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Progress and Issues of the Genome-Wide Association Study for Hypertension

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Abstract: Over the past few years, use of the genome-wide association study (GWAS) has made it possible to identify the primary genetic mechanisms of essential hypertension. GWAS results have helped identify many loci in or near genes that generally were not expected to be associated with blood pressure or essential hypertension. However, considering the great expectations of improving clinical outcomes and the billions of dollars that have been spent on various GWASs, the progress made so far has been slow. There are several factors that could be responsible for the relative lack of success of GWASs. First, it is possible that the number of people enrolled in the various GWASs was not enough, thereby limiting the power to detect additional markers. Second, although the alleles that are associated with a modest increase in risk are constantly being found, their discriminatory ability and use as predictive markers has been quite low. Difficulties with control group selection along with unrepeatability have also been problematic when using GWASs. The current paper summarizes the recent progress attained when using a GWAS of hypertension to identify the many loci associated with essential hypertension. In this review, we discuss the progress and issues of a GWAS for hypertension.

Keywords: Blood Pressure, Genomics, Genome-Wide Association Study, Hypertension.

INTRODUCTION

It has long been accepted that essential hypertension is a polygenic disorder. Three different publications have estimated the heritability in family studies to range from 20% to 68% [1-6]. On the other hand, studies of rare familial forms of hypertension have been extremely successful in identifying causal genes, and illuminating the regulatory pathways of blood pressure [7]. Epidemiological data have also shown that hypertension is a combination of genetic and environmental factors. Moreover, the heritability of blood pressure has been variably estimated to be between 30% and 50% [8]. Furthermore, sibling recurrence risk, defined as a relative risk of hypertension when a sibling is affected, has been estimated at 2.5 to 3.5 [9]. One of the first traits to be studied using the genome-wide association study (GWAS) was hypertension. Today, the prevalence of essential or primary hypertension is high, with this classic quantitative trait for increased systolic and diastolic blood pressures found to be normally distributed in the general population. Our current article provides insights into both the progress and the issues associated with using a GWAS for hypertension.

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INITIAL USE OF GWAS

Although previous blood pressure measurements have lacked standardization [10], there is reliable evidence to indicate that a genetic component from familial and other sources plays an important role in blood pressure, with studies in twins providing some of the most convincing information. Familial studies of blood pressure have demonstrated that parents and children, as well as other siblings, have a high concordance, which contributes to up to 66% of blood pressure traits [11-13]. Twin studies have shown a higher correlation for blood pressure measurements between monozygotic twins (r=0.56-0.62) as compared to dizygotic (r=0.21-28) twin pairs [14-16]. Results from these twin studies are considered the closest to actual heritability estimates, as twins have similar environmental factors. Heritability estimates for blood pressure in many twin studies have exceeded 50%, which indicates that more than half of the variation in blood pressure can be attributed to these genetic effects [17-19]. One twin study also suggests that increases in blood pressure in prehypertension result from a pathway initiated by heritable disturbances displaying joint genetic determination, and then actuated by a series of autonomic and hemodynamic events [20].

However, because blood pressure is a heritable trait, other determinants of blood pressure need to be considered when attempting to interpret results from genetic studies. The prevalence of hypertension differs not only by race and country but also based on lifestyle and environmental fac-

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tors. Rates of hypertension range from 10 to 30% in different populations [21]. Adult black subjects in the United States have higher blood pressure levels and more hypertension than do nonblack subjects [21]. Black subjects of African origin in the United States have a higher rate of hypertension than black subjects outside the United States. [21] Both of these differences [21] were associated with increased body mass index (BMI) and sodium intake among those with higher blood pressure levels. The prevalence of hypertension in Hispanic (Mexican-Americans) and Asian populations is similar to or lower than that in white populations. [21] Furthermore, the prevalence of hypertension has risen in many countries due to the rising prevalence of obesity and an increased consumption of sodium [21], two environmental risk factors that influence blood pressure. One study that compared young lean healthy normotensive males with offspring of parents with essential hypertension and age- and sex-matched offspring of normotensive parents indicated that offspring of hypertensive parents are not only genetically prone to develop hypertension, but they also have a particular propensity to accumulate central body fat, even before a distinct rise in resting blood pressure occurs [22].

Moreover, an increase in daily sodium intake has been reported to be associated with elevated blood pressure in hypertensive patients [23]. In another study, mean sodium intake among white participants in USA with and without high blood pressure was 3330 mg/day and 3600 mg/day (geometric means, 2885 mg/day and 3146 mg/day), respectively. Although participants with hypertension reported lower intake of dietary sodium than those with normal blood pressure, daily intake of sodium was much higher than the recommendations in both groups [24]. These data suggest that sodium sensitivity may differ between people with and without hypertension. Similarly, variations in physical activity levels can also dramatically affect blood pressure. The international physical activity questionnaire (IPAQ) has been used to assess relationships between physical activity and blood pressure in healthy young individuals. [25] Walking, moderate physical activity, and total physical activity scores correlated negatively with systolic blood pressure, diastolic blood pressure, and mean arterial pressure. Additionally, all BP measurements were greater in individuals who were the least physically active [25].

Because genetic affects have such a huge influence on blood pressure, several new study methods have been created to discover genetic variations that influence blood pressure. Of these methods, family-based genetic linkage studies have been widely used to study the molecular genetics of hypertension. Genome-wide linkage scans using microsatellite markers revealed several candidate loci in each chromosome such as chromosome 1 and 18 for white people and chromosome 4 and 15 for African Americans [26]. Another linkage scan identified three loci on chromosome 2q24, 11q23.1–25, and 13q14.11–21.33 for white people [27]. The 2q24 locus was replicated with a logarithm of the odd (LOD) score of 2.68 [28]. A study of white people in the United Kingdom also confirmed the association of hypertension-related phenotypes to the 11q region detected by 2-stage genome screens [29]. Regarding the 13q14.11–21.33 locus, the same location associated with hypertension was reported in a meta-analysis and other genome scans in the literature [30,31]. These studies have shown a large number of variations in blood pressure based on physiological systems.

Prior to the utilization of GWAS, several other methodologies were used to detect hypertension-related genes, which included restriction fragment length polymorphisms (RFLP), variable number of tandem repeats (VNTR), and microsatellite repeats [32-33]. The basis of the current GWASs can be traced to the recent efforts to map patterns of inheritance using the most common form of genomic variations, the single nucleotide polymorphism (SNP) [34-35]. An estimated 10 million common SNPs with a minor-allele frequency of at least 5% are known to be transmitted across generations in blocks. This allows a few particular, or tag, SNPs to capture the majority of the SNP variation within each block [36]. Moreover, rapid advances in technology now permit affordable, reliable genotyping of up to 1 million SNPs in a single scan of a person's DNA [37]. Further work by Ozaki et al. led to the development of GWAS to identify the susceptibility gene of multifactorial disorders such as myocardial infarction [38]. In a large-scale, case-control association study, 92,788 gene-based SNP markers were enrolled [38]. A candidate locus on chromosome 6p21 was first identified as being associated with susceptibility to myocardial infarction. The results of that study further indicated that variants in lymphotoxin-alpha (LTA) gene were risk factors for myocardial infraction and thus implicated LTA in the pathogenesis of the disorder.

GWASs use various study designs, including case-control studies, cohort studies, and clinical trials [39]. However, before any scanning can be done, the strengths and weaknesses of each of the designs must be identified [40-41]. Because GWASs require an enormous number of association tests to be performed (at least one per SNP) in addition to having to meet strict thresholds for statistical significance, very large numbers of samples need to be examined [42]. One frequently used method for managing the size of these studies is the use of a tiered design. With this approach, a subset of the SNPs found to be significant in the GWAS is further genotyped in a second tier. This yields a subsequently smaller subset of significantly associated SNPs that are then in turn tested in a third tier, a fourth tier, etc., with the net result of smaller and smaller subsets being collected [43-44]. Use of this process helps to exclude any false-positive associations. In addition, by enrolling a large number of SNPs that have been identified through a GWAS, subsequent test replications can then minimize any false-negative results [45]. GWAS results are often required to ensure that variants with small effects on the risk of disease can be detected. Similar to all genetic association studies, GWASs also need to be examined and controlled for differences in allele frequency between groups that could possibly cause false-positive associations.

USE OF GWAS FOR HYPERTENSION

Essential or primary hypertension is highly prevalent in humans, and is a classic quantitative trait, with increased systolic and diastolic pressure being normally distributed in the general population (Table **1**). Thus, it is not surprising that one of the first traits to be studied by a GWAS was hypertension. Table **1** shows genes associated with hypertension as determined by GWAS.

Table 1. GWAS susceptibility genes for blood pressure and hypertension by race/ethnicity.

Race-Ethnicity/ Country	Discovery Samples	Traits	Genes Found	Locus	Affecting Variations	Location	Study [Reference #]
Europeans/Europe	Single-stage: GWAS (n = 2000 cases + 3000 controls)	HTN	RYR2	1q43	rs2820037	intergenic	WTCCC [46]
European Americans/United States	Single-stage: GWAS (n = 1260, 1233 and 1327 individuals in different analyses	DBP	CCL20, DAW1	2q36.3	rs7591163	intergenic	Levy *et al.* [47]
		Both SBP and DBP	CDH13	16q23.3	rs3096277	intronic	
		Both SBP and DBP	LPP	3q28	rs6796000	intronic	
		SBP	CCL20, DAW1	2q36.3	rs1721359	intronic	
Europeans/Germany, Estonia, and United Kingdom	Stage 1: GWAS (n = 1017 Germany and 364 cases + 596 controls); Stage 2: Replication (n = 1551 and 447 cases + 1119 Replication (n = 1097 and 596 cases + 650 controls; 2401 cases + 1969 controls)	HTN, SBP, and DBP	CDH13	16q23.3	rs11646213	upstream	Org *et al.* [49]
Europeans/Sweden	Stage 1: GWAS (n = 1621 cases + 1699 controls); Stage 2: Replication (n = 19845 cases + 16541 controls)	HTN	UMOD	16p12.3	rs13333226	upstream	Padmanabhan *et al.* [57]
Europeans/Great Britain	Single-stage: GWAS (n = 2000 cases + 3000 controls)	HTN	GPR39	2q21-q22	rs13420028	intronic	Slavin *et al.* [61]
			XRCC4	5q14.2	rs6452524	intronic	
			MYO6	6q13	rs3798440	intronic	
			ZFAT	8q24.22	rs7827545	intronic	
			MACROD2	20p12.1	rs200752	intronic	
Amish and non-Amish European Americans/United States	Stage 1: GWAS (n = 542 Amish Caucasians); Stage 2: Replication (n = 1367 Amish Caucasians and 5,804 non-Amish Caucasians)	SBP and DBP	STK39	2q24.3	rs6749447	intronic	Wang *et al.* [50]
					rs3754777	intronic	
Japanese/Japan	Stage 1: GWAS (n = 188 cases + 752 controls); Stage 2: Replication (n = 752 cases + 752 controls); Stage 3: Replication (n = 619 cases + 1406 controls)	HTN	ADD2	2p13.3	rs3755351	intronic	Kato *et al.* [52]
			KIAA0789	12q23.3	rs3794260	intronic	
			M6PR	12p13	rs1805762	intronic	
Japanese/Japan	Stage 1: GWAS (n = 936 Japanese); Stage 2: Replication (n = 3228 Japanese); Stage 3: Replication (n = 2895 Japanese)	SBP and DBP	CCBE1	18q21.32	rs1652080	upstream	Hiura *et al.* [58]
			MAP7	6q23.3	rs3778297, rs7747460	intronic, intronic	
			ZFP64	20q13.2	rs6013382	intronic	
			PCDH18	4q31	rs7692053, rs1460138	intergenic, intergenic	
			CDH2	18q11.2	rs9973037	intergenic	
			WWOX	16q23	rs1075609	intergenic	

(Table 1) contd….

Race-Ethnicity/ Country	Discovery Samples	Traits	Genes Found	Locus	Affecting Variations	Location	Study [Reference #]
Korean/Korea	Stage 1: GWAS (n = 8842 Koreans); Stage 2: Replication (n = 7861 Koreans)	SBP and DBP	ATP2B1	12q21.3	rs17249754	intronic	Cho *et al.* [51]
Korean/Korea	Stage 1: GWAS (n = 7551 Koreans); Stage 2: Replication (n = 3703 Koreans)	SBP and DBP	AKAP13	15q24-q25	rs11638762	upstream	Hong *et al.* [60]
Han Chinese/China	Single-stage: GWAS (n = 400 cases + 400 controls)	HTN, SBP	IGF1	12q23.2	rs10860862, rs5742632	intronic enhancer	Yang *et al.* [71]
		HTN, SBP, and DBP	SLC4A4	4q21	ND		
		HTN, MBP, sibTDT	WWOX	16q23.3-q24.1	ND		
		HTN, MBP	SFMBT1	3p21.31	ND		
Han Chinese/China	Stage 1: GWAS (n = 175 cases + 175 controls); Stage 2: Replication (n = 1008 cases + 1008 controls)	HTN	SFMBT1	3p21.1	ND		Yang *et al.* [71]
			GRB14	2q22-q24	ND		
			TMEM56	1p21.3	ND		
			FOCAD (KIAA1797)	9p21	ND		
Han Chinese/China	Stage 1: GWAS (n = 175 cases + 175 controls); Stage 2: Replication (n = 833 cases + 833 controls)	HTN	LOC344371	2p22.3	rs9308945-rs6711736 -rs6729869-rs10495809	intergenic-intergenic-intergenic-intergenic-	Yang *et al.* [54]
			MYADML	2p22.3	ND		
			FAM98A	2p22.3	ND		
			RASGRP3	2p22.3	ND		
			IMPG1	6q14.2-q15	rs1886985	intronic	
Han Chinese/China	Single-stage: GWAS (n = 111 cases + 217 controls)	HTN, SBP, and DBP	TPR, PDC	1q31.1	rs3817586, rs11812050	intronic	Guo *et al.* [65]
African Americans/United States and West Africans/Africa	Stage 1: GWAS (n = 509 African American cases + 508 controls); Stage 2: Replication (n = 366 West African cases + 614 controls)	SBP	SLC24A4	14q32.12	rs11160059	intronic	Adeyemo *et al.* [53]
			CACNA1H	16p13.3	rs3751664	non-synonymous coding SNP	
African Americans/United States	Stage 1: GWAS (n = 8591 African Americans); Stage 2: Replication (n = 11882 African Americans); Stage 3: Replication (n = 69899 European Americans)	DBP	GPR98-ARRDC3	5q14	rs10474346	intergenic	Fox *et al.* [62]
		SBP	C21orf91	21q21	rs2258119, rs2824495	intronic, missense	
		SBP	SLC25A42, SUGP2	19p13.11	rs2012318, rs4808907	intronic, missense	
		DBP	HLA-B	6p21.3	rs2523586	upstream	

GWAS, genome-wide association study; DBP, diastolic blood pressure; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; sibTDT, sib-based transmission disequilibrium test; ND, not described

In 2007, the Wellcome Trust Case Control Consortium (WTCCC) reported the first genome-wide association results for hypertension. Although this study identified several risk variants for hypertension, none reached the required statistical significance level ($P<5\times10^{-7}$) [46]. However, the WTCCC study did identify SNPs associated with heart disease, which included 9p21 with odds ratios ranging from 1.10 to 1.40. These results led investigators to suggest that genetic variants for hypertension were likely to have smaller effect sizes than those observed for genetic variants associ-

ated with heart disease. As a result, it was advocated that larger sample sizes through meta-analysis be applied along with a denser coverage of the genome. During the same year, Levy et al. reported the loci near CCL20, WDR69, CDH13, and the LPP gene could be associated with blood pressure [47].

From 2008 to 2009, results of several GWASs on hypertension were published. In the GWAS study by Sabatti et al., which examined the blood pressure in 4,763 participants from the 1996 Northern Finland Birth Cohort study, the authors failed to identify any genetic variants associated with blood pressure [48]. A European study by Org et al. indicated there was an SNP upstream of the T-cadherin gene (CDM13) on chromosome 16q 23.3 that was associated with diastolic blood pressure (initial: $P=5.55\times10^{-5}$; replication: $P=5.3\times10^{-8}$) [49]. Wang et al. reported finding a novel genetic variant that was associated with essential hypertension in a study of 542 subjects from the Amish Family Diabetes Study (AFDS) [50]. Some variants across the STK39 (serine threonine kinase 39) were found to be associated with systolic blood pressure, with p values ranging from $P=8.9\times10^{-6}$ to 9.1×10^{-5}. However, after Bonferroni correction, these p values were not significant. Combining data from three Amish population studies, Wang et al. reported a 3 mmHg higher systolic blood pressure and a 1 mmHg higher diastolic blood pressure in subjects carrying a minor allele copy of the rs6749447 variant. In a combined meta-analysis of Amish and non-Amish cohorts (Diabetes Genetics Initiative and Framingham Heart Study) an effect size of 1.9 (1.2–2.6) mmHg increase in systolic blood pressure was observed ($P=1.6\times10^{-7}$, rs6749447). Moreover, Cho et al. described an SNP (rs17249754) in intron of the ATPase calcium ion transporting plasma membrane 1 gene (ATP2B1) with $P=1.3\times10^{-7}$ [51]. This gene encodes the protein ATPase calcium ion transporting plasma membrane 1, which is known to be involved in calcium homeostasis. Over the past 2 years, several GWASs have also reported finding other new loci [52-54].

In 2009, two large-scale meta-analyses of GWASs for blood pressure and hypertension were published [55-56] (Table **2**). These studies examined the Global BPGen Consortium and the Cohorts for Heart and Ageing Research in Genome Epidemiology Blood Pressure (BP) Consortium performed a meta-analysis of the GWAS data from two other reports that previously examined 34,433 and 29,136 individuals, respectively. Both consortia identified genome-wide significant associations ($P<5\times10^{-8}$) at 8 loci, with 3 of the loci being common to both studies (Table **3**). Two of 13 genomic regions identified by these two meta-analyses contained genes that had been previously implicated in hypertension susceptibility. These include the chromosome 1p32 with atrial natriuretic peptide A- and B-type natriuretic peptide genes (NPPA and NPPB) and chromosome 10q24 with a strong candidate gene, the CYP17A1. Mutations in the latter gene cause 17-α-hydroxylase congenital adrenal hyperplasia, an autosomal recessive mineralocorticoid hypertension.

In 2010 and 2011, several small or middle-sized studies found loci that appeared to be associated with blood pressure and hypertension. Padmanabhan et al. used an extreme case-control design to conduct a GWAS that examined 1,621 hypertensive cases and 1,699 controls [57]. They identified a locus on chromosome 16 in the 59 region of Uromodulin of SNP rs13333226 with a significant P value (3.6×10^{-11}). Hiura et al. examined 936 participants who were recruited from the Suita Study and performed a GWAS with 538,732 SNPs [58]. This mid-sized GWAS indicated that there was no master gene in the Japanese population that profoundly affects blood pressure-related phenotypes. Over the past 2 years, there have been other GWASs that have also provided insight into the research of loci associated with blood pressure and hypertension [59-62].

In 2011, two other large-scale meta-analyses of GWASs were conducted, with one international consortium using a multi-stage design to examine blood pressure of 69,395 individuals of European descent [63] (Table **2**). This particular study identified 16 novel loci, of which 6 contained genes previously known or suspected to regulate blood pressure, GUCY1A3–GUCY1B3 (rs13139571), NPR3–C5orf23 (rs1173771), ADM (rs7129220), FURIN–FES (rs2521501), GOSR2 (rs17608766), and GNAS–EDN3 (rs6015450) (Table **3**). In the other study, the AGEN-BP Consortium examined 19,608 subjects of East Asian ancestry [64]. In that study, samples underwent de novo genotyping (n = 10,518) and further replication (n = 20,247) (Table **2**). The meta-analysis identified genome-wide significant associations ($P < 5 \times 10^{-8}$) with systolic or diastolic blood pressure, which included variants at 4 new loci, ST7L-CAPZA1 (rs17030613), FIGN-GRB14 (rs16849225), ENPEP (rs6825911), and NPR3 (rs1173766), and a newly discovered variant near TBX3 (rs35444). Among the 5 newly discovered variants, significant replication in independent samples was obtained for all of the loci except NPR3 (Table **3**). This study also confirmed the presence of 7 loci previously identified in populations of European descent: CASZ1 (rs880315), MTHFR (rs17367504), ITGA9 (rs155524), FGF5 (rs16998073), CNNM2-NT5C2 (rs11191548), ATP2B1 (rs2681472), and CSK-ULK3 (rs1378942). Moreover, at 12q24.13 near ALDH2 (rs11066280), strong association signals ($P=7.9\times10^{-31}$ and $P=1.3\times10^{-35}$ for systolic and diastolic blood pressure, respectively) were observed with ethnic specificity. However, it is difficult to characterize uniformity of function for these molecules.

In 2012, Guo et al. conducted a combined linkage and association study using over 500,000 SNPs genotyped in a group of 328 individuals comprising 111 hypertensive probands and their siblings [65]. Using a family-based association test, their results demonstrated an association with hypertension for SNPs on chromosome 5q31.1 (rs6596140). The nearest gene at this SNP is follistatin like 4 (FSTL4) gene, a member of the follistatin gene family of transforming growth factor (TGF)-beta superfamily inhibitors, which are widely expressed in neurons, cardiac muscle cells, smooth muscle cells, and intestinal epithelium [66]. Furthermore, one candidate gene, PDC, encoding phosducin, which was identified in retina and brain as a 33-kDa protein and binds to the subunits of heterotrimeric GTP binding proteins [67-68], is a potential candidate gene for retinitis pigmentosa [69]. The investigators indicated the role of the G protein regulator PDC in hypertension and found that the SNP (rs12402521) in the PDC gene was significantly associated with both wake and stress-response blood pressure phenotypes [70]. It was demonstrated that PDC is an important modulator of sympathetic activity and blood pressure and

Table 2. Brief comparison of five genome-wide association meta-analyses.

Study	CHARGE	Global BPgen	UMOD locus	ICBP-GWAS	AGEN-BP
Reference	55	56	57	63	64
Sample size	29,136	34,433	79,133	69,395	19,608
Ancestry	European	European	European	European	East Asian
Number of studies	6	13	15	29	8
Studies included (abbreviation)	AGES, ARIC, CHS, FHS, RS, RES	WTCCC, DGI, EPIC-Norfolk-GWAS,	Swedish BP Extremes, MONICA/PAMELA, MPP,	YFS, EPIC-TURIN, FLEMENGHO, Nigerians,	CAGE, GenSalt, KARE, Shanghai-
		KORA, MIGen, CoLaus, NFBC1966, SHIP,	MDC, BRIGHT/ASCOT, PREVEND,	ARYA, ELSA, PREVEND, Prospect-EPIC,	Ruijin, SiMES, SP2, Taiwan, Suita,
		Fenland Study, FUSION, InCHIANTI,	CoLaus, KORA, SHIP, 58BC,TwinsUK,	INTERGENE, MRC NSHD, YMCA, COBRA,	
		PROCARDIS, SU.VI.MAX, TwinsUK	MIGen, DGI, Fenland, NESDA	HYPEST, BRIGHT, EAS, NPHS-II, etc.	
Age range	38–72	31–59	55.4	19–74	48–66
HTN (%)	17–60%	15–52%	—	1–74%	0–67%
Treated HTN	5–35%	2–24%	—	0–50%	0–37%
Primary traits	SBP, DBP, and HTN	SBP, DBP	SBP, DBP, and HTN	SBP, DBP, and HTN	SBP, DBP
Genome-wide significance	$P<5\times10^{-8}$	$P<5\times10^{-8}$	3.66×10^{-11}	$P<5\times10^{-8}$	$P<5\times10^{-8}$

DBP, diastolic blood pressure; HTN, hypertension; SBP, systolic blood pressure.

may thus represent a promising target for treatment of stress-dependent hypertension. The association study using rs3817586 located in intron of the TPR gene encoding the translocated promoter region, nuclear basket protein, close to the PDC gene, was replicated in the within-family test for diastolic blood pressure and mean arterial pressure [66].

Yang et al. examined a Han Chinese population and used 400 matched pairs of young-onset hypertensive patients and normotensive controls to conduct the first genome-wide gene-based association scan for hypertension in this particular population [71]. Their results indicated IGF1, SLC4A4, WWOX, and SFMBT1 were hypertension susceptibility genes. Serum IGF1 level was associated with the level of blood pressure [72], and reported as a candidate gene for cardiovascular disease in Japanese subjects [73-74]. The other three genes have not been examined in detail to estimate the association between those polymorphisms and hypertension in any population.

CURRENT OUTSTANDING PROBLEMS AND PROSPECTS

For a genome-wide testing approach to achieve success, it needs to genotype a large numbers of markers for every individual (1-stage design). Despite the prices for SNP arrays going down, costs for a GWAS are still high. The investment in a GWAS is estimated to be $125,000 per discovered locus, with a total investment of $250 million over 5 years during 2006 to 2011 [75]. A study of with the final goal of identifying the human genome for hypertension will improve the expenses related directly or indirectly to the treatment and detection of hypertension (approximately $10 billion yearly in the United States) [76]. However, although remarkable progress has been achieved in some monogenic diseases, progress has been much slower for more common complicated diseases such as hypertension [77]. Extensive media coverage has unfortunately led to unrealistically high expectations, which have therefore led to disappointment when the results of such studies are not as dramatic or rapid as expected. There are several factors that could be responsible for this relative lack of success.

First, as we reported in the beginning of this article, the number of people enrolled in most GWASs is less than 1000. Moreover, even a relatively large GWAS (one with 5000 case subjects and 5000 control subjects) still has a somewhat low power when it comes to detecting specific markers. The least stringent accepted measure of genome wide significance in such studies is 0.9% at a P value of 10^{-7} for an allele with a frequency of 25% and a relative risk per allele of 1.1. However, because there are so many susceptibility loci for hypertension on chromosomes, the probability of detecting at least one is quite good, with an 83% chance of detection if there are 200 risk loci.

Second, the majority of newly identified risk alleles have very small relative risks, ranging from 1.1 to 1.5 [78], even though such analyses have small P values and hence are unlikely to be false positives. However, even when alleles that

Table 3. Loci associated with blood pressure traits in CHARGE, Global Bpgen, AGEN-BP, and ICBP-GWAS meta-analyses.

Study	Total Number	SNP	Trait: P Value	Loci	Nearby Genes	Biological function					
AGEN-BP	49,952	rs17030613	DBP: 1×10^{-8}	1p13	ST7L, CAPZA1	Similarity to tumor suppressor gene	Regulates growth of the actin filament				
Global Bpgen	82,973	rs17367504	SBP: 2×10^{-13}	1p36	MTHFR, CLCN6, NPPA, NPPB, AGTRAP	5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, occlusive vascular disease	Voltage-dependent chloride channel protein family	Natriuretic peptide family	Natriuretic peptide family	Negatively regulates angiotensin II signalin.	
AGEN-BP	32,611	rs880315	DBP: 3×10^{-10}	1p36	CASZ1	Zinc finger transcription factor, tumor suppressor					
AGEN-BP	49,511	rs16849225	SBP: 4×10^{-9}	2q24	FIGN, GRB14	Unknown	Growth factor receptor-binding protein				
CHARGE	63,569	rs9815354	DBP: 3×10^{-9}	3p22	ULK4	Unknown					
Global Bpgen and AGEN-BP	134,234	rs16998073	DBP: 1×10^{-21}	4q21	PRDM8, FGF5, c4orf22	PR domain zinc finger protein 8	Fibroblast growth factor (FGF) family	Unknown			
AGEN-BP	49,515	rs6825911	DBP: 9×10^{-9}	4q25	ENPEP	Glutamyl aminopeptidase					
ICBP-GWAS	69,395	rs13139571	DBP: 2×10^{-10}	4q31	GUCY1A3, GUCY1B3	Soluble guanylate cyclases, GTP to 3',5'-cyclic GMP and pyrophosphate	The beta subunit of the soluble guanylate cyclase (sGC)				
AGEN-BP	49,970	rs1173766	SBP: 2×10^{-8}	5p14-p13	NPR3	Natriuretic peptide receptors					
ICBP-GWAS	69,395	rs1173771	SBP: 2×10^{-16}; DBP: 9×10^{-12}; HTN: 3×10^{-10}	5p14-p13	NPR3, C5orf23	Natriuretic peptide receptors					
CHARGE	63,569	rs11014166	DBP: 1×10^{-8}	10p12	CACNB2	A subunit of a voltage-dependent calcium channel protein					
Global Bpgen	87,273	rs1530440	DBP: 1×10^{-9}	10q21	c10orf107, TMEM26, RTKN2, RHOBTB1, ARID5B	Unknown	Transmembrane protein 26	Rhotekin 2, associated with rheumatoid arthritis in Japanese	Rho family of the small GTPase superfamily, the organization of the actin filament system	AT-rich interaction domain (ARID) family of DNA binding proteins	
Global Bpgen, CHARGE and AGEN-BP	173,867	rs11191548	SBP: 7×10^{-24}	10q24	CYP17A1, AS3MT, CNNM2, NT5C2	Cytochrome P450 superfamily of enzymes	S-adenosyl-L-methionine (AdoMet) to trivalent arsenical	Cyclin box motif, magnesium homeostasis	Important role in cellular purine metabolism		
Global Bpgen and CHARGE	63,569	rs1004467	SBP: 1×10^{-10}	10q24	CYP17A1, AS3MT, CNNM2, NT5C2	Cytochrome P450 superfamily of enzymes					
CHARGE	63,569	rs381815	SBP: 2×10^{-9}	11p15	PLEKHA7	Unknown					

(Table 3) contd….

Study	Total Number	SNP	Trait: P Value	Loci	Nearby Genes	Biological function						
ICBP-GWAS	69,395	rs7129220	SBP: 3×10^{-12}	11p15	ADM	Adrenomedullin and proadrenomedullin N-terminal 20 peptide						
CHARGE	63,569	rs2681492	SBP: 4×10^{-11}	12q21	ATP2B1	Family of P-type primary ion transport ATPases						
CHARGE	63,569	rs2681472	DBP: 2×10^{-9}; HTN: 2×10^{-11}	12q21	ATP2B1	Family of P-type primary ion transport ATPases						
AGEN-BP	40,719	rs17249754	DBP; 2×10^{-13}	12q21	ATP2B1	Family of P-type primary ion transport ATPases						
Global Bpgen and CHARGE	79,661	rs653178	DBP: 3×10^{-18}	12q24	SH2B3, ATXN2	SH2B adaptor family, cytokine signaling	The autosomal dominant cerebellar ataxias (ADCA), spinocerebellar ataxia type 2 (SCA2)					
Global Bpgen and CHARGE	63,569	rs3184504	SBP: 5×10^{-9}; DBP: 3×10^{-14}	12q24	SH2B3, ATXN2							
AGEN-BP	49,984	rs35444	DBP: 1×10^{-10}	12q24	TBX3	Phylogenetically conserved family of genes that share a common DNA-binding domain, the T-box						
AGEN-BP	46,957	rs11066280	SBP: 8×10^{-31}; DBP: 1×10^{-35}	12q24	RPL6, PTPN11, ALDH2	Ribosomal protein, 60S subunit	Protein tyrosine phosphatase (PTP) family	Aldehyde dehydrogenase family of proteins, alcohol metabolism				
CHARGE	63,569	rs2384550	DBP: 4×10^{-8}	12q24	TBX3, TBX5	Phylogenetically conserved family of genes that share a common DNA-binding domain, the T-box	Phylogenetically conserved family of genes that share a common DNA-binding domain, the T-box					
Global Bpgen and CHARGE	134,258	rs1378942	DBP: 1×10^{-23}	15q24	CYP1A1, CYP1A2, CSK, LMAN1L, CPLX3, ARID3B, ULK3	CYP1A1, encodes a member of the cytochrome P450	Cytochrome P450 superfamily of enzymes	c-src tyrosine kinase	Lectin, mannose-binding, 1 like	complexin 3 (unknown)	ARID (AT-rich interaction domain) family of DNA-binding proteins	unc-51 like kinase 3 (unknown)
Global Bpgen and CHARGE	63,569	rs6495122	DBP: 2×10^{-10}		CYP1A1, CYP1A2, CSK, LMAN1L, CPLX3, ARID3B, ULK3	CYP1A1, encodes a member of the cytochrome P450	Cytochrome P450 superfamily of enzymes	c-src tyrosine kinase	Lectin, mannose-binding, 1 like	complexin 3 (unknown)	ARID (AT-rich interaction domain) family of DNA-binding proteins	unc-51 like kinase 3 (unknown)

(Table 3) contd….

Study	Total Number	SNP	Trait: P Value	Loci	Nearby Genes	Biological function						
ICBP-GWAS	69,395	rs2521501	SBP: 5×10^{-19}; DBP:2×10^{-15}	15q26	FURIN, FES	A member of the subtilisin-like proprotein convertase family	Cytochrome P450 superfamily of enzymes					
NESDA	39,706	rs13333226	HTN: 3.60×10^{-11}	16p12.3	UMOD	Inhibitor of calcium crystallization in renal fluids						
Global Bpgen	82,441	rs16948048	DBP: 5×10^{-9}	17q21	ZNF652, PHB	Zinc finger protein 652	Human cellular senescence and tumor suppression					
Global Bpgen	77,690	rs12946454	SBP: 1×10^{-8}	17q21	PLCD3, ABCD4, HEXIM1, HEXIM2	A member of the phospholipase C family	Superfamily of ATP-binding cassette (ABC) transporters	Hexamethylene-bis-acetamide in vascular smooth muscle cells	Unknown			
ICBP-GWAS	69,395	rs17608766	SBP: 1×10^{-10}	17q21	GOSR2	Transports proteins among the medial- and trans-Golgi compartments.						
ICBP-GWAS	69,395	rs6015450	SBP: 4×10^{-23}; DBP: 6×10^{-23}; HTN: 4×10^{-14}	20q13	GNAS, EDN3	G-protein alpha subunit	Endothelin family					

SNP, single nucleotide polymorphism; DBP, diastolic blood pressure; HTN, hypertension; SBP, systolic blood pressure.

are associated with a modest increase in risk are constantly found, their ability to be discriminatory and predictive markers is low [79]. Heritability and the prevalence of disease are important selective markers that influence the GWAS [80]. Therefore, studies should be performed with these conditions in mind. Genetic variants that have the highest relative risks are almost always presented during the first wave of GWAS findings. These genetic markers are almost certainly identified first due to the statistical power. However, one of the striking facts about these first findings is that they usually only explain a small proportion of the underlying genetic contribution to hypertension. Perhaps more unfortunate is that some of the previous studies were subsequently proven to have found associated risk factors with no diagnostic and prognostic relevance. Moreover, many of these markers were later determined to be located in non-coding sequences. Loss of function of the gene encoding the plasma membrane Ca(2+) pumps (PMCA) have been associated with a risk allele for hypertension [81]. These risk alleles have to be confirmed as true susceptibility alleles of hypertension in humans.

Third, the most reliable evidence of a true genetic association, short of defining the causal variant functionally, is replication of the association, especially if it appears in multiple populations [82-83]. However, determinations of genetic risk factors of essential hypertension through the use of GWASs have reported contradictory results. For example, Kidambi et al. attempted to replicate the previous finding reported for an independent sample of 2474 unrelated African Americans in the Milwaukee metropolitan area [84]. When they performed a GWAS for all 24 SNPs, they found no statistically significant differences for the minor allele frequencies between subjects and controls. Only one SNP (rs2146204) showed any borderline association (P=0.006). Niu et al. used the ligase detection reaction method and attempted to confirm a previous GWAS that had examined 548 patients diagnosed with essential hypertension and 560 age- and gender-matched controls [85]. Their results found that only two Han Chinese patients had any susceptibility to hypertension. Overall, these prior findings demonstrate the difficulties that can be encountered when trying to confirm previously reported GWAS results.

Fourth, another major concern when performing a GWAS is the makeup of the case and control groups. In some of the previously reported studies, experiments were not designed to look for any specific type of disease. For example, if the mean age of controls in a study was very young, the control subjects were not suitable, because phenotypes of cardiovascular and cerebrovascular diseases are related to aging, as many such diseases occur late in life. Therefore, sometimes the case-control association studies are performed using a super-control group comprised of older subjects without a disease [86-87].

Although GWASs have thus far failed to unlock the genetics of hypertension, it is possible that this methodology could identify rare variants associated with an increased risk for essential hypertension, thereby leading to new insights

for developing better methods for prevention or treatment of hypertension [88]. Although a common noncoding SNP might only have a small effect, the underlying gene/protein/mechanism could very well become an important drug target. In the future, novel designs, such as a GWAS of blood pressure traits that respond to drug therapy, may play a larger role in the diagnosis and treatment of hypertension [89].

CONCLUSION

In this article we reviewed the progress and issues of using GWAS for hypertension. GWAS data accumulated during this past decade have brought the biggest gain in our knowledge of the pathophysiology of hypertension. However, there are many outstanding problems to identify the susceptibility variants for hypertension. GWAS seems to be replacing next-generation whole-genome or exome sequencing studies. However, nobody knows whether GWAS methods will survive or become obsolete in the next decade. Hopefully, in the future, we will be able to use advanced technology to elucidate the genetic background of hypertension.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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