Genome-wide scan identifies *CDH13* as a novel susceptibility locus contributing to blood pressure determination in two European populations

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Hypertension is a complex disease that affects a large proportion of adult population. Although approximately half of the inter-individual variance in blood pressure (BP) level is heritable, identification of genes responsible for its regulation has remained challenging. Genome-wide association study (GWAS) is a novel approach to search for genetic variants contributing to complex diseases. We conducted GWAS for three BP traits [systolic and diastolic blood pressure (SBP and DBP); hypertension (HYP)] in the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) S3 cohort (*n* = 1644) recruited from general population in Southern Germany. GWAS with 395 912 single nucleotide polymorphisms (SNPs) identified an association between BP traits and a common variant rs11646213 (T/A) upstream of the *CDH13* gene at 16q23.3. The initial associations with HYP and DBP were confirmed in two other European population-based cohorts: KORA S4 (Germans) and HYPEST (Estonians). The associations between rs11646213 and

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three BP traits were replicated in combined analyses (dominant model: DBP, $P = 5.55 \times 10^{-5}$, effect -1.40 mmHg; SBP, $P = 0.007$, effect -1.56 mmHg; HYP, $P = 5.30 \times 10^{-8}$, OR = 0.67). Carriers of the minor allele A had a decreased risk of hypertension. A non-significant trend for association was also detected with severe family based hypertension in the BRIGHT sample (British). The novel susceptibility locus, *CDH13*, encodes for an adhesion glycoprotein T-cadherin, a regulator of vascular wall remodeling and angiogenesis. Its function is compatible with the BP biology and may improve the understanding of the pathogenesis of hypertension.

INTRODUCTION

Hypertension is a common disease affecting 25% of the adult population (1). Individuals with high blood pressure (BP) can develop complications of the cardiovascular and kidney function. Continuously high BP is a complex physiological condition where environmental and life-style factors such as increased body mass, smoking, alcohol and salt consumption as well as multiple genetic factors contribute to the development of the disease (2). Twin studies have shown that up to half of the inter-individual variance in BP level is heritable (3). The heritability estimate for long-term average systolic blood pressure (SBP) has been calculated to be 0.66 and for diastolic blood pressure (DBP) 0.60 (4). A number of genes and their mutations have been described to be responsible for rare monogenetic traits that severely affect BP regulation in families carrying these gene variants (5). Linkage scans and candidate gene-based association studies have mapped a large number (>160) of genomic regions to be potentially associated with SBP, DBP or the clinical diagnosis of hypertension (HYP) (6). In general, replication of the initially identified associations has been challenging. Genome-wide association studies (GWASs) are currently an alternative methodology to search for novel genetic variants associated with complex diseases (7). We conducted a GWAS for three BP traits (SBP, DBP and HYP) in the Kooperative Gesundheitsforschung in der Region Augsburg (KORA) S3 epidemiological cohort ($n = 1644$) recruited from a general population in Southern Germany. The association of one common single nucleotide polymorphism (SNP) located upstream of the *CDH13* gene was replicated with HYP and DBP in two independent study populations from Germany and Estonia. The *CDH13* gene encodes for a cell–cell adhesion glycoprotein T-cadherin involved in vascular wall remodeling and angiogenesis consistent with its potential role in BP regulation.

RESULTS

Genome-wide association study

The design of this study involved three stages: stage 1—genome-wide analysis for the three BP traits in KORA S3, a population-based cohort (Augsburg, Southern Germany) (8); stage 2—replication in the same population, but in a different independent cohort (KORA S4); stage 3—replication in two further sample sets from European populations (HYPEST, Estonians; BRIGHT, British) (Table 1; Supplementary Material, Fig. S1). In stage 1, the KORA S3 sample of 1644 individuals was genotyped using the Affymetrix 500K Gene

Chips. A total of 395 912 SNPs were included for the association analysis. These SNPs satisfied the following quality control criteria: minor allele frequency (MAF) $\geq 1\%$, call rate $>93\%$ and Hardy-Weinberg Equilibrium (HWE) P-value >0.001. Associations were assessed by fitting linear or logistic regression to SBP and DBP or hypertension, respectively. Although none of the detected P-values were below the genome-wide significance level of 1.26×10^{-7} after Bonferroni correction, the strength of some detected associations was close to this level. The strongest associations ($P < 5 \times 10^{-6}$; additive model) were detected between SBP and six loci: 5q34 (rs12153297; $P = 3.46 \times 10^{-7}$), 2p24 (rs10195618; $P = 6.42 \times 10^{-7}$), 10p12 (rs7898888; $P = 8.30 \times 10^{-7}$), 17p11.2 (rs1242502; $P = 1.72 \times 10^{-6}$), 10q22.1 (rs11814843; $P = 2.46 \times 10^{-6}$) and 12q14.3 (rs11176419; $P = 4.94 \times 10^{-6}$). These associations were also reflected at the quantile–quantile (QQ)-plot of SBP revealing a small excess of low P-values (Fig. 1). In addition, three loci showed strong association with HYP: 16q23.3 (rs11646213; $P = 2.34 \times 10^{-6}$, dominant model), 5q32.1 (rs2972345; $P = 3.17 \times 10^{-6}$, additive model) and 18q22.3 (rs2052662; $P = 3.69 \times 10^{-6}$, dominant model). Respective QQ-plots are shown in Supplementary Material, Fig. S2. In total, our genome-wide scan revealed 80 autosomal regions associated with BP traits with $P < 5.5 \times 10^{-5}$ (Supplementary Material, Table S1). These regions contained either a single SNP or a cluster of SNPs associated with SBP ($n = 42$), DBP ($n = 22$) or HYP ($n = 16$). Half of the 80 top SNPs ($n = 41$) exhibited association signal ($P < 0.01$) with more than one of the BP phenotype.

Replication in population-based cohorts KORA S4 and HYPEST

To confirm the associations found in GWAS and to minimize the probability of identifying a false positive, we sought replication in three independent European samples. First, replication was attempted using a sample recruited separately from the same population as in stage 1 with identical sampling design (8) ($n = 1830$; KORA S4 cohort; Germans; Table 1). We included SNPs ($n = 77$; 54 genomic regions) from stage 1 based on P-value, potential biological candidacy or location near previously reported linkage peaks. All selected loci contained more than one SNP with the evidence of association in GWAS with tested BP traits ($P < 10^{-3}$). In addition, SNPs that appeared to be associated with more than one BP traits were considered as higher priority to be followed up in the replication stages (for a detailed description see Materials and Methods section). Only a single SNP (rs11646213 at

Table 1. Characteristics of the study samples

Study stage	Country	Source of study samples	Sample size (M/F)	Age in years [mean (±SD)] cases/controls	Number of cases/controls[a]	BMI [mean (±SD)] cases/controls	SBP (mmHg) [mean (±SD)] cases/controls	DBP (mmHg) [mean (±SD)] cases/controls	n for antihypertensive treatment cases/controls
Study sample for association with hypertension									
Stage 1	Germany	KORA S3	960 (479/481)	57.4 (6.2)/52.9 (7.2)	364/596	26.5 (2.3)/25.1 (2.6)	148.6 (16.9)/119.6 (11.2)	89.7 (10.2)/75.8 (7.6)	140 (38.5%)/0
Stage 2	Germany	KORA S4	1566 (748/818)	55.6 (9.6)/51.2 (8.5)	447/1119	26.7 (2.4)/25.0 (2.7)	147.6 (19.1)/118.6 (11.4)	91.3 (11.4)/76.3 (7.2)	257 (57.5%)/0
Stage 3	Estonia	HYPEST	1246 (397/849)	44.3 (14.1)/40.3 (10.7)	596/650	28.7 (3.8)/24.4 (3.8)	144.2 (18.0)/128.0 (8.2)	87.6 (10.4)/80.6 (6.32)	389 (65.3%)/0
	UK	BRIGHT	4370 (1742/2627)	57.5 (11.1)/59.0 (9.0)	2401/1969	27.3 (3.5)/25.3 (3.3)	154.2 (20.1)/123.1 (10.5)	94.1 (11.0)/76.5 (7.1)	2401 (100%)/0
All			8142		3808/4334				
Study sample for association with systolic and diastolic blood pressure									
Stage 1	Germany	KORA S3	1017 (502/515)	54.3 (7.14)	na	25.7 (2.6)	132.31 (19.61)	82.16 (10.48)	0
Stage 2	Germany	KORA S4	1551 (764/787)	51.9 (8.57)	na	24.7 (2.83)	127.06 (18.32)	80.70 (10.40)	0
Stage 3	Estonia	HYPEST	1097 (373/724)	42.7 (12.7)	na	25.3 (5.8)	139.61 (18.38)	86.34 (11.13)	0
All			3665						

M, males; F, females; BMI, body mass index (kg/m^2); SD, standard deviation; SBP and DBP, systolic and diastolic blood pressure; na, not applicable.
[a]Detailed definition of hypertensive cases and normotensive controls is given in Materials and Methods section.
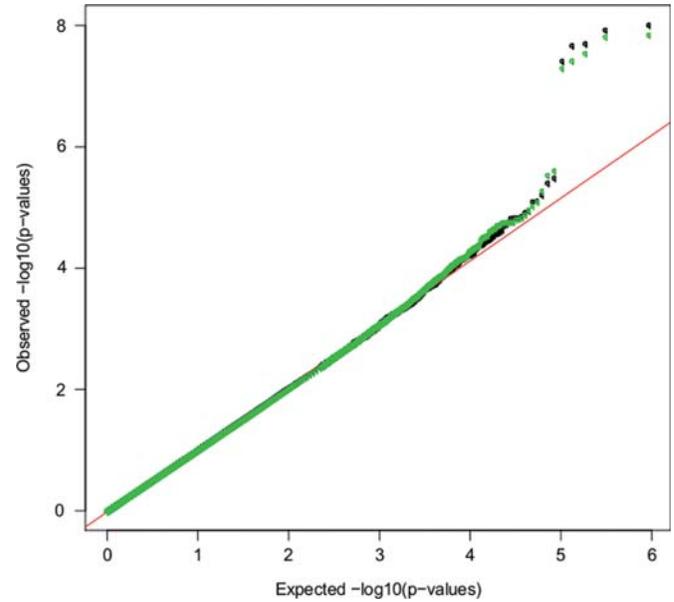
Figure 1. Quantile–quantile plots of the χ^2 statistic obtained from GWA analysis for SBP under the additive genetic model in the KORA S3 500K sample. The distribution of $\log_{10}(P)$ of association tests is shown before (black dots) and after (green dots) correction for population stratification performed by principal component analysis using the EIGENSOFT software (21). A genomic control analysis led to an inflation factor (λ) of 1.02.

16q23.3) was associated with all three BP traits (SBP, DBP and HYP) in both the KORA S3 and KORA S4 samples (Table 2; Supplementary Material, Tables S2–S4). In GWA scan, evidence of association ($P < 10^{-3}$) was also seen with several other SNPs across a >1 Mb region, covering the upstream and coding sequence of the *CDH13* gene (Fig. 2). Due to low linkage disequilibrium (LD), almost no correlation is seen between the top SNP rs11646213 and the other associated markers in the close-by region. The associations of four further SNPs selected for stage 2 (rs6784190, rs448559, rs1994547 and rs9948310) were replicated with HYP and/or SBP. The effect size estimates of the five SNPs have the same sign and similar magnitude as in KORA S3. The loci that failed replication and did not pass to stage 3 are shown in Supplementary Material, Table S4.

A set of nine SNPs was carried on to the third stage and was genotyped in another European population-based sample, HYPEST ($n = 1823$, Estonians; Table 1; Supplementary Material, Tables S2–S3). Five SNPs were chosen based on positive replication from stage 2 along with three neighboring markers (for a detailed description see Materials and Methods section). In addition, we included rs12731181 with the strongest P-value ($P = 2.5 \times 10^{-4}$, HYP) in stage 2, but seen with opposite effects in KORA S3 and KORA S4 (Supplementary Material, Table S2). As in KORA S4, the most consistent association signals in HYPEST were detected between rs11646213 and DBP ($P = 0.017$) as well as HYP ($P = 0.048$). The association with SBP was not confirmed in HYPEST (Table 2).

We performed a weighted meta-analysis ($n = 3665$ for SBP, DBP; $n = 1407$ cases/2365 controls for HYP) to combine the results in three population-based cohort samples (KORA S3, S4 and HYPEST) (Table 2). Combined analysis improved

Table 2. Association of rs11646213 (T/A; 16q23.3) with hypertension (HYP), systolic (SBP) and diastolic (DBP) blood pressure

	Sample	MAF cohort (case/control)[a]	HYP[a] P-value, OR (95% CI) Add	Dom	SBP P-value, beta (SE) Add	Dom	DBP P-value, beta (SE) Add	Dom
Population-based cohorts	KORA S3	41% (36/45%)	1.39×10^{-4}, 0.67 (0.53, 0.82)	2.34×10^{-6}, 0.49 (0.36, 0.66)	0.165, −1.08 (0.78)	0.02, −2.64 (1.13)	0.047, −0.95 (0.47)	0.0034, −2.02 (0.69)
	KORA S4	39% (34/40%)	0.001, 0.75 (0.63, 0.89)	0.002, 0.70 (0.55, 0.87)	0.008, −1.67 (0.63)	0.096, −1.50 (0.90)	0.007, −1.01 (0.37)	0.065, −0.98 (0.53)
	HYPEST	35% (36/38%)	0.438, 0.93 (0.78, 1.11)	0.048, 0.79 (0.62, 0.99)	0.348, −0.68 (0.73)	0.42, −0.80 (0.99)	0.033, −0.97 (0.46)	0.017, −1.47 (0.62)
	Combined: KORA S3/S4/ HYPEST		8.27×10^{-6}, 0.78 (0.70, 0.87)	5.30×10^{-8}, 0.67 (0.58, 0.77)	0.003, −1.20 (0.17)	0.007, −1.56 (0.33)	7.50×10^{-5}, −0.98 (0.06)	5.55×10^{-5}, −1.40 (0.12)
Family-based case–control sample	BRIGHT[b]	na (38/40%)	0.082, 0.92 (0.83, 1.01)	0.141, 0.90 (0.78, 1.04)	na	na	na	na
	All combined		1.85×10^{-5}, 0.85 (0.79, 0.92)	1.39×10^{-6}, 0.78 (0.71, 0.86)	na	na	na	na

MAF, minor allele frequency; Add, additive, Dom, dominant genetic models; OR, odds ratio; CI, confidence interval; SE, standard error.
[a]Definition of hypertensive cases and normotensive controls is given in Materials and Methods section.
[b]The BRIGHT sample-included WTCCC severe hypertension cases (n = 1754), and additional BRIGHT cases (n = 493) and controls (n = 1947) genotyped in this study. P-values and OR or effect sizes for HYP and SBP/DBP were calculated using logistic and linear regressions implicated in PLINK (25), respectively. Results were combined using the inverse-variance method under fixed-effects model.

the statistical significance of the association between rs11646213 and DBP ($P = 5.55 \times 10^{-5}$, effect −1.40, SE 0.12, dominant model) as well as HYP ($P = 5.30 \times 10^{-8}$, OR = 0.67, 95% CI: 0.58−0.77, dominant model). For both phenotypes, the dominant model had a stronger support compared with the additive effect model (Table 2). Meta-analysis also replicated the initial association between rs11646213 and SBP ($P < 0.008$) (Table 2). Carriers of the minor allele A exhibited lower BP and reduced risk to develop hypertension compared with the carriers of major allele homozygotes. In KORA, carriers of the minor allele A (AA+AT) of rs11646213 were measured in average 2.25 mmHg lower SBP and 1.54 mmHg lower DBP compared with the major allele TT homozygotes (Fig. 3A and B). The 'protective' effect of the minor allele increased after 50 years of age: carriers of the A allele were measured in average 3.24 and 2.08 mmHg lower SBP and DBP, respectively. In HYPEST, the carriers of the A allele have an average 1.72 mmHg lower DBP across all age groups (Fig. 3D).

In addition to rs11646213 (*CDH13*), the meta-analysis across KORA S3, KORA S4 and HYPEST provided evidence for tentative associations with BP for rs1994547 (*TMEM16C*), rs6784190 (*SLITRK3*) and rs9948310, rs506038 (both *KCTD1*) (Supplementary Material, Tables S2–S3). The associations between rs1994547 and HYP ($P = 1.84 \times 10^{-4}$, OR = 1.38) and between rs6784190 and SBP ($P = 3.34 \times 10^{-5}$, effect 1.78) reached $P < 5 \times 10^{-4}$ under the additive model.

Replication in a family based hypertension case–control sample BRIGHT

In BRIGHT, the original sampling design (9) allowed to test association only with HYP, comparing cases from severely hypertensive families matched with normotensive controls. Among the nine SNPs genotyped in stage 3, the rs9948310 (18q11.2) located within the *KCTD1* gene resulted in significant association ($P = 5.70 \times 10^{-4}$; OR = 0.78, additive model) (Supplementary Material, Table S2). However, this locus needs further replication efforts as the effects in KORA S3 ($P = 3.07 \times 10^{-2}$, OR = 1.35) and KORA S4 ($P = 2.71 \times 10^{-2}$; OR = 1.28) were in opposite direction. The SNP rs11646213, which revealed consistent association with BP traits in population-based cohorts KORA S3, KORA S4 and HYPEST, showed also a non-significant trend for association with HYP in the BRIGHT cases from severely hypertensive families ($P = 8.22 \times 10^{-2}$, OR = 0.92, additive model; $P = 1.41 \times 10^{-1}$, OR = 0.90, dominant model).

DISCUSSION

Our genome-wide association screen with BP traits in the KORA S3 cohort identified a common SNP rs11646213 (MAF = 0.41), which revealed association signals with all three BP traits (HYP $P = 2.34 \times 10^{-6}$; DBP $P = 0.0034$; SBP $P = 0.02$, dominant model) (Table 2). This SNP was ranked among the top loci in GWAS ($P < 5 \times 10^{-6}$). Associations with HYP and DBP were replicated in two further population-based cohort samples, representing western
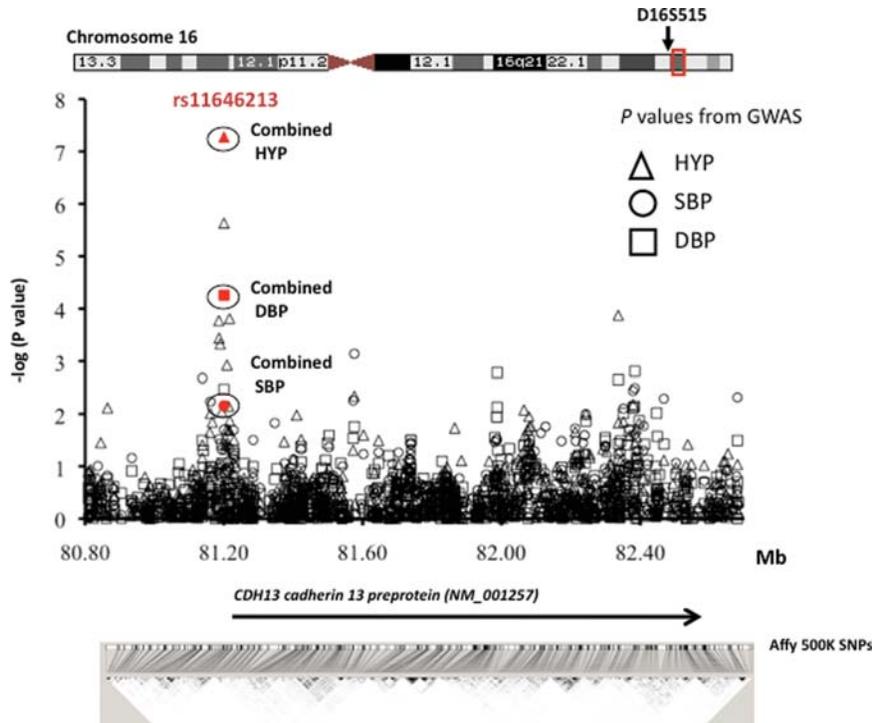
Figure 2. Genomic region of the rs11646213 associated with BP traits at 16q23.3. The upper part of the panel shows the chromosomal location of the SNP along with a flanking microsatellite D16S515 reported to be linked with BP traits (11,12). The middle part of the panel represents a plot of *P*-values from the genome-wide scan (654 SNPs) in KORA S3 with HYP (open triangle; logistic regression), SBP and DBP (open circle and open square, linear regression), and from the combined analysis of the population-based cohort samples KORA S3 (GWAS), KORA S4 and HYPEST under the dominant genetic model. The *x*-axis represents the genomic position (NCBI build 35) and the *y*-axis shows $-\log_{10}$ (*P*-value). The lower part of the panel shows the gene content and linkage disequilibrium (LD) pattern (pairwise r^2; KORA S3) of the genomic region. HYP, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; GWAS, genome-wide association study.

(Germans, KORA S4) and eastern Europeans (Estonians, HYPEST). In addition, a trend for association was observed with severe family based hypertension in the British population (BRIGHT).

The SNP rs11646213 is located 17.9 kb upstream of the large *cadherin 13 preprotein* (*CDH13*) gene (1.2 Mb, 14 exons; Fig. 2). Although the association of the upstream region of *CDH13* with BP was not ultimately confirmed in all sample sets of this study, the available published data on this gene and its functions warrant further examination. Independent evidence of the involvement of *CDH13* in BP regulation comes from GWAS conducted in another population-based cohort, Framingham Heart Study using Affy100K Gene Chip. In particular, the SNP rs3096277, located within *CDH13* intron 11 (1.12 Mb from rs11646213), showed association *P*-values of $P = 9.90 \times 10^{-8}$ and $P = 1.40 \times 10^{-4}$ with long-range SBP and DBP, respectively (10). Furthermore, rs254340 located in the vicinity of rs3096277 (29.9 kb) showed a borderline association with SBP in the HYPEST sample ($P = 0.076$) (Supplementary Material, Table S3). Consistent with the evidence of these two GWAS, the genomic region 16q23.1 has been also linked with BP traits in linkage scans (Fig. 2) (11,12).

Although the detected variant explains a small fraction of the total variation in BP level, the identified gene adapts well to the BP biology. *CDH13* encodes for a calcium-dependent cell–cell adhesion glycoprotein T-cadherin that has the following

properties. It is predominantly expressed in nervous and cardiovascular system, with the highest expression observed in the aorta, the arteries and in the heart (13). T-cadherin interacts in vascular endothelial and smooth muscle cells with two different ligands, which both play an important role in cardiovascular physiology, low-density lipoproteins (14) and adiponectins (15). It has been implicated as a regulator of vascular wall remodeling and angiogenesis, and is up-regulated in vascular endothelial cells under pathological conditions like within atherosclerotic lesions (13,16). Consistent with the contribution of T-cadherin in tumor angiogenesis, the *CDH13* gene has been associated with different cancer types (lung, breast and prostate) (17–19). All these features make this gene a promising candidate for BP regulation. Further fine-mapping or re-sequencing experiments will be required to clarify the signal of association and to identify likely causal variants.

The three stages of the study provided supportive evidence for the contribution of the identified locus to BP variability, but the strength of replication varied among samples. This may result from the phenotypic heterogeneity among sample collections due to differences in study design and/or insufficient power to detect the modest associations. Recently, a report by the family blood pressure program suggested the effect of different study designs to explain the non-replicability of the six most-significant SNPs from the WTCCC GWAS of essential hypertension (20). The KORA S3, KORA S4 and HYPEST samples were recruited from
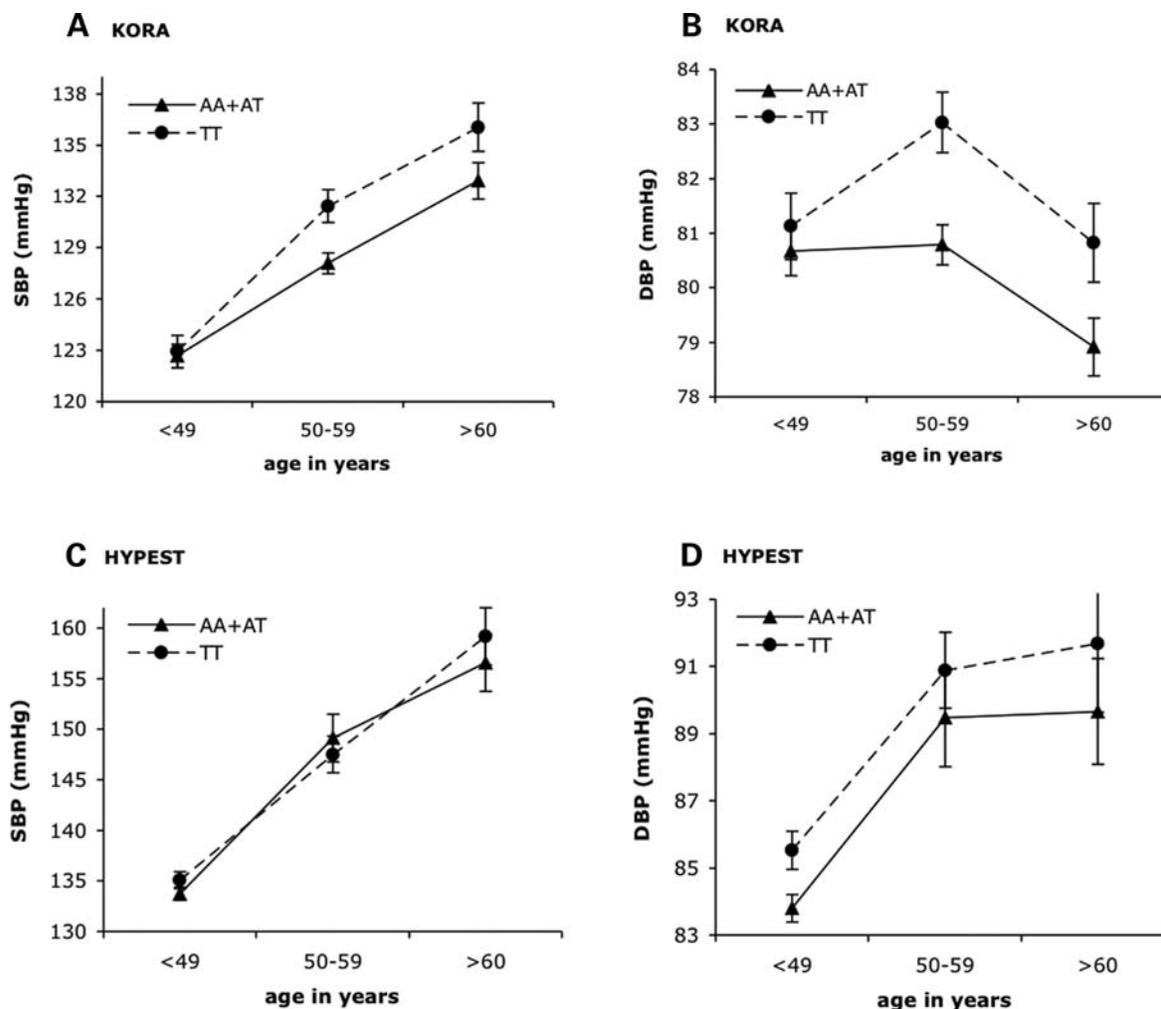
Figure 3. Mean and SEM of SBP (**A** and **C**) and DBP (**B** and **D**) among minor allele carriers and non-carriers of rs11646213 in combined KORA S3 + S4 cohorts (total $n = 2497$) and HYPEST samples (total $n = 1060$) stratified by age. In KORA (A and B), the sample sizes in different age groups ranged from 351 to 739 for minor allele carriers (AA+AT) and from 189 to 367 for major allele homozygotes (TT). In HYPEST (C and D), the sample sizes in different age groups ranged from 58 to 455 for minor allele carriers (AA+AT) and from 53 to 297 for major allele homozygotes (TT).

the general population and have multiple BP records available over different periods of time. Thus, close phenotypic criteria could be used for defining samples entering association analysis with SBP and DBP, as well as cases and controls in the hypertension study. The MRC BRIGHT study provides a resource of severely hypertensive families with a diagnosis of hypertension (9). The combination of inheritance factors responsible for familiar clustering of hypertension in the British population may differ from the palette of genetic variants contributing to BP regulation in the general populations of Germany and Estonia. Also, population-specific genetic variation may play a role in determining the power of the association study. For example, the rs11646213 highlighted in this study had lower MAF in the Estonian population compared with the German population (Table 2), possibly weakening the power of the analysis with the HYPEST sample. Despite the positive replication signals in two populations and a trend for association in the third, we are aware of the tentative nature of the detected association. One also has to

keep in mind alternative scenarios that the discovery samples may have overestimated the effect size or the detected significant associations in study populations may still be by chance alone.

In summary, our study provides evidence that GWAS scan has a potential to identify novel genetic components contributing to a complex multifactorial trait such as BP. However, it highlights the importance to take into account the potential heterogeneity in study design among discovery and replication samples. In addition, population-specific gene variants and/or gene–environment interactions may have a contribution in increasing or reducing the risk to develop hypertension. Thus, the ideal replication samples should be recruited from a genetically close population, practicing similar traditional life-style as the original study population. The identification of a novel susceptibility locus *CDH13* contributing to BP determination encourages further research to clarify the functional basis of the identified association.

MATERIALS AND METHODS

Subjects and study design

An overview of the study design is shown in Supplementary Material, Fig. S1. A detailed description of the recruitment of the GWAS sample (KORA S3 cohort) and replication samples KORA S4, HYPEST and BRIGHT is given below and in Supplementary Material. All studies have been approved by the local Ethics Committee and all participants have given informed consent. All participants from the KORA, the HYPEST and the BRIGHT studies are of white European ancestry.

KORA S3 500K and replication sample KORA S4

The subjects in genome-wide (stage 1) and replication (stage 2) analyses were selected from KORA S3 and S4 surveys, respectively. Both represent independent samples of unrelated subjects from the general population from the Augsburg Area (Southern Germany) recruited in 1994–1995 (S3) and 1999–2001 (S4) (8). A fraction of the KORA S3 ($n = 4856$) participants were followed up in 2003–2004 during the KORA F3 survey ($n = 2974$). Subjects who participated in both S3 and F3 surveys ($n = 1644$, ages 25–69) were selected for genotyping on the Affymetrix 500K platform in the framework of the KORA 500K consortium (http://epi.gsf.de/kora-gen/seiten/kora500k_e.php). The standardized examinations applied in both surveys have been described elsewhere (8). The characteristics of both cohorts and determination of BP measurements are given in Supplementary Material. GWAS with SBP and DBP was conducted with BP data from the S3 survey, including only individuals not receiving BP lowering medication ($n = 1017$; Table 1). The distribution of SBP and DBP measurements for the study group in the GWAS is shown in Fig. S3. For case–control analysis, the groups of hypertensives (1) ($n = 364$) consist of: (i) individuals under antihypertensive medication during both S3 and F3 surveys; (ii) untreated subjects with SBP $\geq$ 160 mmHg and/or DBP $\geq$ 100 mmHg (Grade 2 hypertension) in the S3 and F3 surveys and (iii) untreated individuals with SBP $\geq$ 140 mmHg (Grade 1 hypertension) in S3 that developed 10 years later to Grade 2 or severe hypertension (F3 survey). Control subjects ($n = 596$) were selected to have optimal ($<$120/80 mmHg) or normal ($<$130/85 mmHg) BP measured during both S3 (original) and F3 (10 years later) surveys, and had never been prescribed antihypertensive medication. We excluded individuals with diabetes and/or with obesity (BMI $>$ 30 kg/m^2). In replication stage 2, we selected the individuals from KORA S4 survey (full cohort $n = 4261$) that satisfied the same phenotype criteria as in GWAS with S3 ($n = 1830$; Table 1). A total of 1551 subjects entered the association analysis with SBP and DBP. Case–control association analysis (HYP) was performed with 447 hypertensives/1119 normotensives.

HYPEST replication sample

The Estonian sample consists of unrelated subjects recruited between 2004 and 2007 across Estonia in the framework of the HYPEST sample collection (1823 individuals; recruitment

details in Supplementary Material). The aim of HYPEST study is to find hypertension risk factors in the Estonian population. The recruited individuals have detailed epidemiological data and a documented history of multiple SBP and DBP readings (on average of 4.31 readings per individual) during mean 3.17 years. The selection of HYPEST individuals into the replication stage followed as close as possible the phenotype criteria used for KORA S3 and KORA S4 subjects. Association analysis with SBP and DBP was performed using 1097 untreated individuals derived from the population-based cohort consisting of long-term blood donors across Estonia (Table 1). The distribution of BP values in the study group is shown in Supplementary Material, Fig. S3. For binary analysis with hypertension (HYP), cases ($n = 596$) were defined as subjects with either BP readings $\geq$160/100 mmHg based on the median of several measurements or receiving antihypertensive therapy. Normotensive controls ($n = 650$; BP $<$130/85 mmHg based on the mean of two independent readings across mean 4.1 years or $<$140/90 mmHg based on the mean of more than or equal to three readings) were selected from the population-based HYPEST cohort among the subjects who have never been prescribed antihypertensive treatment.

BRIGHT replication sample

The MRC British Genetics of Hypertension (BRIGHT) case–control samples have been recruited across UK (http://www.brightstudy.ac.uk). Case ascertainment and phenotyping has been described elsewhere (9). Briefly, cases originated from severely hypertensive families (1700 sib-pairs and 800 families collected for transmission disequilibrium test) were defined as patients under antihypertensive treatment and with BP readings $\geq$150/100 mmHg based on one reading or $\geq$145/95 mmHg based on the mean of three readings. Healthy normotensive controls ($n = 2000$; BP $<$140/90 mmHg, no antihypertensive medication and no diagnosed diseases) were recruited by matching age, sex and geographical distribution across UK. In replication stage 3b, 493 unrelated BRIGHT cases and 2000 controls were genotyped. For association analysis with HYP, we also included BRIGHT hypertensive cases genotyped in the framework of the WTCCC (7). The association analysis (cases $n = 2401$/controls $n = 1969$) was performed with subjects with BMI $\leq$ 35 and no diabetes (Table 1). Since BRIGHT cases (severe hypertension; all subjects treated with antihypertension medication) were collected as extremes, we were unable to test associations with SBP and DBP.

KORA S3 genotyping and SNP quality

Genotyping for KORA S3 500K was performed using the Affymetrix Gene Chip Human Mapping 500K Array Set consisting of two chips (Sty I and Nsp I). Genotyping experiments were carried out according to the manufacturer's instructions, and the genotypes were determined using BRLMM clustering algorithm. Genotyping laboratory experiments, call of genotypes and genotyping quality control are described in detail elsewhere (http://epi.gsf.de/kora-gen/seiten/kora500k_e.php). We excluded SNPs with signals of unreliable genotyping

quality (call rate $<93\%$), deviation from HWE ($P < 0.001$) and rare SNPs (MAF $< 1\%$). Exclusion details are given in Supplementary Material. The number of autosomal SNPs entering the statistical analysis was 395 912.

SNP selection for replication

SNPs selected to be genotyped in the replication stage 2 (in total $n = 77$; Supplementary Material, Tables S2–S4) satisfied one of the following conditions: (i) SNPs with the strongest evidence for association ($n = 4$; P-value $< 10^{-6}$); (ii) SNPs with P-value $< 5.5 \times 10^{-5}$ from regions revealing multiple associations ($n = 19$) and/or near reported linkage peaks ($n = 15$) or (iii) exonic SNPs ($n = 4$; P-value $< 10^{-3}$); (iv) significant SNPs exhibiting overlapping association signals with SBP, DBP and HYP and/or close to potential BP candidate genes ($n = 33$; P-value $< 10^{-2}$). Top SNPs located within or in the vicinity (± 100 kb) of a known copy number variation regions ($n = 18$; Supplementary Material, Table S1) were not followed up due to the inability to distinguish between true and spurious associations.

In stage 3, we selected SNPs, which reached significant ($n = 6$ SNPs; $P < 0.05$) or borderline P-values ($n = 1$; $P < 0.07$), in association tests with SBP, DBP or HYP in stage 2 (Supplementary Material, Tables S2 and S3). In addition, we carried on two SNPs within the *CDH13* gene, rs3784990 and rs254340. Despite negative replication in KORA S4, these SNPs were considered for further testing in other populations as they are flanking rs3096277 with prior evidence for association with BP from Framinghm Heart Study 100K GWAS (10). In GWAS sample KORA S3, the marker rs3784990 was among top SNPs (HYP, additive model, $P = 2.21 \times 10^{-5}$) and rs254340 showed association signals with SBP and DBP ($P < 9 \times 10^{-3}$) (Supplementary Material, Table S1).

SNP genotyping in the replication samples

Stage 2 genotyping (KORA S4 cohort, $n = 1830$) of 74 SNPs was performed using the iPLEX assay (Sequenom; primers are given in Supplementary Material, Table S5). Three SNPs (rs11646213, rs254340 and rs3784990) with insufficient performance of iPLEX assay were genotyped using 5′-nuclease allelic discrimination (Taqman) assay (Applied Biosystems). Stage 3 genotyping was performed using the KASPar chemistry, a competitive allele-specific PCR SNP genotyping system using fluorescence resonance energy transfer quencher cassette oligos (the Genotyping Unit of the William Harvey Research Institute; call rate $>97\%$). All SNPs genotyped were in HWE, and overall genotyping call rate was $>97\%$ for HYPEST and $>95\%$ for BRIGHT samples (Supplementary Material, Table S6). Replication experiments are detailed in Supplementary Material.

Statistical analysis of genetic effects

The possible effect of population stratification in the KORA S3 500K sample was assessed using the EIGENSOFT software (21,22) as described (23). A principal component analysis showed a negligible effect of population stratification (Fig. 1; Supplementary Material, Fig. S2). The genomic control analysis (24) led to an inflation factor (λ) of 1.02 and 1.05 for quantitative (SBP) and case–control (HYP) phenotypes, respectively.

The significance of the locus-trait associations was tested, and odds ratios/effect sizes and confidence interval were obtained by fitting linear (with SBP and DBP) and logistic (with HYP) regressions implemented in the PLINK software (25). All analyses were adjusted for age and sex. In all study stages, the association analyses were performed under additive and dominant genetic models. Additive genetic models assume a trend per copy of the minor allele to contribute to SBP and DBP measurements on genotype categories, whereas dominant genetic models assume that heterozygotes have the same increased risk as minor homozygous genotypes. We considered the Bonferroni ($P = 0.05/395912 = 1.26 \times 10^{-7}$) threshold that takes into account that multiple hypotheses test are being performed. In addition, we estimated the effective number of independent test performed genome-wide as the number of tagging SNPs. The tagging SNPs were selected using Carlson *et al.* (26) method implemented using in-house 'perl' code (threshold $r^2 > 0.4$). The estimated number of effective tests was 141 870, which gives the significance level after Bonferroni correction of $P = 3.52 \times 10^{-7}$ ($P = 0.05/141\,870$). LD structure of the highlighted regions was visualized using HAPLOVIEW (27). Results were combined in a meta-analysis using the inverse-variance method under fixed-effects models using the R software.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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