirty single nucleotide polymorphisms (SNPs) were selected for genotyping and association analysis with BP. These SNPs were selected based on the previous association

evidence with BP from GWASs or admixture mapping analysis (23, 25–29) (Supplementary Table 1).

DNA genotyping and quality control:

Complete phenotype information was obtained in 2,881 participants. Genomic DNA was extracted from whole blood drawn from these participants using Gentra Puregene kits from QIAGEN. Genotyping was performed using the PlexSeq process (Plexseq Diagnostics, Cleveland, OH). Primers were designed to amplify regions surrounding each SNP using a multiplexed approach. Each primer design included additional sequence at the 5' end that was then used to anneal universal barcoded Illumina primers in a secondary amplification reaction. All samples (including negative and positive controls) were uniquely barcoded and sequenced simultaneously using a MiSeq instrument (Illumina, San Diego, CA). Fastq files were analyzed using Plexcall software (PlexSeq Diagnostics, Cleveland, OH) which provides genotype calls for all SNP's in each sample.

SNP genotyping quality was evaluated for departures from Hardy-Weinberg equilibrium (HWE) and call rate. One SNP had significant departure from the HWE with $P = 0.001$ and was excluded from analysis. Two SNPs with a missing rate larger than 90% were also excluded from analysis. Two hundred and fourteen samples with genotype call rate less than 85% were excluded from the analysis. After QCs, 27 SNPs and 2667 individuals were left for further association analysis.

Association analysis

All data management was carried out using R (30). Genotype and phenotype association analysis was carried out using software PLINK (31). Systolic blood pressure (SBP) and diastolic and blood pressure (DBP) values were obtained as an average of the closest two measurements. Pulse pressure (PP) was calculated as the difference of SBP and DBP. Only 12.5% of individuals with hypertension were treated with pharmacotherapy. However treatment information was sparse and incomplete and therefore BP traits were not imputed for hypertension treatment. Hypertension status (HTN) was defined by either $SBP > 140\text{mm Hg}$ or $DBP > 90\text{mm Hg}$. Only individuals with complete phenotype and covariate data were included in analysis. Linear regression was applied for SBP, DBP, MAP and PP while logistic regression was applied for HTN. In all the analysis the covariates: age, age², gender and BMI were included. The SNP effect was coded additively. Since all the 27 SNPs passed QCs were selected from previous confirmed BP variants, a significance level of 0.05 was considered as replication in this study.

Multi-trait statistical analyses using CPASSOC

We applied the CPASSOC package to combine association evidence of SBP, DBP and HTN. CPASSOC provided two statistics, S_{Hom} and S_{Het} as previously described (32). S_{Hom} is similar to the fixed effect meta-analysis method (33) but accounts for the correlation of summary statistics of the correlated traits. S_{Hom} uses a sample size of a trait as a weight. S_{Het} is an extension of S_{Hom} but power can be improved when the effect sizes are different for different traits. The distribution of S_{Het} under the null hypothesis was obtained through an estimated beta distribution. To calculate statistics S_{Hom} and S_{Het} a correlation matrix is

required to account for the correlation among traits or induced by overlapped or related samples from different cohorts. In this study, we directly use the correlation matrix calculated from the residual of three blood pressure traits after adjusting for gender, age, age² and BMI.

RESULTS

Baseline population characteristics

The prevalence of hypertension among the MEPI-CVD study was 1237/5183 (24%). Among these 782 cases and 2099 controls were selected for genotyping). Table 1 presents the demographic characteristics of study participants. Participants with hypertension had a higher BMI and waist hip ratio compared to those with normal blood pressure. Participants with hypertension also had higher total cholesterol and low density lipoprotein cholesterol concentrations compared to participants with normal blood pressure. High density lipoprotein was lower among hypertensive patients.

Association analysis among Ugandan samples with four blood pressure traits

Thirty SNPs which are known to be associated with blood pressure or hypertension were genotyped (Supplementary Table 1). Three of them failed QCs and were therefore were not further included for analysis. Table 2 lists the 11 SNPS that were significantly associated with at least one BP trait ($P < 0.05$). To control for the effects of multiple testing, we used Li and Ji's approach which assumes that correlated tests are independent and adjusts them according to an 'effective number' (M_{eff}) of independent tests (34). These M_{eff} based tests have been shown to control error rate accurately, resulting in an increase in power. We estimated 56 independent tests for the 27 SNPs and the four BP traits. Under null hypothesis that none of 56 independent tests were significant, we can expect $56 \times 0.05 = 2.7$, around 3 SNPs with $P < 0.05$. We observed 11 significant SNPs, which are substantially higher than expected.

Among the significant SNPs, four SNPs were associated with SBP: rs2004776, rs7726475, rs11837544 and rs2681492 and the nearest genes are *AGT*, *NPR3/SUB1*, *PLXNC1* and *ATP2B1*, respectively. Six SNPS; rs2004776, rs11977526, rs11191548, rs381815, rs2681492 and rs1327235 and the nearest genes are *AGT*, *IGFBP3*, *CYP17A1*, *PLEKHA7*, *ATP2B1* and *JAG*, respectively, were associated with DBP. Two SNPs located within *AGT* and *IGFBP-3* genes respectively were associated with HTN. For PP, four variants rs1458038, rs11725861, rs7726475 and rs11953630 whose corresponding genes are *FGF5*, *CHIC2*, *SUB1/NPR3* and *EBF1* reached significance ($P < 0.05$). Since combining evidence of the four BP traits will improve the power of association evidence, we performed CPASSOC analysis (32). Eight of the 11 variants were significantly associated with all the BP traits (Table 2).

We next calculated a risk score for each individual by $\sum \beta_i g_i$, where β_i and g_i are the effect size and genotype value of the i^{th} variant of the 27 variants we selected and tested the association between the risk score and each of BP traits. The effect size β_i was obtained from the published data (Supplementary Table 1). Significant linear regression association

evidence was observed for SBP (effect size=0.64, $P = 0.0026$), DBP (effect size=0.45, $P = 0.0214$). However, there was no significant association for HTN (effect size= 1.28, $P= 0.089$). We further compared the effect sizes estimated from this study and that from the published data (Supplementary Table 1) and observed that the effect sizes were highly correlated (Figure 1).

DISCUSSION

Replication of genetic variants identified in BP GWAS in different populations is necessary to improve generalizability of findings to diverse populations. In particular, replication of findings in samples of populations with lower LD, notably African populations, usually leads to more refined estimates of positions of causal variants (18, 35). In this replication study, the first of its kind among East African population, we were able to replicate eight of the selected 30 SNPs for SBP, DBP, PP and HTN status in a direction consistent with the primary studies. These were higher than the three variants expected under the null hypothesis. Another three SNPs were significantly associated with any one of the four blood pressure traits, albeit in an effect direction different to the primary studies. These included rs2681492 and rs7726475, the SNPs with the strongest association evidence to the studied BP traits. However, there were still a substantial number of SNPs which failed to reach the 0.05 significance level. We then performed power analysis with the sample size 2,881. Using the effect size of 0.1% BP variation accounted for by a single BP variant, as suggested by GWASs (36, 37), our study has 31% statistical power to detect a BP variant at the 0.05 significance level, which is consistent with what we observed. The genetic score defined by the 27 variants are also significantly associated with both SBP and DBP. We further demonstrated that the effect sizes estimated in this study are consistent with those obtained from published literature of different ethnic populations (Figure 1). This is consistent with our previous study (37) which shows that BP loci may have universal effects across studied populations, and further demonstrates that multi-ethnic samples are critical for the identification, fine mapping and understanding trait variability.

Among the 8 replicated SNPs, rs2004776 near the *AGT* had the strongest association evidence with BP traits in this study and was replicated for SBP, DBP and HTN. The *AGT* gene has been implicated in both the pathogenesis of hypertension and blood pressure response to ACE inhibitors and angiotensin receptor blockers (38). Multiple consortium-based GWAS have confirmed this SNP -BP trait association in samples derived from population of European and East Asian ancestry (38–40). The effect direction of this association in European samples is same to that found in our samples (41) (Supplemental Table 1). Many candidate studies also suggested the variants in *AGT* are associated with BP traits (42, 43). Previously, other *AGT* variants associated with hypertension in Caucasians could not be replicated in indigenous Africans(44).

The SNP rs381815 found near the *PLEKHA7* gene was replicated for DBP; whereas rs11977526 which is near *IGFBP-3* was replicated for DBP and hypertension. These two variants have consistent association evidence in multi-ethnic populations such as Europeans, South East Asians and African Americans and now in East Africans, suggesting that this association may have a broad role in BP regulation across ethnicities (45–47). Post- GWAS

evaluation for the mechanistic contribution of *PLEKHA7* to BP traits and cardiovascular function has revealed a role in the regulation of ion calcium, nitric oxide bioavailability, and vasculature responsiveness (48). Zhu et al, using the summary statistics from COGENT African ancestry GWAS, identified rs11977526 as the most significant IGFBP3-related SNP in association with multiple BP traits among SBP, DBP and HTN (49). IGFBP-3 binds IGF-1 thereby reducing action at the cellular level which subsequently decreases vascular resistance through stimulation of nitric oxide synthesis (50).

The SNPs rs1458038, rs11725861 and *rs11953630* found in the proximity of *FGF5*, *CHIC2* and *EBF1* respectively were significantly associated with PP. In addition, rs11725861 and *rs11953630* were significant for all the traits in the combined analysis. These SNPs have demonstrated interethnic replication among diverse ethnicities (49, 51, 52). More importantly, the three variants have been associated with blood pressure responsiveness to beta-blocker and thiazide diuretic monotherapy with among Caucasians(53). The SNP rs1458038 near *FGF5*, for example, was associated with significant BP responses to both atenolol and HCTZ, albeit in opposite directions. Whereas the aim of this study was not to assess variants for pharmacological response to BP medication, the multi-ethnic replication for other BP traits may have similar effects on BP response to pharmacological treatment.

The two SNPs with the most significant associations had an effect direction opposite to that demonstrated among European and Southeast Asian GWAS (45, 54, 55) The SNP rs2681492 located in *ATP2B1* gene was associated with SBP and DBP. The ATP gene encodes the plasma membrane calcium ATPase isoform 1 that plays a critical role in intracellular calcium homeostasis by removing bivalent calcium ions from eukaryotic cells against very large concentration (56) The variant rs7726475 which is located on chromosome 5 between the *SUB1* and *NPR3* genes was associated with SBP and PP. Among African American samples, using admixture mapping, Zhu et al showed an association between rs7726475 and SBP and DBP (26). The *NPR3* gene was reported to be significantly associated with BP traits with another SNP, rs1173771 with same direction, in previous interethnic GWAS in East Asians and Europeans (55, 57). The difference in effect direction of the SNPs compared to the primary populations associations suggests that the same locus may have varying effects among different ethnic groups. True genetic architectural variation among populations and differences in linkage disequilibrium may be responsible for these differential effect directions although the effect of environmental cannot be completely ruled out (58).

The clinical implications of identified variants are limited by available data on cardiovascular outcomes in populations of African ancestry. However, three of the replicated SNPs have demonstrated association with responsiveness to antihypertensive medication. This may have implications for pharmacological control of blood pressure among diverse ancestries. While it is clear that many variants display trans-ethnic replication, the finding that our most significant variants have an opposite effect direction to that found in the primary studies calls for further studies to elucidate the effect of these variants on blood pressure traits in the African.

CONCLUSION:

We were able to replicate multiple BP variants identified from previous BP GWAS of Europeans, African Americans and Eastern Asians or admixture mapping analysis in our Eastern African population, which has not been studied previously. However, we also failed to replicate many variants possibly because of insufficient statistical power of the current sample size. We also observed that some variants have different effect directions in different ethnic populations which may suggest that some genes, while regulating blood pressure across diverse populations, may have a differential effect for various BP traits. Our study also suggests BP variants may have universal effects across ethnic populations, which was suggested by Franceschini et al.(37).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Scatter plots of effects size for Uganda study with the reference studies of the 28 SNPs for SBP, DBP and HTN.

Table 1.

Demographic characteristics of study subjects

	Whole study (n=2881)	Normotensive (n=2099)	Hypertensive (n= 782)	p-value
Age (years)	30 (25-43)	22 (24-38)	42 (30-55)	<0.0001
Males n (%)	831 (28.8)	598 (28.5)	233 (29.8)	0.492
BMI (Kg m ⁻²)	24.4 ± 5.1	23.95 ± 4.7	25.59 ± 5.95	<0.0001
SBP	126.4 ± 21.1	117.0 ± 11.6	151.6 ± 20.3	<0.0001
DBP	80.0 ± 12.3	74.9 ± 7.7	93.5 ± 12.4	<0.0001
Waist	80.8 ± 12.2	78.7 ± 11.5	83.6 ± 13.2	<0.0001
WHR	0.82 ± 0.07	0.82 ± 0.07	0.84 ± 0.07	<0.0001
Total cholesterol (mmol/l)	2.84 (2.2-3.86)	2.76 (2.18-3.49)	2.88 (2.2-4.0)	0.009
HDL(mmol/l)	0.84 (0.46-1.71)	0.79 (0.43-1.71)	0.99 (0.56-1.73)	0.0002
LDL(mmol/l)	1.08 (0.84-1.34)	1.01 (0.8-1.3)	1.15 (0.9-1.44)	<0.0001
Triglycerides(mmol/l)	0.97 (0.66-1.47)	0.94 (0.63-1.42)	1.06 (0.74-1.56)	<0.0001

Table 2.

The SNPs whose association with BP traits was significant in the MEPI-CVD samples

SNP	Chr	Gene	Effect Allele/Other Allele	EAF	Trait	N	BETA	SE
rs2004776	1	AGT	C/T	0.46	SBP	2659	1.38	0.53
					DBP	2659	0.79	0.32
					PP	2659	0.59	0.37
					HTN	2602	0.16	0.07
					CPASSOC S _{Hom}	2659/ 2659/ 2602	NA	NA
					CPASSOC S _{Het}	2659/ 2659/ 2602	NA	NA
rs1458038	4	FGF5	T/C	0.04	SBP	2665	2.25	1.36
					DBP	2665	0.31	0.83
					PP	2665	1.94	0.97
					HTN	2601	0.17	0.17
					CPASSOC S _{Hom}	2665/ 2665/ 2601	NA	NA
					CPASSOC S _{Het}	2665/ 2665/ 2601	NA	NA
rs11725861	4	CHIC2	C/T	0.22	SBP	2663	-0.28	0.63
					DBP	2663	0.66	0.38
					PP	2663	0.94	0.45
					HTN	2598	0.14	0.08
					CPASSOC S _{Hom}	2663/ 2663/ 2598	NA	NA
					CPASSOC S _{Het}	2663/ 2663/ 2598	NA	NA
rs7726475	5	SUB1/NPR3	A/G	0.01	SBP	2667	-10.41	3.30
					DBP	2667	-3.62	2.01
					PP	2667	-6.79	2.35
					HTN	2602	-0.44	0.48
					CPASSOC S _{Hom}	2667/ 2667/ 2602	NA	NA
					CPASSOC S _{Het}	2667/ 2667/ 2602	NA	NA
rs11953630	5	EBF1	A/G	0.12	SBP	2665	0.80	0.79
					DBP	2665	-0.47	0.48
					PP	2665	1.27	0.56
					HTN	2600	-0.09	0.10

SNP	Chr	Gene	Effect Allele/Other Allele	EAF	Trait	N	BETA	SE	
					CPASSOC S _{Hom}	2665/ 2665/ 2600	NA	NA	0.67
					CPASSOC S _{Het}	2665/ 2665/ 2600	NA	NA	0.02
rs11977526	7	IGFBP-3	T/C	0.33	SBP	2666	1.04	0.59	0.08
					DBP	2666	0.76	0.36	0.03
					PP	2666	0.28	0.42	0.51
					HTN	2601	0.18	0.08	0.02
					CPASSOC S _{Hom}	2666/ 2666/ 2601	NA	NA	0.01
					CPASSOC S _{Het}	2666/ 2666/ 2601	NA	NA	0.06
					rs11191548	10	CYP17A1	G/A	0.03
DBP	1998	-2.47	1.13	0.03					
PP	1998	1.91	1.32	0.15					
HTN	1984	-0.11	0.25	0.67					
CPASSOC S _{Hom}	1998/ 1998 1984	NA	NA	0.26					
CPASSOC S _{Het}	1998/ 1998/ 1984	NA	NA	0.13					
rs381815	11	PLEKHA7	T/C	0.28					
					DBP	2664	0.70	0.35	0.04
					PP	2664	0.18	0.41	0.66
					HTN	2600	0.00	0.09	0.99
					CPASSOC S _{Hom}	2664/ 2664 2600	NA	NA	0.21
					CPASSOC S _{Het}	2664/ 2664/ 2600	NA	NA	0.18
					rs11837544	12	PLXNC1	T/A	0.20
DBP	2665	-0.68	0.39	0.09					
PP	2665	-0.73	0.46	0.12					
HTN	2601	-0.16	0.09	0.07					
CPASSOC S _{Hom}	2665/ 2665 2601	NA	NA	0.03					
CPASSOC S _{Het}	2665/ 2665/ 2601	NA	NA	0.12					
rs2681492	12	ATP2B1	G/A	0.14					
					DBP	2661	-1.34	0.45	0.0029
					PP	2661	-0.58	0.53	0.27

SNP	Chr	Gene	Effect Allele/Other Allele	EAF	Trait	N	BETA	SE
rs1327235	20	JAG 1	A/G	0.42	HTN	2598	-0.12	0.10 0.21
					CPASSOC S _{Hom}	2661/ 2661/ 2598	NA	NA 0.01
					CPASSOC S _{Het}	2661/ 2661/ 2598	NA	NA 0.02
					SBP	2666	-0.82	0.52 0.12
					DBP	2666	-0.70	0.32 0.03
					PP	2666	-0.12	0.37 0.75
					HTN	2601	-0.10	0.07 0.15
					CPASSOC S _{Hom}	2666/ 2666/ 2601	NA	NA 0.05
					CPASSOC S _{Het}	2666/ 2666/ 2601	NA	NA 0.12