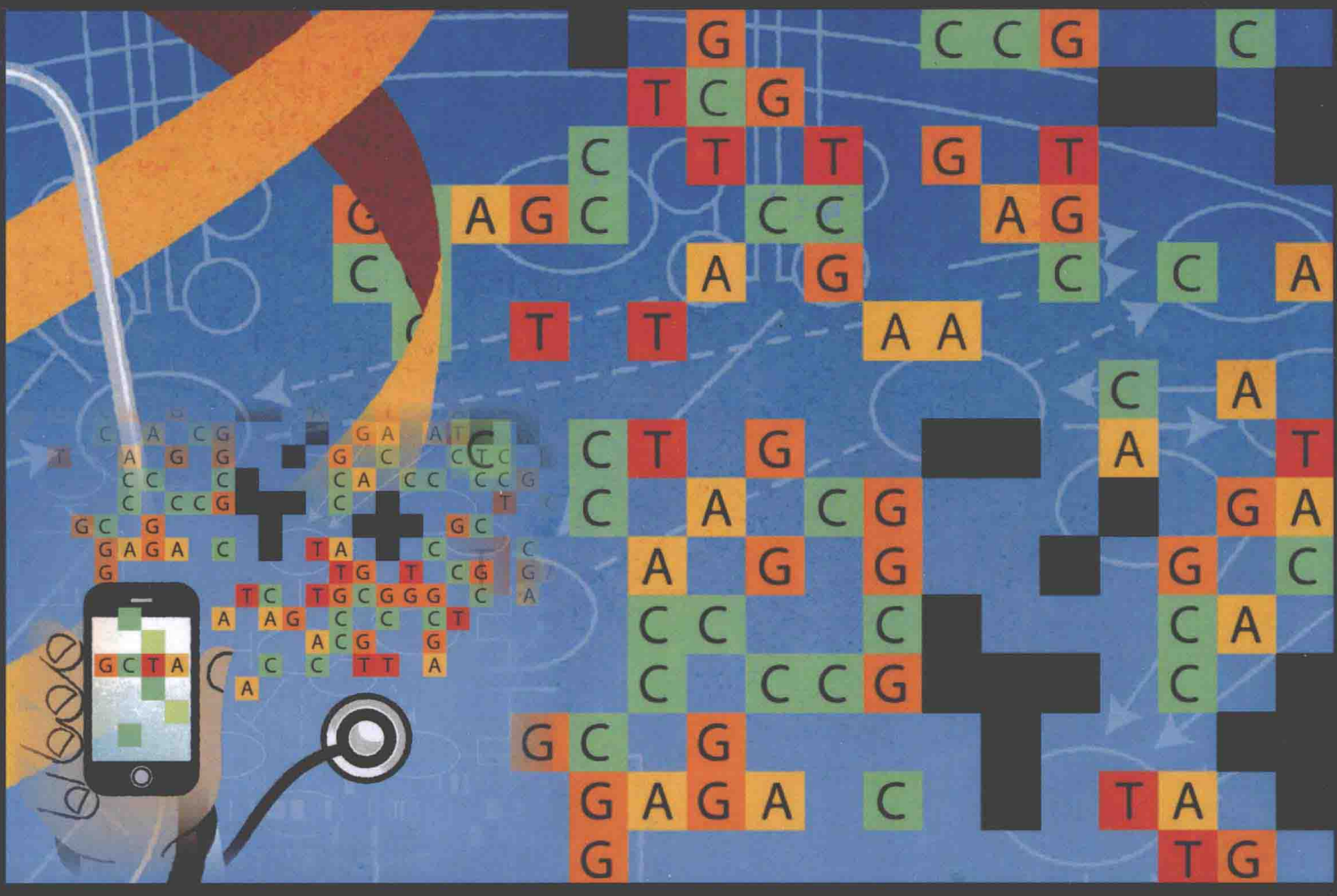
Genomic and Personalized Medicine

Second Edition

Edited by

Geoffrey S. Ginsburg & Huntington F. Willard



VOLUME

2

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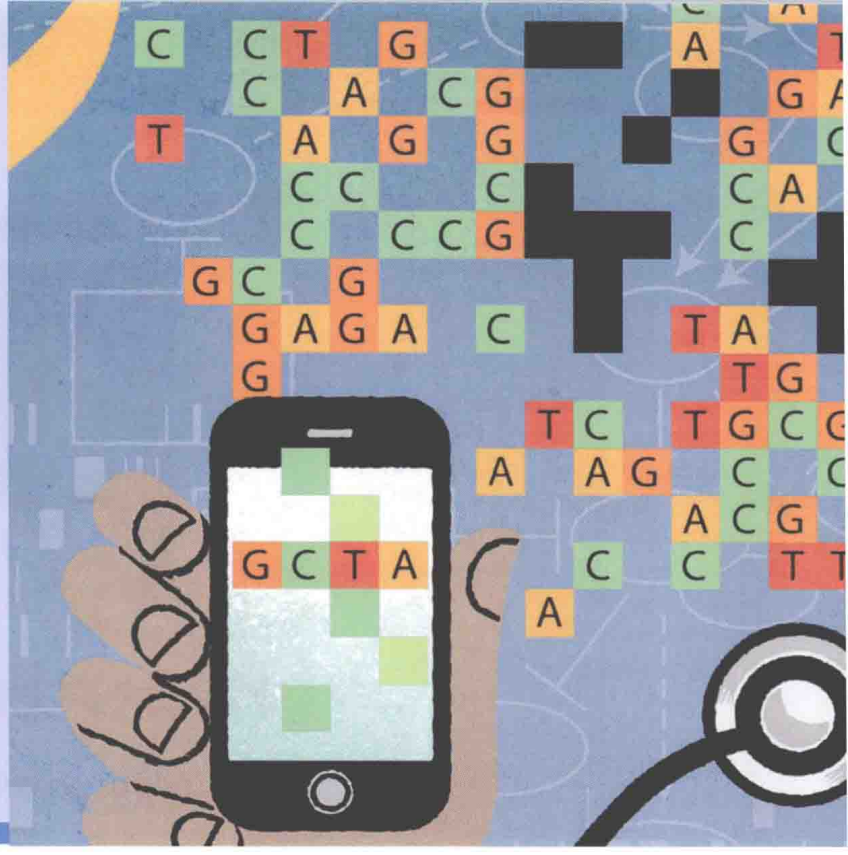

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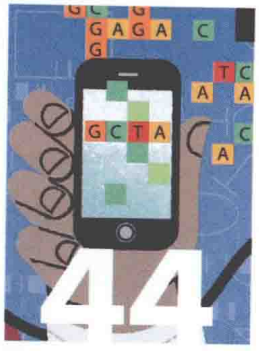

Hypertension

Patricia B. Munroe and Toby Johnson

INTRODUCTION

Elevated blood pressure (BP) or hypertension [≥140 mmHg systolic blood pressure (SBP) and/or ≥90 mmHg diastolic blood pressure (DBP)] is highly prevalent, affecting 26.4% of people aged 20 or older worldwide (Kearney et al., 2005), and is the leading global cause of preventable death. The World Health Organization (WHO) estimated that in 2004 hypertension accounted for 13% of deaths worldwide; 16.8% in high-income countries and 7.5% in low-income countries, due mainly to coronary heart disease (CHD) and stroke (WHO, 2009). Other complications associated with hypertension include heart failure, peripheral vascular disease, renal impairment, retinal hemorrhage, and visual impairment (WHO, 2002). Prospective observational studies show that the risk of cardiovascular disease (CVD) increases in a roughly linear fashion across the normal population range of BP (Chobanian et al., 2003), and that in older age groups, the risk of cardiovascular disease doubles for each increment of 20 mmHg SBP and 10 mmHg DBP, starting as low as 115 mmHg SBP and 75 mmHg DBP.

Existing anti-hypertensive treatments are effective at the population level, reducing the risk of developing CHD events (fatal and nonfatal) by 25% and the risk of stroke by 33%, per 10 mmHg reduction in SBP and 5 mmHg reduction in DBP achieved, independent of pre-treatment BP level (Law et al., 2009). However, at the individual level, BP is often poorly controlled, and many patients do not achieve <140 mmHg SBP and <90 mmHg DBP targets. In the 2006 Health Survey for England, only 28% of patients achieved this target, even though most were prescribed two or more anti-hypertensive drugs (Falaschetti et al., 2009). For many years, the first-choice therapies were beta-blockers and thiazide diuretics, which were developed more than 50 years ago (Borhani, 1959; Prichard, 1966). More recently angiotensin-converting enzyme inhibitors (ACEi), calcium channel blockers (CCBs), and angiotensin II receptor antagonists (ARBs) have become increasingly preferred, and current guidelines in the United Kingdom recommend ACEi or ARBs as first-line treatment for individuals <55 years, and CCBs prescribed for individuals >55 years and those of African or Caribbean ancestry (National Institute for Health and Clinical Excellence, 2011). The ACEi, ARBs, and thiazide diuretics all target the renin-angiotensin-aldosterone system (RAAS), which is a hormonal pathway that maintains normal BP and blood volume (Nguyen Dinh Cat and Touyz, 2011). There are very few new drugs in development for lowering BP in the general population, and only one new therapy has been granted regulatory approval in the past three years. Aliskirin is a first-in-class oral renin inhibitor that also targets the RAAS, and clinical trial data indicate effects on BP comparable to ACEi and ARBs (Musini et al., 2008).

The main causes of hypertension are well known. Lifestyle and genetic effects are both influential. The most important lifestyle risk factors are excess dietary sodium intake, body weight, alcohol consumption, stress, and lack of exercise (Chiong, 2008). Evidence for a genetic component comes from studies of families and twins, and suggests that the heritability (the fraction of BP variance contributed by genetic factors) for both SBP and DBP is between 30% and 50% (Havlik et al.,

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1979). However, heritability studies do not identify which genetic differences are important or by what mechanisms they exert their effects on BP. Recent advances in human genetics offer the opportunity to discover hitherto-unknown mechanisms and pathways affecting BP, which could, in principle, be targeted by novel therapeutic approaches and thus improve treatment of hypertension and prevention of CVD.

FINDING BLOOD PRESSURE GENES

Identification of the genetic basis of BP has been a longstanding and challenging research objective (Wallace et al., 2007). Mutations in specific genes causing rare, monogenic forms of hypertension have been successfully identified using linkage analysis and positional cloning. These mutations are in genes primarily expressed in the kidney, and affect salt/water homeostasis (Lifton et al., 2001). Although linkage analysis has had some success in mapping genes for other complex diseases, including type 1 diabetes and Crohn's disease (Brant and Shugart, 2004; Concannon et al., 2005), the use of linkage-based methods has not been successful for finding genes causing essential hypertension (high BP with no obvious medical cause), despite studies of sibling pairs and families with large sample sizes, and up to 400 microsatellite markers across the genome being analyzed (Caulfield et al., 2003; Cowley, 2006; Wu et al., 2006). Alongside genome-wide linkage studies, numerous candidate gene association studies for hypertension have also been carried out, albeit mostly with relatively small sample sizes, and with a general lack of consistency of the replicability of findings (Dominiczak et al., 2004).

However, over the past three years there has been substantial progress, and this success is largely attributable to the advent and rapid technological advances of genome-wide association studies (GWAS) (Hirschhorn and Daly, 2005). Modern GWAS use mass-produced DNA microarrays to genotype 300,000 or more single nucleotide polymorphisms (SNPs), distributed across the whole genome, in each individual. SNPs not directly genotyped can be imputed using correlation structures between multiple SNPs observed in reference panels densely genotyped by the HapMap (International HapMap Consortium, 2003) or 1000G studies (1000 Genomes Project Consortium, 2010). Typically a discovery analysis is conducted, in which hundreds or thousands of individuals are genotyped, and at each SNP (genotyped or imputed) the allele present is tested for association with hypertensive status and/or continuous BP phenotypes. The "BP phenotype" commonly used for GWAS studies is an untreated BP value, or an imputed BP value if the individual is taking anti-hypertensive medication (Tobin et al., 2005). Gender, age, age^2, and body mass index (BMI) are then included as covariates in an analysis, and a correction for population stratification is included if necessary. Associations that are suggestive or significant after multiple testing correction (genome-wide significant) are typically followed up by genotyping in further independent samples of individuals to establish definitive genome-wide significance (McCarthy et al., 2008). Most GWAS have used unrelated individuals, for which tests of association require straightforward contingency table or linear regression analyses, although it is also possible to apply GWAS methodology in samples of related individuals (Scuteri et al., 2007).

The Wellcome Trust Case Control Consortium (WTCCC) published the first GWAS results for hypertension in 2007 (Wellcome Trust Case Control Consortium, 2007). This study tested for association by comparing 2000 unrelated hypertensive cases, versus 3000 "common controls." The WTCCC study design used the same "common controls" for seven different disease comparisons, which had a potentially important disadvantage because the controls were from general population samples and therefore were not all non-hypertensive. The WTCCC did not find any genome-wide significant SNPs for hypertension, suggesting common genetic variants affecting risk for hypertension were likely to have relatively small effect sizes and/or were not covered by the Affymetrix 500K genotyping arrays used.

A number of subsequent GWAS tested association with either hypertension or with SBP and DBP as continuous outcomes, and reported a significant association for SBP at SNPs on chromosome 2q24.3, in the *STK39* (serine threonine kinase 39) gene (Wang et al., 2009), a significant association for DBP and hypertension at a SNP on chromosome 16q23.3, upstream of the *CDH13* (adhesion glycoprotein T-cadherin) gene (Org et al., 2009), and a significant association for SBP and DBP at a SNP near the *ATP2B1* (ATPase calcium transporting, plasma membrane 1) gene (ATP = adenosine triphosphate), reported by the Korean Association Resource (KARE) project (Cho et al., 2009). Only variants near *ATP2B1* have been replicated in subsequent studies with much larger sample sizes, suggesting that the associations near *STK39* and near *CDH13* are either false positives or are population-specific effects.

At the same time, Newton-Cheh and colleagues reported results from a candidate gene association study for two BP biomarkers, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which are peptides with known vasodilatory properties. They tested SNPs near the atrial natriuretic peptide precursor (*NPPA*) and brain natriuretic peptide precursor (*NPPB*) genes, and for two SNPs they found significant association with increased plasma ANP and BNP levels, and, at the same two SNPs, association with decreased SBP and DBP (Newton-Cheh et al., 2009b). This study highlights a more general idea that studying intermediate quantitative phenotypes may increase power to detect associations for the disease phenotype(s) that are ultimately of interest (Plomin et al., 2009).

The modest findings of individual GWAS for BP traits meant that a natural next step was to meta-analyze results from multiple GWAS, and thus achieve effectively much larger sample sizes. The greater power of such studies has led to the identification of 40 distinct loci, each harboring one or more genetic variants with robust and validated association with BP traits (Table 44.1).

TABLE 44.1 Summary of genetic variants robustly associated with blood pressure from peer-reviewed publications

SNP	Locus nickname	Chr	Base pair position (NCBI build 36)	MAF	Discovery cohort ethnicity	Reference
GWAS of SBP and DBP						
*rs17249754	*ATP2B1*	12	88,584,717	0.37	East Asian	Cho et al., 2009
Intermediate phenotype analysis						
*rs5068	*NPPA/NPPB*	1	11,828,511	0.06	European	Newton-Cheh et al., 2009b
Meta-analyses of GWAS of SBP, DBP, and hypertension						
Global BPgen Consortium						
rs17367504	*MTHFR-NPPB*	1	11,785,365	0.14	European	Newton-Cheh et al., 2009a
*rs11191548	*CYP17A1-NT5C2*	10	104,836,168	0.09	European	
*rs16998073	*FGF5*	4	81,541,520	0.21	European	
*rs1530440	*c10orf107*	10	63,194,597	0.19	European	
*rs653178	*SH2B3*	12	110,470,476	0.47	European	
*rs1378942	*CYP1A1-CSK*	15	72,864,420	0.35	European	
*rs16948048	*ZNF652*	17	44,795,465	0.39	European	
CHARGE Consortium						
rs1004467	*CYP17A1*	10	104,584,497	0.10	European	Levy et al., 2009
*rs381815	*PLEKHA7*	11	16,858,844	0.26	European	
rs2681492	*ATP2B1*	12	88,537,220	0.20	European	
rs3184504	*SH2B3*	12	110,368,991	0.47	European	
*rs9815354	*ULK4*	3	41,887,655	0.17	European	
*rs11014166	*CACNB2*	10	18,748,804	0.34	European	
*rs2384550	*TBX3-TBX5*	12	113,837,114	0.35	European	
rs6495122	*CSK-ULK3*	15	72,912,698	0.42	European	
*rs880315	*CASZ1*	1	10,719,453	0.36	East Asian	Takeuchi et al., 2010
AGEN-BP study						
rs17030613	*ST7L-CAPZA1*	1	112,971,190	0.47	East Asian	Kato et al., 2011
*rs16849225	*FIGN-GRB14*	2	164,615,066	0.40	East Asian	
*rs6825911	*ENPEP*	4	111,601,087	0.48	East Asian	
*rs1173766	*NPR3*	5	32,840,285	0.38	East Asian	
*rs11066280	*RPL6-ALDH2*	12	111,302,166	0.22	East Asian	
rs35444	*TBX3*	12	114,036,820	0.25	East Asian	

(*continued*)

TABLE 44.1 (Continued)

SNP	Locus nickname	Chr	Base pair position (NCBI build 36)	MAF	Discovery cohort ethnicity	Reference
ICBP-GWAS for SBP and DBP						
*rs2932538	*MOV10*	1	113,018,066	0.25	European	Ehret et al., 2011
*rs13082711	*SLC4A7*	3	27,512,913	0.22	European	
*rs419076	*MECOM*	3	170,583,580	0.47	European	
*rs13107325	*SLC39A8*	4	103,407,732	0.05	European	
*rs13139571	*GUCY1A3-GUCY1B3*	4	156,864,963	0.24	European	
rs1173771	*NPR3-C5orf23*	5	32,840,285	0.40	European	
*rs11953630	*EBF1*	5	157,777,980	0.37	European	
*rs1799945	*HFE*	6	26,199,158	0.14	European	
*rs805303	*BAT2-BAT5*	6	31,724,345	0.41	European	
rs1813353	*CACNB2(3')*	10	18,747,454	0.45	European	
*rs932764	*PLCE1*	10	95,885,930	0.44	European	
*rs7129220	*ADM*	11	10,307,114	0.19	European	
*rs633185	*FLJ32810-TMEM133*	11	100,098,748	0.28	European	
*rs2521501	*FES-FURIN*	15	89,238,392	0.31	European	
*rs17608766	*GOSR2*	17	42,368,270	0.14	European	
*rs1327235	*JAG1*	20	10,917,030	0.46	European	
*rs6015450	*GNAS-EDN3*	20	57,184,512	0.12	European	
rs17367504	*MTHFR-NPPB*	1	11,785,365	0.15	European	
rs3774372	*ULK4*	3	41,852,418	0.17	European	
rs1458038	*FGF5*	4	81,383,747	0.29	European	
rs4373814	*CACNB2 (5')*	10	18,459,978	0.45	European	
rs4590817	*C10orf107*	10	63,137,559	0.16	European	
rs11191548	*CYP17A1-NT5C2*	10	104,836,168	0.09	European	
rs381815	*PLEKHA7*	11	16,858,844	0.26	European	
Rs17249754	*ATP2B1*	12	88,584,717	0.16	European	
rs3184504	*SH2B3*	12	110,368,991	0.47	European	
rs10850411	*TBX5-TBX3*	12	113,872,179	0.30	European	
rs1378942	*CYP1A1-ULK3*	15	72,864,420	0.35	European	
rs12940887	*ZNF652*	17	44,757,806	0.38	European	

(*continued*)

TABLE 44.1 (Continued)

SNP	Locus nickname	Chr	Base pair position (NCBI build 36)	MAF	Discovery cohort ethnicity	Reference
ICBP-GWAS for PP and MAP						
rs13002573	*FIGN*	2	164,623,454	0.20	European	Wain et al., 2011
rs1446468	*FIGN*	2	164,671,732	0.47	European	
*rs319690	*MAP4*	3	47,902,488	0.49	European	
*rs871606	*CHIC2*	4	54,494,002	0.15	European	
*rs2071518	*NOV*	8	120,504,993	0.17	European	
*rs17477177	*PIK3CG*	7	106,199,094	0.28	European	
rs2782980	*ADRB1*	10	115,771,517	0.19	European	
*rs11222084	*ADAMTS8*	11	129,778,440	0.37	European	
Extreme case/control design						
*rs13333226	*UMOD*	16	20,273,155	0.17	European	Padmanabhan et al., 2010
Women's Genome Health Study						
*rs2898290	*BLK-GATA4*	8	11,471,318	0.47	European	Ho et al., 2011
Candidate genes – subset of GWAS						
*rs2004776	*AGT*	1	228,915,325	0.24	European	Johnson et al., 2011
*492	*ADRB1*	10	115,795,046	0.27	European	

This table lists all significantly associated SNPs with blood pressure per study. * indicates the study which reported the first association at this locus, the "40 independent blood pressure loci." Some studies found associations at the same locus, but the index SNP reported differed. In some cases the reported SNP was in high LD, for others it was an independent signal at a locus. The "locus nickname" indicates the genes nearest to the associated genetic variant as described in the original papers. Chr = chromosome, bp = base pair, MAF = minor allele frequency.

Meta-analyses of GWAS to Discover Blood Pressure Genes

The first large-scale meta-analyses of GWAS results for SBP, DBP, and case/control hypertension were published in May 2009 by two large international consortia, Global BPgen (GBPG) and the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) (Levy et al., 2009; Newton-Cheh et al., 2009a). Both consortia analyzed approximately 2.5 million SNPs (directly genotyped and imputed) in large numbers of individuals of European ancestry (GBPG N = 34,433 and CHARGE N = 29,136), and both followed up their top 10 independent signals from each scan by performing a simultaneous reciprocal exchange of association results, with the GBPG consortium also following up 12 significant signals by direct genotyping in a further 71,225 individuals of European ancestry.

Each consortium identified genome-wide significant ($P < 5 \times 10^{-8}$) SNP associations at eight distinct loci. Of these, three loci were simultaneously discovered by both consortia, and one association identified by CHARGE was the same as the previously discovered association at the *ATP2B1* locus (Cho et al., 2009); hence, in total, 12 novel associations were discovered (Table 44.1). The majority of the genome-wide significant SNPs were associated with both SBP and DBP and odds of hypertension, with the same direction of effect. The associated SNPs were common, with a minor allele frequency (MAF) >5%, and the effect sizes were ≤1.0 mmHg for SBP and ≤0.5 mmHg for DBP.

Since the GBPG and CHARGE meta-analyses, there has been a steady flow of new BP loci discoveries. Association at a SNP located near *CASZ1*, which had showed suggestive association in the CHARGE meta-analysis results, was replicated in samples of East Asian (Japanese) ancestry (Takeuchi et al., 2010). Combining the CHARGE meta-analysis results with data from a single, extremely large cohort of 23,019 individuals from the Women's Genome Health Study (WGHS) led to the discovery of an association near the *BLK-GATA4* genes (Ho et al., 2011). This association is located on chromosome 8 in a large polymorphic inversion, in a linkage disequilibrium (LD) block spanning many genes, and allelic imbalance has also been reported at this locus (Nusbaum et al., 2006; Wagner et al., 2010). The Asian Genetic Epidemiology Network Blood Pressure (AGEN-BP) GWAS study meta-analyzed GWAS results from 19,608 individuals of East

Asian ancestry, followed up top hits in a further 31,000 individuals also of East Asian ancestry, and discovered six significant associations with BP, near *ST7L/CAPZA1*, *FIGN/GRB14*, *ENPEP*, *NPR3*, *ALDH2*, and *TBX3* (Kato et al., 2011). The association at the *TBX3* locus was independent ($r^2 = 0.001$ in Utah residents with Northern and Western European ancestry (CEU)) to the variant previously reported by CHARGE (Levy et al., 2009).

Padmanabhan and colleagues performed an "extreme case/control" GWAS comparing highly selected hypertensive cases (top 2% of the population) versus controls selected for low BP and low occurrence of cardiovascular events (bottom 9% of the population) (Padmanabhan et al., 2010). Despite genotyping a discovery sample of fewer than 4000 individuals, the stringency of the ascertainment scheme meant that this study discovered a novel SNP at *UMOD*, which was validated by follow-up in a further 36,386 individuals. The SNP allele associated with higher risk of hypertension had previously been observed to be also associated with impaired renal function (as measured by estimated glomerular filtration rate) and higher risk for chronic kidney disease (Kottgen et al., 2009). Johnson and colleagues selected 30 genes that were known targets for anti-hypertensive drugs and tested SNPs in this "candidate gene" set for association with BP and hypertension, using the CHARGE meta-analysis results. With follow-up using GBPG meta-analysis and WGHS GWAS results, genome-wide significant associations at two loci, angiotensinogen (*AGT)* and the beta-adrenergic receptor 1 (*ADRB1*) were discovered (Johnson et al., 2011). The association at *AGT* was the same as the one previously reported by Watkins and colleagues in a smaller candidate gene study (Watkins et al., 2010).

Although there is wide variation in study designs (e.g., studies in populations with different ethnic ancestries, cases/controls ascertained from the extremes of the population BP distribution, or testing only "candidate gene" subsets of GWAS data), it is unclear whether the new discoveries are being made as a direct consequence of the different study designs, or are merely a reflection of the fact that all genetic association studies for BP have been somewhat underpowered. Hence, each study (regardless of design) would detect a more-or-less random subset of the likely hundreds of genetic associations with individually small effect sizes (Park et al., 2010). The latter hypothesis is supported by the observation that GBPG and CHARGE, two large studies with very similar overall designs, had relatively little overlap in the loci that reached discovery significance thresholds (Munroe et al., 2009).

Many of the genetic associations just described have subsequently been replicated in samples of different ancestry from where they were initially discovered, including in East Asian (Hong et al., 2010a, b; Kato et al., 2011; Liu et al., 2011; Niu et al., 2010; Takeuchi et al., 2010), South Asian (Newton-Cheh et al., 2009a), and African ancestries (Fox et al., 2011; Zhu et al., 2011).

International Consortium for Blood Pressure Genome-wide Association Studies

In the autumn of 2008, GBPG and CHARGE joined forces, and with additional GWAS studies formed a new BP genetics consortium, the International Consortium for Blood Pressure Genome-wide Association Studies (ICBP-GWAS). This consortium evaluated associations between 2.5 million SNPs and SBP and DBP, and a second project focused on mean arterial pressure (MAP) and pulse pressure (PP). MAP and PP are calculated from SBP and DBP, but testing for association with MAP may increase power to detect genetic variants that influence both SBP and DBP with concordant effects, and testing for association with PP increases power to detect some genetic variants that influence SBP and DBP with discordant effects.

The meta-analysis of GWAS for SBP and DBP was performed in 69,395 individuals of European ancestry, and was followed by a three-staged validation study using 133,661 additional individuals of European ancestry (Ehret et al., 2011). A total of 29 SNPs at 28 loci were found to be significantly associated with SBP and DBP (all with $P < 5 \times 10^{-9}$), of which 16 of the associations were novel findings (Table 44.1), with one of the associations (at *NPR3*) discovered simultaneously by AGEN-BP (Kato et al., 2011). Analyses in this enlarged dataset did not support the association at the *PLCD3* locus that was reported previously by GBPG, illustrating that even large GWAS meta-analyses are susceptible to false positive results (which are controlled at 5% *per phenotype* per study by the conventional genome-wide significance threshold). The effect sizes of the new variants were similar to findings from other GWAS BP studies (mostly $\leq$1 mmHg for SBP and $\leq$0.5 mmHg for DBP), and observed directions of effect were concordant for SBP, DBP, and odds of hypertension.

A meta-analysis of GWAS for MAP and PP was performed in 74,064 individuals of European ancestry (Wain et al., 2011). Using "look-ups" in GWAS results from a further 48,607 individuals meant that a larger number of SNPs (99 in total) selected at a less stringent threshold ($P < 1 \times 10^{-5}$) could be followed up and potentially validated. This strategy revealed eight genome-wide significant associations; five for PP near *FIGN*, *CHIC2*, *PIK3CG*, *NOV*, and *ADAMTS8*, and three for MAP near *FIGN*, *ADRB1*, and *MAP4*. The SNPs near *FIGN* associated with PP and with MAP are two independent SNPs not in linkage disequilibrium (LD) in Europeans ($r^2 = 0.054$ in HapMap CEU), suggesting possible multiple causal variants. One of the SNPs near *FIGN* is the same as recently reported by Kato and colleagues (2011). The association at *ADRB1* is not in strong LD ($r^2 = 0.14$ in CEU) with the variant reported previously by Johnson and colleagues (2011).

Previous work had shown that some of the 13 significant associations discovered by GBPG and CHARGE in European ancestry samples also showed significant associations in samples of non-European ancestries (Hong et al., 2010a, b; Kato et al., 2011; Liu et al., 2011; Niu et al., 2010; Takeuchi et al., 2010; Zhu et al., 2011). To study this systematically in the largest sample sizes available, the ICBP-GWAS consortium tested the 29 SNPs for association with SBP and DBP in meta-analyses of results from non-European ancestries. Some of the individual SNPs showed significant associations in populations of East- or South Asian ancestry (or both) after

correction for multiple testing (Ehret et al., 2011). The general lack of statistically significant associations likely reflects a lack of power due to the fact that the available sample sizes were small compared to the discovery sample size in Europeans. Arguably, the most meaningful analysis of ancestry-specific effects (or lack of effects) is to test whether each SNP is associated with different effect sizes (on a mmHg-per-allele scale) in different ancestries, which is equivalent to testing for a genotype-by-ancestry interaction effect and allows for allele frequency differences between populations (because, for example, SNPs monomorphic in a given population have infinitely wide confidence limits on a mmHg-per-allele scale). Using effect size estimates for European ancestry samples that are free of winners' curse bias, there is no evidence of such ancestry-specific effects (Figure 44.1A and B). This analysis addresses the most biologically informative question, whether there is evidence that an allele would be associated with a different effect size if it was segregating in multiple populations. Hence, on the data currently available, although genotype frequencies do differ between populations of different ancestries, there is no evidence that the expected phenotype given a particular genotype depends on population ancestry (i.e., the biological mechanisms that determine phenotype as a function of genotype are the same for all populations). If this is true more generally (beyond the 29 SNPs studied here by ICBP-GWAS for SBP and DBP), future studies could increase power by combining data from populations of multiple ancestries in a single meta-analysis, as has been done for other phenotypes [Chambers et al., 2010; Coronary Artery Disease (C4D) Genetics Consortium, 2011].

NEW INSIGHTS INTO BLOOD PRESSURE BIOLOGY

To date, large-scale meta-analyses of GWAS and other approaches have revealed 40 genetic loci for BP and hypertension. The genetic variants discovered are all common (MAF > 5%), which is expected because such variants are better covered by GWAS genotyping arrays and because association tests are more powerful for common variants. Of the 29 associations with SBP and DBP reported by ICBP-GWAS, for example, many of the associated SNPs are intergenic, with the nearest gene located several kb (kilobasepairs) away, whereas eight are within genes and are potentially functional as encoding amino acid changes in the protein sequence (non-synonymous SNPs; nsSNPs). Functional mechanisms involving gene regulation are suggested for at least 5/29 SNPs, which are *cis*-acting expression SNPs (eSNPs) associated with the expressed transcript levels for nearby genes (Ehret et al., 2011). Of all the robustly associated BP loci (Table 44.1), many have at least one biologically plausible gene in the associated interval (e.g., *NPPA*, *NPPB*, *CYP17A1*, *GUCY1A3*, *GUC1B3*, *NPR3*, *ADM*, *GNAS*, *EDN3*, *ENPEP*, *GATA4*, *ADRB1*, and *AGT*).

Some of the recently reported genetic associations are in or near genes previously suspected to affect BP on the basis of prior functional and physiological experiments. However, despite *AGT* being a known component of the RAAS and one of the most intensively studied candidate genes, *ADRB1* being a known target for beta-blockers, and *ADM* encoding the adrenomedullin peptide with known vasodilatory properties, the small apparent effect sizes (for all genetic variants tested so far) have meant that very large sample sizes were needed to obtain robust confirmation of an association between BP and common genetic variation at these loci. The GWAS also identified genetic variants associated with BP that are in or near several genes that are part of the natriuretic peptide-guanylate cyclase-nitric oxide signaling pathway. Three distinct SNPs have now been reported near *NPPA* and *NPPB* (Newton-Cheh et al., 2009a, b; Tomaszewski et al., 2010), one SNP near the guanylate cyclase α and β subunits (*GUCY1A3-GUCY1B3*) (Ehret et al., 2011) and two SNPs near the C-type natriuretic peptide receptor (*NPR3*) (Ehret et al., 2011; Kato et al., 2011).

Many of the newly discovered BP loci do not contain genes that have previously been implicated in BP or cardiovascular disease. However, several of the genes have been studied in other contexts and have known functions that engender novel and potentially testable mechanistic hypotheses. One such example is the hemochromatosis (*HFE*) gene, where known mutations cause hereditary hemochromatosis, a disorder of iron overload that leads to hepatic cirrhosis and other complications (Pietrangelo, 2010). A second example is solute carrier family 39, member 8 (*SLC39A8*), which encodes a zinc transporter previously shown to play a role in cellular importation of zinc at the onset of inflammation. Its expression can be induced by tumor necrosis factor alpha (TNF-α) (Besecker et al., 2008), and this protein has also been implicated in cadmium and manganese transport (Himeno et al., 2009). The SNPs in these genes associated with BP are both nsSNPs: H63D in *HFE* is a low-penetrance allele for hereditary hemochromatosis, and A391T in *SLC39A8* has also been associated with high-density lipoprotein levels (Teslovich et al., 2010) and body mass index (Speliotes et al., 2010). Functional studies of these genes and their effects on BP and CVD, using, for example, transgenic knockout mouse model systems, will be an interesting area for future research.

DEVELOPING NEW THERAPIES FOR CARDIOVASCULAR DISEASE

Although each genetic variant exhibits only a modest effect on BP (mostly <1 mmHg for SBP and <0.5 mmHg DBP per risk allele), this does not *necessarily* correlate with efficacy of a therapeutic agent at the level of the gene product. In this context, the examples of HMG CoA reductase (*HMGCR*) and cholesterol esterase transfer protein (*CETP*) are widely cited. Genetic variants in or near these genes are associated with small effects on low-density lipoprotein (LDL) cholesterol and