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The Cardiovascular Risk in Young Finns Study

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Abstract—Clinical relevance of a genetic predisposition to elevated blood pressure was quantified during the transition from childhood to adulthood in a population-based Finnish cohort (N=2357). Blood pressure was measured at baseline in 1980 (age 3–18 years) and in follow-ups in 1983, 1986, 2001, and 2007. Thirteen single nucleotide polymorphisms associated with blood pressure were genotyped, and 3 genetic risk scores associated with systolic and diastolic blood pressures and their combination were derived for all of the participants. Effects of the genetic risk score were 0.47 mm Hg for systolic and 0.53 mm Hg for diastolic blood pressures (both $P<0.01$). The combination genetic risk score was associated with diastolic blood pressure from age 9 years onward ($\beta=0.68$ mm Hg; $P=0.015$). Replications in 1194 participants of the Bogalusa Heart Study showed essentially similar results. The participants in the highest quintile of the combination genetic risk score had a 1.82-fold risk of hypertension in adulthood ($P<0.0001$) compared with the lowest quintile, independent of a family history of premature hypertension. These findings show that genetic variants are associated with preclinical blood pressure traits in childhood; individuals with several susceptibility alleles have, on average, a 0.5-mm Hg higher blood pressure, and this trajectory continues from childhood to adulthood. **(*Hypertension*. 2011;58:1079-1085.) ● Online Data Supplement**

Key Words: epidemiological study ■ genetic risk score ■ blood pressure ■ cardiovascular disease

High blood pressure (BP), especially systolic BP, increases the risk of heart disease and stroke by affecting arterial structure and function in adults. Raised BP is the cause of ≈13% of total mortality and is responsible for >7.5 million deaths annually worldwide.[1] Atherosclerosis begins in early childhood, and elevated BP is an important risk factor in the pathogenesis of atherosclerosis and cardiovascular events.[2–5] Globally, childhood hypertension is an expanding health issue and is largely attributed to the obesity epidemic, as well as high dietary salt intake.[6] Genetic factors are clearly implicated in the pathogenesis of elevated BP, because the heritability of BP and hypertension has been estimated to be 31% to 68%.[7] Genome-wide association studies (GWASs) have revealed several variants associated with adult BP, supporting the idea that BP is a complex polygenic trait.[8–11] Our aim was to examine whether the genetic variants known to be associated with BP levels or hypertension in adults are also associated with higher systolic and diastolic BPs in children and early adolescence, thus exposing the carriers to lifelong effects of elevated BP. To this end, we identified 13 common single-nucleotide polymorphisms (SNPs) independently associated with BP levels in recently published

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GWAS.[12,13] We used BP and lifestyle data from the Young Finns Study, a randomly selected population of Finns with a long clinical follow-up beginning in childhood and adolescence.[5,14] The analyses were replicated in the multiethnic population of the Bogalusa Heart Study with long clinical follow-up data starting from early childhood.[15]

Methods

Study Cohort and Clinical Measurements

The participants were 2357 persons (1234 women and 1123 men), all ethnically homogenous Finns, participating in the ongoing population-based cohort follow-up study, the Cardiovascular Risk in Young Finns. The first cross-sectional survey was conducted in 1980 in 5 Finnish cities (Helsinki, Kuopio, Oulu, Tampere, and Turku). Participants were randomly selected from the cities and their rural vicinities to form 6 cohorts aged 3, 6, 9, 12, 15, and 18 years in 1980. Follow-up studies of the cohorts were carried out in 1983, 1986, 2001, and 2007. The study was approved by an institutional ethics committee, and the subjects gave an informed consent. Details of the study protocol and population have been published previously.[5,14,15] Women who were pregnant in 2001 or 2007 were excluded from the present study.

BP, weight, and height measurements were made according to a standardized protocol.[14–16] Detailed methods regarding BP measurements are given in the online Data Supplement (please see http://hyper.ahajournals.org). Body mass index (BMI) was calculated as participant weight in kilograms divided by the square of height in meters. In adulthood, data on antihypertensive treatment, a family history of premature hypertension (before the age of 55 years in either parent), current smoking status (regular cigarette smoking on a weekly basis or more often or nonsmoker), and alcohol use (in standard drinks per day) were obtained via a self-administered questionnaire in 2001 and 2007. Dietary sodium intake was assessed in 2007 using a modified 131-item food frequency questionnaire developed by the Finnish National Institute for Health and Welfare.[17] Pediatric hypertension for subjects between the ages of 3 and 15 years was defined according to the 95th percentile thresholds for systolic and diastolic BPs (International Pediatric Hypertension Association 2006). A binary variable for prevalent hypertension in adulthood (age ≥18 years) was defined as the use of antihypertensive medication or systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg in 2007. We applied a substitution method for those subjects who were under antihypertensive treatment in 2001 (N=58) or 2007 (N=143) by adding 15 mm Hg to the systolic and 10 mm Hg to the diastolic BP measured in 2001 or 2007.[18]

Genotyping and Calculation of the Genetic Risk Scores

Common genetic variants associated with BP levels were identified from recently published GWASs.[10,11] To ascertain that there were no interactions between the SNPs in the genetic risk scores (GRSs), the SNPs were first paired according to the highest linkage disequilibrium values, and from each pair the SNP was chosen that had the lowest *P* value in the original GWASs. Finally, we chose 13 BP-associated SNPs that contributed independently to BP (please see Table S1, in the online Data Supplement, at http://hyper.ahajournals.org). Whole-genome SNP data of the study subjects were obtained using an Illumina BeadChip Human 670K including 546 677 SNPs. We included all of the 2357 subjects with complete data on the 13 SNPs. All of the SNPs were in Hardy-Weinberg equilibrium. The allele count for the 5 directly genotyped SNPs was coded 0/1/2, and the expected allele count for the 8 imputed SNPs ranged from 0.0 to 2.0. The GRSs were calculated for each individual as weighted sums of allele count (expected or real), weighted with the effect sizes from reported GWASs.[12,13] Weighted GRS model is an approach for evaluating multiple genetic markers simultaneously in association testing for clinical phenotypes. Aggregation of polygenic information in a GRS weighted by the estimated effect of each genetic marker on the risk phenotype is an effective means to encapsulate risk-associated genetic information.[19,20] To explore additive effects of the loci associated more strongly with systolic or diastolic BPs in the discovery studies, 3 different GRSs were derived, the combination GRS from all 13 of the BP-associated alleles, an 8-SNP diastolic GRS (rs16998073, rs1530440, rs3184504, rs1378942, rs16948048, rs9815354, rs11014166, and rs2384550) that associated more strongly with diastolic BP, and a 5-SNP systolic GRS (rs17367504, rs11191548, rs12946454, rs381815, and rs2681492). Subjects were divided into 5 groups according to quintiles of the combination GRS. The combination GRS had a normal distribution in the Young Finns population (please see Figure S1 in the online Data Supplement).

Replication Study

The analyses were replicated in the Bogalusa Heart Study, an independent follow-up study with existing knowledge of the genetic composition of the participants and BP measurements starting from childhood. Details of the project have been described elsewhere.[15] Briefly, between 1973 and 2010, 9 cross-sectional surveys of children aged 4 to 17 years and 10 cross-sectional surveys of adults aged 18 to 50 years, examined previously as children, were conducted in Bogalusa, LA. This panel design of repeated cross-sectional examinations has resulted in serial observations from childhood to adulthood, with 3929 individuals (2374 of European ancestry and 1555 of black ancestry) having 4 to 16 serial measurements.

Statistical Methods

The primary phenotype of interest was the longitudinal trend in BP from 1980 through 1983, 1986, 2001, and 2007. The GRSs were standardized (mean=0; SD=1), and the effect estimates (β) indicate the change in BP in millimeters of mercury per a 1-SD change in GRS. Models were adjusted for age, sex, and BMI. The longitudinal mixed models were adjusted for each study year separately to account for a secular trend in BP and the change in measurement device. The longitudinal models for systolic BP included a term for age squared, because the association with age was nonlinear. Repeated measurements from same persons are likely to be autocorrelated, which must be taken into account using appropriate longitudinal data analysis methods. The strength of autocorrelation is assumed to be inversely associated with time between measurements. In the longitudinal analysis, we took into account the various time intervals between measurements (3 years between 1980, 1983, and 1986; 15 years between 1986 and 2001; and 6 years between 2001 and 2007) by using a continuous autoregressive covariance structure.

Using the same analysis methods and a first-order autoregressive covariance structure in the longitudinal models, we tested whether the GRS was associated with BP in the Bogalusa Study. All of the SNPs included in the original analyses were available in the Bogalusa Heart Study population of European ancestry, but SNPs rs3184504 and rs1378942 were not available for the black population, so that the racial groups were analyzed separately. Imputed genotype dosages were used when direct genotype data were not available. The participants of the Bogalusa Heart Study were divided into 5 age groups with equal numbers of participants. Only individuals with complete allelic information of the SNPs in the GRSs were included, and the final population from the Bogalusa Heart Study included 1194 individuals (826 European and 368 black).

As secondary analyses, we performed cross-sectional linear regression analyses at baseline and at the 2007 follow-up for all of the GRSs and the individual SNPs. Because the analyses of selected individual SNPs are of an exploratory nature, we present nominal *P* values without correction for multiple testing. Age-stratified linear regression analyses were performed to ascertain the age when a genetic effect was first detectable. The effects of the GRSs and the SNPs on the risk of hypertension in adulthood were studied with logistic regression. We analyzed the hypertension risk for the GRSs, for quintiles of the combination GRS (assuming a linear effect across quintiles of the combination GRS), and for the individual SNPs adjusting for the family history of premature hypertension. We evaluated the predicted probability of adult hypertension with 2

Table 1. Age-Specific Mean Systolic and Diastolic BPs and BMI and Sizes of the Different Age Groups

	Age Group, y														
Variable	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45
Systolic BP, mm Hg	104	107	108	110	117	121	123	119	115	118	117	118	120	123	124
Diastolic BP, mm Hg	NA	64	64	64	67	70	71	70	68	72	72	74	75	78	78
BMI, kg/m^2	15.6	15.5	16.7	18.3	20.2	21.4	22.2	23.2	24.5	25.2	25.4	25.7	26.2	26.2	26.6
Subjects with systolic BP data, N	342	710	1086	1100	1119	988	528	531	316	637	662	684	680	364	314
Subjects with diastolic BP data, N	0	704	1077	1096	1112	987	527	530	316	636	662	684	680	364	314

Total No. of measurements was 11 785; total No. of subjects was 2357. NA indicates not available; BP, blood pressure; BMI, body mass index.

logistic regression models. One was fitted with the GRS, age, sex, and BMI and the other with only age, sex, and BMI. We calculated the areas under the receiver operating characteristic curves and compared their difference by using the C statistics.[21] Finally, we studied to what extent sex and dietary salt intake could modify the results by including the interaction terms "sex*genetic variable" and "sodium intake*genetic variable" in the models for adult hypertension and systolic and diastolic BPs in 2007. The statistical analyses were performed with R version 2.11.1 and SAS version 9.2.

Results

At baseline in 1980, the median and interquartile range (IQR) for systolic BP were 112 and 15, respectively; diastolic BP median was 69 (IQR=13), and BMI median was 17.3 (IQR=4.4), and at the 2007 follow-up, the median and IQR for systolic BP were 120 and 20, respectively; diastolic BP median was 76 (IQR=15), and BMI median was 25.3 (IQR=5.7). In 2007, the smoking prevalence was 19% and average alcohol use was 1 drink per day. There were no differences in 2001 or 2007 smoking prevalence or alcohol consumption according to the GRS, and smoking and alcohol use were omitted from additional analyses. The age-specific average BP and BMI and the sizes of the age groups are described in Table 1.

The GRSs were significantly associated with BP both in the longitudinal analyses spanning from childhood and adolescence to adult age and in the cross-sectional analyses at baseline and at 2007 (Table 2). In the longitudinal analyses, systolic BP was associated with the combination GRS (β=0.47 mm Hg; P=0.008) and diastolic BP with both the combination GRS (β=0.53 mm Hg; P=0.0003) and the diastolic GRS (β=0.50 mm Hg; P=0.0005). The effect sizes were 0.39 to 0.49 mm Hg per 1-SD change in the GRS, explaining 0.1% to 0.2% of the variation in BP. In the cross-sectional analyses, the combination GRS explained

Table 2. Longitudinal and Cross-Sectional Effects of the GRSs on Blood Pressure Traits (in Millimeters of Mercury per a 1-SD Change in GRS) and the Longitudinal OR of Adult Hypertension Between the Extreme Quintiles of the GRS and Replications in Bogalusa Data

	Longitudinal Effect From 1980 Through 2007*		Replication in Bogalusa: Longitudinal Effect				Cross-Sectional Effect at Baseline in 1980		Cross-Sectional Effect at 2007		Longitudinal OR for Hypertension in Adulthood†	Replication in Bogalusa: OR for Hypertension in Adulthood	
			European Ancestry		Black Ancestry								
GRS	Systolic BP, mm Hg§	Diastolic BP, mm Hg	Systolic BP, mm Hg	Diastolic BP, mm Hg	Systolic BP, mm Hg	Diastolic BP, mm Hg	Systolic BP, mm Hg	Diastolic BP, mm Hg	Systolic BP, mm Hg	Diastolic BP, mm Hg		European Ancestry	Black Ancestry
13-SNP Combination GRS‡	β=0.47 P=0.008‖	β=0.53 P=0.0003	β=0.18 P=0.30	β=0.01 P=0.94	β=1.22 P=0.002	β=0.78 P=0.005	β=0.11 P=0.61	β=0.35 P=0.10	β=1.0 P=0.0006	β=0.76 P=0.002	OR=1.82, CI=1.53–2.17, P<0.0001	OR=0.95, CI=0.80–1.13, P=0.57	OR=1.10, CI=0.87–1.40, P=0.44
5-SNP Systolic BP GRS	β=0.21 P=0.23		β=0.42 P=0.048		β=1.02 P=0.09		β=0.08 P=0.69	β=0.08 P=0.70	β=0.71 P=0.02	β=0.38 P=0.12	OR=1.40, CI=1.18–1.64, P=0.001	OR=0.965, CI=0.78–1.20, P=0.75	OR=0.889, CI=0.61–1.29, P=0.54
8-SNP Diastolic BP GRS		β=0.50 P=0.0005		β=0.10 P=0.60		β=1.76 P=0.002	β=0.07 P=0.74	β=0.37 P=0.08	β=0.78 P=0.01	β=0.65 P=0.008	OR=1.25, CI=1.06–1.49, P=0.03	OR=0.94, CI=0.72- 1.23, P=0.64	OR=1.84, CI=1.13–3.02 P=0.02

GRS indicates genetic risk score; SNP, single nucleotide polymorphism; BP, blood pressure; OR, odds ratio; BMI, body mass index.

*Longitudinal models adjusted for measurement year, age (and age squared for systolic blood pressure), sex, and BMI; cross-sectional and logistic models adjusted for age, sex, BMI, and family history.

†OR indicates odds ratio between the extreme quintiles of the GRS.

‡SNP indicates single nucleotide polymorphism. The SNPs in each GRS were as follows: rs16998073, rs1530440, rs3184504, rs1378942, rs16948048, rs9815354, rs11014166, rs2384550, rs17367504, rs11191548, rs12946454, rs38181, and rs2681492 in the 13-SNP combination GRS; rs17367504, rs11191548, rs12946454, rs381815, and rs2681492 in the 5-SNP systolic BP GRS and rs16998073, rs1530440, rs3184504, rs1378942, rs16948048, rs9815354, rs11014166, and rs2384550 in the 8-SNP diastolic BP GRS. SNPs rs3184504 and rs1378942 were not available for the black population of the Bogalusa Heart Study.

§BP indicates blood pressure, a substitution method was used for subjects under antihypertensive treatment in 2001 (N=58) or 2007 (N=143) by adding 15 mm Hg to the systolic and 10 mm Hg to the diastolic BP measured in 2001 or 2007.

‖β indicates effect estimate (in millimeters of mercury per a 1-SD change in the GRS).

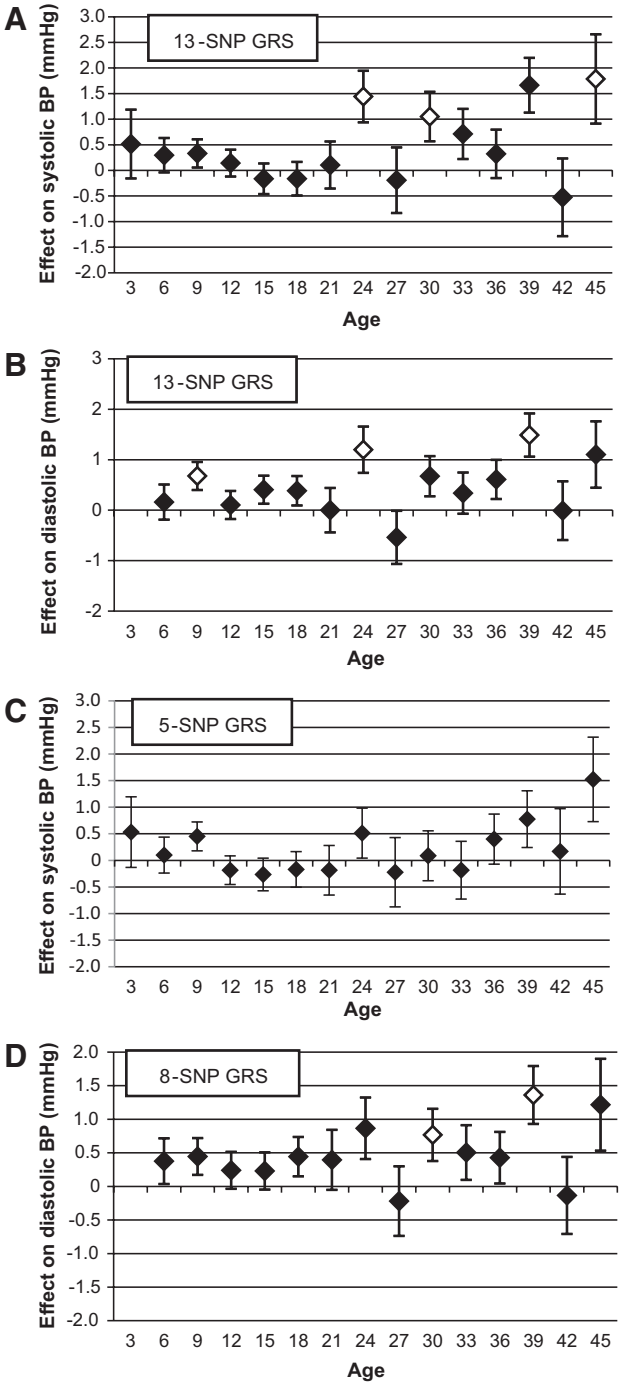

Figure 1. Effects of the genetic risk scores (GRSs) on blood pressure traits (in millimeters of mercury per a 1-SD change in the GRS) in all of the age groups. Open symbols denote linear regression; $P<0.05$. Error bars denote SE. Diastolic blood pressure was measured from age 6 years onward. Effect of (**A**) the combination GRS on systolic blood pressure (BP); **B**, the combination GRS on diastolic BP; **C**, the systolic GRS on systolic BP; and **D**, the diastolic GRS on diastolic BP.

0.5% of the variation in diastolic BP in the age group of 9-year olds. The individual SNPs rs16948048, rs11014166, and rs11191548 were also associated with BP traits in the longitudinal models (please see Figure S2).

In age-stratified analyses, the combination GRS was significantly associated with diastolic BP at the age of 9 years

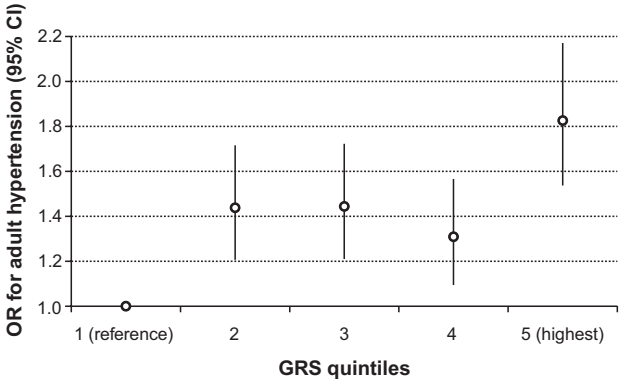

Figure 2. Odds ratio (OR) and 95% CI of adult hypertension in the quintiles of the combination genetic risk score (GRS) in a logistic regression model adjusted for age, sex, and body mass index (BMI). The ranges of the quintiles of the weighted combination GRS were 1=less than −0.01023 (lowest quintile, reference), 2=−0.01023 to −0.00597, 3=−0.00598 to −0.00272, 4=−0.00273 to 0.00104, and 5=>0.00105 (highest).

(β=0.68 mm Hg; P=0.02; Figure 1), and the SNPs rs11014166, rs16948048, and rs11191548 had the most significant effects (please see Table S2). The cross-sectional effects on BP at baseline and the 2007 follow-up, as well as the odds ratio (OR) for adult hypertension for all of the GRSs, are presented in Table 2 (for the individual SNPs, please see Table S2).

The GRSs were independent risk factors for hypertension in adulthood, even when adjusted for a family history of premature hypertension (Table 2). There was a significant difference in adulthood between the extreme quintiles of the combination GRS in systolic BP (119 mm Hg in the lowest and 123 mm Hg in the highest quintile; P=0.0008) and diastolic BP (76 and 78 mm Hg; P=0.003).

In age-, sex-, and BMI-adjusted logistic regression, the risk of hypertension in adulthood was significantly higher in the highest quintile compared with the lowest (OR: 1.82 [95% CI=1.53–2.16]; $P<0.0001$). To avoid overstating effect sizes associated with a specified subject category, we also compared the highest quintile with the quintile containing the mean value of GRS of the population. In such a comparison, the risk of hypertension remained elevated in the highest quintile (OR: 1.44 [95% CI: 1.20–1.71]; $P<0.0001$; Figure 2). The proportions of normotensive subjects in the highest and lowest quintiles of the combination GRS from childhood and adolescence through adulthood are shown in the online Data Supplement (please see Figure S3). To explore the discriminative power of the combination GRS, we compared receiver operating characteristic curves for 2 models. Model 1 included age, sex, and BMI, and model 2 also included the combination GRS (please see Figure S4). The model that included the combination GRS had a nonsignificantly higher area under the receiver operating characteristic curve value than the model that included only age, sex, and BMI (areas under the receiver operating characteristic curves: 0.72 versus 0.71; P=0.33).

We tested the possibility of a sex difference by including an SNP*sex or GRS*sex interaction term in the models. There was a significant difference between men and women for the SNP rs11191548 (SNP*sex: P=0.005). In sex-stratified regression analysis, the effect estimate for women

was 0.9 mm Hg per risk allele (P=0.003). In men, the effect was not significant (β=−0.7; P=0.13). For the GRSs or other individual SNPs with significant effects we did not find evidence for sex differences. Similarly, the dietary sodium intake measured in 2007 did not modify the relation between BP/hypertension and individual SNPs of GRSs (interaction term P values always P>0.5).

In the longitudinal replication analyses with the Bogalusa Heart Study participants, the combination GRS was directly and significantly associated with systolic and diastolic BPs in blacks (β=1.22 mm Hg, P=0.002 and β=0.78 mm Hg, P=0.005, respectively) and nonsignificantly in Europeans (β=0.18, P=0.30 and β=0.01, P=0.94; Table 2). Systolic GRS was directly associated with systolic BP both in blacks (β=1.02; P=0.09) and Europeans (β=0.42 mm Hg; P=0.048; Table 2). Diastolic GRS was directly associated with diastolic BP in blacks (β=1.76 mm Hg; P=0.002) but not in Europeans (β=−0.1; P=0.60; Table 2). The effects of individual SNPs in Bogalusa data are shown in Table S2. Among the SNP effects, 12 of 13 markers in Europeans of the Bogalusa Heart Study showed the same direction of effect on diastolic and 10 of 13 showed the same direction of effect on systolic BP as in the Young Finns Study. The age-stratified associations of the GRSs in the Bogalusa data are presented in Figure S7. In blacks, the diastolic GRS predicted hypertension in adulthood (OR: 1.84 [95% CI=1.13–3.02]; P=0.02; Table 2).

Discussion

These longitudinal data demonstrate that the combination GRS associated with higher BP levels in youth and early adulthood. Individuals with the highest combination GRS had significantly higher diastolic BP at the age of 9 years, and the effect was persistent from childhood through adult age. Common variants generally have a small effect size, but in adults an effect size of 1 mm Hg could translate to a 10% higher mortality risk.[22] The original GWASs were conducted in older populations, and the extent to which their findings can be validated in the early age groups has not been clarified. Therefore, we conducted individual replications for the effects on BP of the GRSs and each of the 13 SNPs in young individuals between 3 and 18 years of age. In older adults, systolic BP may be directly associated with cardiovascular risk and mortality,[23] but in a recent analysis of BP data collected from Swedish conscripts, elevated diastolic BP in late adolescence contributed more to subsequent mortality in middle age compared with systolic BP.[24]

Thanassoulis and Vasan[25] regarded family history as the best marker for the genetic risk of complex traits. In our cohort, individuals with family history of premature hypertension had 2.46-fold increased odds for hypertension (P<0.0001), demonstrating the importance of both lifestyle and unknown gene-environment interactions. In our study, the GRSs were independent risk factors for hypertension in adulthood, even when adjusted for family history of premature hypertension. A similar finding was reported recently for GRS based on coronary heart disease loci.[26] These results show that, although effects of individual SNPs are small, they jointly add precision to the risk profiling of individuals over family history.

Several important genomic regions are connected to the SNPs used for calculation of the GRSs in this study. The individual SNPs with nominally significant effect sizes in this study were rs11191548, rs16948048, rs17367504, rs9815354, and rs2681492. In secondary analyses, 2 SNPs, rs11014166 (OR: 1.21 [95% CI=1.01–1.45]; P=0.04) and rs11191548 (OR: 1.39 [95% CI=1.01–1.93]; P=0.046) were associated with an increased risk of adult hypertension. We also found a sex difference in the effect of the rs11191548, where the effect estimate was 0.9 mm Hg per risk allele in women but there was no significant effect in men in this sample. The rs11191548 is located near the gene *CYP17A1* encoding cytosolic purine 5′-nucleotidase, an enzyme in the cytochrome P450 family involved in catalyzing the synthesis of cholesterol, steroids, and other lipids. However, this locus contains many genes other than *CYP17A1,* such as the *CYP17* gene, where mutations cause congenital adrenal hyperplasia, a rare (mendelian) cause of hypertension. Little else is known about how this locus might influence BP in humans. Therefore, we calculated the level of linkage disequilibrium of rs11191548 with other SNPs located within *CYP17A1* in our population (please see Table S3). rs11191548 was in strong linkage disequilibrium with 2 other SNPs located within CYP17A1. However, because none of the SNPs in this study have a known functional role and there are no missense variants, no expression quantitative trait loci mapping was available to further support the possible involvement of *CYP17A1*, and, therefore, variants in other nearby genes could underlie the observed association between rs11191548 and adult hypertension.

The SNP rs16948048 was also associated with diastolic BP in women in our study cohort. The exact mechanism by which this variant can increase BP remains as yet unknown. The rs16948048 is a variant of the *ZNF652* gene encoding the zinc finger protein 652, which belongs to a family of transcription-modulating proteins, able to switch genes on and off.[27] Zinc finger proteins are widespread in nature and composed some 3% of the human genome, and because they are uniquely suited to specifically recognizing large sequences of the genome, they are currently a target of intensive research for genetic engineering and gene therapy. Interestingly, the same variant (rs16948048) was associated with a significantly lower risk of hypertension in a recent Chinese Han population-based study by Niu et al,[28] highlighting the need for more research on the pathophysiological mechanisms by which minor genetic differences may act on the variation of BP both between individuals and populations.

The SNP rs17367504 near the gene encoding natriuretic peptide B was significantly associated with BP in this study. Common genetic variants at the *NPPA-NPPB* loci (natriuretic peptide precursor A and B) have been associated previously with interindividual variation in plasma natriuretic peptides and BP.[9] The natriuretic peptides may lower BP by promoting the urinary excretion of sodium or through vasodilation, and they are synthesized in response to high BP or volume overload. The SNPs rs9815354 near the gene *ULK4* (Unc-51-like kinase 4; serine/threonine protein kinase) and rs2681492 near *ATP2B1* (ATPase, Ca^{++} transporting, plasma membrane 1) were also associated with BP traits in the cross-sectional analyses.

A limitation of this study is that the combination of risk alleles was based on statistical analysis, and the biological significance of the genetic variants needs further clarification before assigning a clinical value to a GRS. Not all of the individual SNPs proved to be significantly associated with BP in our data, and the effect of GRS may be "diluted" by the inclusion of SNPs that have little or no effect. Because the GRSs in this study included SNPs that have been discovered in very large international consortia, no correction for multiple testing was performed, and the significance of individual SNPs should be interpreted with caution. Although they were statistically not significant, the smaller effect sizes at ages 27 and 42 years possibly arose from cohort effects. We did not include the potential confounders or effect modifiers, such as smoking or alcohol consumption, in adulthood in this study, but antihypertensive treatment was accounted for by a substitution method. Although the combination GRS was statistically significantly associated with hypertension in multivariable models, it did not significantly improve areas under the receiver operating characteristic curves in addition to age, sex, and BMI, as judged by the C statistics.

Several gene-environment interactions and physiological pathways through which the genetic effects on BP could be mediated are currently under investigation, including salt sensitivity, the renin-angiotensin-aldosterone system, and endothelial dysfunction.[29–32] We had cross-sectional data on sodium intakes collected with food frequency questionnaires in adulthood, but modification of the association between the genetic markers and BP by salt intake was not detectable. However, the used food frequency questionnaires were not designed to precisely capture salt intake or cumulative salt exposure, and with a more valid assessment method, such as repeated 24-hour urine collection, the intake of salt could possibly have been shown to play a more significant role in these analyses.[33]

The disease probability of complex polygenic traits, such as BP, can be studied with whole-genome prediction methods, because more SNPs will probably be recognized in the future.[34–37] Recently, Ho et al[38] used a novel approach combining both GWAS-derived SNPs and a gene expression-guided approach in the identification of a novel locus associated with BP in a large cohort of middle-aged women. Their strategy highlights the importance of identifying biologically functional SNPs and looking for associations beyond the conventional threshold for genome-wide significance ($P<5*10^{-8}$).

Perspectives

The genetic variants had a quantifiable effect on BP traits from an early age, and this was independent of age, sex, and BMI in our randomly selected population-based cohort. The combination GRS was an independent predictor of hypertension in adulthood. Our population consisted entirely of ethnically homogenous Finns, and, therefore, the analyses were repeated in the racially mixed population of the Bogalusa Heart Study. In participants of European ancestry, the relations between GRSs and BP were in the same direction in the Bogalusa data, but somewhat stronger genetic effects were seen among blacks. Based on our findings, population-based public health measures, such as preventive lifestyle advice, BP monitoring, and, if needed, early treatment, could be targeted to high-risk individuals if the genetic risk could be detected more effectively in childhood. However, given the very low proportion of variance in BP explained by these genetic markers, it may well be that population-wide measures, such as reducing the salt content of the food supply, would prove more effective in reducing the burden of disease attributed to high BP.[39] Comparative analyses beyond the replication study included here are needed to assess whether our observations are applicable to other more heterogenous groups of young whites and other ethnicities before genetic testing can be recommended to predict future risk of hypertension.

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Disclosures

None.

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ONLINE SUPPLEMENT

GENETIC VARIANTS AND BLOOD PRESSURE IN A POPULATION-BASED COHORT: THE CARDIOVASCULAR RISK IN YOUNG FINNS STUDY

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Online supplement: Methods: Detailed blood pressure (BP) measurements

BP was measured from the right arm, between 8 and 10 a.m. from fasting subjects in the sitting position after 5 minutes of rest. Three measurements were done at 2-3 min intervals and the average of three measurements from each examination was used as the final value. Readings were made to the nearest 2 mmHg. In individuals who were 3 years old at baseline in 1980, systolic BP was recorded with an ultrasound device (Arteriosonde, Roche) using a cuff size of 5.5*14 cm (1). The 3-year old children were sitting in their mother's lap and the measurement was made only after the child had become relaxed and calm. The ultrasound device was calibrated daily with the mercury gravity sphygmomanometer. Systolic and diastolic BP were measured from age 6 onwards with a standard mercury gravity sphygmomanometer in 1980 and 1983 and with a random-zero sphygmomanometer (Hawksley & Sons, Lancin, UK) in 1986, 2001 and 2007. The cuff sizes for children were 9.5*28 cm and 13*40 cm, and the cuffs were selected so that the cuff covered at least 2/3 of the upper arm surface. Three different cuff sizes were used for the measurements in adulthood, of which the most appropriate was chosen according to arm diameter: for an arm diameter between 26 and 32 cm the small adult cuff was 12 cm wide, for arm diameters 33-41 cm the medium adult cuff was 14 or 15 cm wide and for arms >41 cm the large adult cuff was 18 cm wide.

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Table S1. Description of the SNPs used for calculation of the GRSs [2,3].

SNPnumber	Nearby gene and description	Chromosome number and position	Primary trait	Impu-ted	Impu-tation quality	CA/ A2	CAF	From original GWAS **studies** [2,3] Effect (mmHg/coded allele for primary trait)	P value
rs16948048	*ZNF652:* zinc finger protein 652	chr17:47440466	DBP	Yes	0.995	G/A	0.43	0.31	$5x10^{-9}$
rs11014166	*CACNB2:* Calcium channel voltage-dependent, beta 2 subunit	chr10:18708798	DBP	Yes	0.9945	A/T	0.68	0.37	$1x10^{-8}$
rs16998073	*FGF5:* fibroblast growth factor 5	chr4:81184341	DBP	Yes	0.785	T/A	0.28	0.50	$1x10^{-21}$
rs1530440	*c10orf107:* Chromosome 10 open reading frame 107	chr10:63524591	DBP	Yes	1.00	T/C	0.20	-0.39	$1x10^{-9}$
rs3184504	*SH2B3,ATXN2:* SH2B adaptor protein 3, ataxin 2	chr12:111884608	DBP	No	1.00	T/C	0.40	0.46	$3x10^{-18}$
rs1378942	*CSK-CYP1A2:* cytochrome P450, family 1, subfamily A, polypeptide 2	chr15:75077367	DBP	No	1.00	C/A	0.45	0.43	$1x10^{-23}$
rs9815354	*ULK4:* Unc-51-like kinase 4; serine/ threonine protein kinase	chr3:41912651	DBP	Yes	0.9602	A/G	0.22	0.49	$3x10^{-9}$
rs2384550	*TBX3/TBX5:* T-box 3 / T-box 5	chr12:115352731	DBP	No	1.00	A/G	0.32	-0.35	$4x10^{-8}$
rs2681492	*ATP2B1:* ATPase, Ca^{++} transporting, plasma membrane 1	chr12:90013089	SBP	No	1.00	T/C	0.93	0.85	$4x10^{-11}$
rs11191548	*CYP17A1* Cytochrome P450, family 17 subfamily A, polypeptide1	chr10:104846178	SBP	Yes	0.997	T/C	0.92	1.16	$7x10^{-24}$
rs17367504	*MTHFR-NPPB:* methylenetetrahydrofolate reductase (NAD(P)H) / natriuretic peptide B	chr1:11862778	SBP	No	1.00	G/A	0.14	-0.85	$2x10^{-13}$
rs12946454	*PLCD3:* Phospholipase C, delta 3	chr17:43208121	SBP	Yes	0.9623	T/A	0.26	0.57	$1x10^{-8}$
rs381815	*PLEKHA7:* Pleckstrin homology domain containing family A, member 7	chr11:16902268	SBP	Yes	0.9969	T/C	0.24	0.65	$2x10^{-9}$

SNP=single nucleotide polymorphism, CA=coded allele, A2=other allele, CAF=coded allele frequency, DBP=Diastolic blood pressure, SBP=systolic blood pressure

Table S2. Longitudinal and cross-sectional effects of the SNPs on blood pressure traits and the risk of adult hypertension. Longitudinal models adjusted for age, sex and BMI; cross-sectional and logistic models for age, sex, BMI and family history (see text for statistical method details).

SNP (nearby gene)	Longitudinal effect from 1980 through 2007		Longitudinal effect in Bogalusa data: European ancestry		Longitudinal effect in Bogalusa data: African-American ancestry		Cross-sectional effect at baseline in 1980		Cross-sectional effect at 2007		OR for hyper-tension in adult-hood (between alleles)	Hypertension risk in Bogalusa data	
	Systolic BP (mmHg)	Diastolic BP (mmHg)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Systolic BP (mmHg)	Diastolic BP (mmHg)		European ancestry	African-American ancestry
rs16948048 (*ZNF652*)	β=0.31 p=0.22	β=0.67† p=0.001	β= -0.0494 p=0.84	β=0.0500 p=0.79	β=-0.3494 p=0.41	β=0.4725 p=0.15	β=0.06 p=0.84	β=0.72 p=0.02	β=0.68 p=0.12	β=0.69 p=0.048	OR=1.09, CI=0.92-1.28, p=0.32	OR=1.29, CI=0.96-1.72, p= 0.09	OR=1.11, CI=0.79-1.55, p=0.54
rs11014166 (*CACNB2*)	β=0.74 p=0.005	β=0.52† p=0.02	β=0.1439 p=0.57	β=0.0372 p=0.85	β=-0.4377 p=0.41	β=0.1495 p=0.72	β=0.13 p=0.67	β=0.11 p=0.74	β=0.90 p=0.05	β=0.67 p=0.07	OR=1.21, CI=1.01-1.45, p=0.04	OR=0.93, CI=0.69-1.26 p=0.65	OR=1.11, CI=0.73-1.67, p=0.64
rs16998073 (*FGF5*)	β=0.20 p=0.51	β=0.25† p=0.31	β=0.4288 p=0.13	β=0.3076 p=0.16	β=2.2966 p=0.004	β=0.3615 p=0.56	β=0.36 p=0.32	β=0.16 p=0.66	β=-0-09 p=0.86	β=0.05 p=0.91	OR=1.12, CI=0.91-1.38, p=0.27	OR=1.16, CI=0.83, 1.62, p=0.44	OR=1.84, CI=1.01-3.38, p=0.047
rs1530440 (*c10orf107*)	β=0.39 p=0.20	β=0.02‡ p=0.94	β=0.1356 p=0.66	β=0.2496 p=0.30	β=-08732 p=0.29	β=-1.0126 p=0.11	β=0.06 p=0.86	β=0.48 p=0.19	β=0.55 p=0.30	β=0.36 p=0.39	OR=1.03, CI=0.84-1.26, p=0.79	OR=1.03, CI=0.70-1.49, p=0.90	OR=0.88, CI=0.45-1.70, p=0.69
rs3184504 (*SH2B3,ATXN2*)	β=0.06 p=0.80	β=0.09† p=0.67	β=-1.0499 p<0.0001	β=-0.9396 p<0.0001	missing	missing	β=-0.24 p=0.41	β=-0.08 p=0.78	β=0.56 p=0.21	β=0.52 p=0.13	OR=1.05, CI=0.89-1.24, p=0.60	OR=0.75, CI=0.56-1.01, p=0.05	missing
rs1378942 (*CSK-CYP1A2*)	β=0.35 p=0.16	β=0.26† p=0.20	β=-0.2330 p=0.35	β=-0.1490 p=0.44	missing	missing	β=0.25 p=0.39	β=-0.06 p=0.83	β=0.58 p=0.18	β=0.37 p=0.28	OR=1.01, CI=0.86-1.19, p=0.92	OR=1.08, CI=0.80-1.45, p=0.62	missing
rs9815354 (*ULK4*)	β=0.35 p=0.25	β=0.13† p=0.61	β=0.1348 p=0.68	β=0.1514 p=0.56	β=1.7294 p=0.001	β=0.6306 p=0.13	β=0.40 p=0.26	β=0.09 p=0.80	β=1.17 p=0.03	β=0.74 p=0.08	OR=0.90, CI=0.74-1.10, p=0.32	OR=1.01, CI=0.67-1.50, p=0.98	OR=1.35, CI=0.90-2.04, p=0.16
rs2384550 (*TBX3/TBX5*)	β=0.004 p=0.99	β=-0.24† p=0.27	β=-0.4145 p=0.10	β=-0.5751 p=0.003	β=-0.8950 p=0.04	β=-0.5109 p=0.13	β=-0.08 p=0.81	β=-0.55 p=0.09	β=0.32 p=0.50	β=0.16 p=0.66	OR=1.14, CI=0.95-1.36, p=0.15	OR=1.28, CI=0.95-1.72, p=0.11	OR=0.90, CI=0.65-1.27, p=0.56
rs2681492 (*ATP2B1*)	β=-0.58‡ p=0.22	β=-0.69 p=0.08	β=0.2469 p=0.44	β=-0.0777 p=0.76	β=0.7221 p=0.28	β=1.0980 p=0.03	β=0.66 p=0.24	β=1.41 p=0.02	β=-0.02 p=0.98	β=0.44 p=0.51	OR=1.08, CI=0.79-1.48, p=0.62	OR=1.08, CI=0.73-1.60, p=0.69	OR= 1.27, CI=0.73-2.19, p=0.40
rs11191548 (*CYP17A1*)	β=0.63† p=0.17	β=1.05 p=0.005	β=1.0756 p=0.02	β=0.2557 p=0.46	β=-0.0595 p=0.94	β=-0.5124 p=0.37	β=-0.12 p=0.82	β=0.77 p=0.15	β=2.39 p=0.003	β=1.63 p=0.01	OR=1.39, CI=1.01-1.93, p=0.046	OR=0.82, CI=0.49-1.38, p=0.46	OR= 0.81, CI=0.47-1.42, p=0.47
rs17367504 (*MTHFR-NPPB*)	β=-0.50† p=0.16	β=-0.50 p=0.09	β=-0.2776 p=0.38	β=-0.0159 p=0.95	β=-1.6467 p=0.007	β=-1.3328 p=0.005	β=0.67 p=0.11	β=0.71 p=0.09	β=0.51 p=0.42	β=0.42 p=0.40	OR=1.22, CI=0.95-1.57, p=0.12	OR= 0.99, CI=0.67-1.45, p=0.94	OR= 0.94, CI=0.58-1.50, p=0.78
rs12946454 (*PLCD3*)	β=0.30† p=0.31	β=-0.14 p=0.57	β=0.5940 p=0.03	β=0.4660 p=0.03	β=0.4958 p=0.46	β=-0.2767 p=0.59	β=-0.33 p=0.33	β=0.03 p=0.94	β=-0.30 p=0.56	β=0.24 p=0.56	OR=0.98, CI=0.81-1.20, p=0.87	OR= 1.04, CI=0.75-1.43, p=0.82	OR= 0.94, CI=0.56-1.60, p=0.83
rs381815 (*PLEKHA7*)	β=-0.03‡ p=0.92	β=-0.005 p=0.98	β=-0.1287 p=0.65	β=-0.2739 p=0.22	β=0.0057 p=0.99	β=0.0755 p=0.85	β=-0.12 p=0.74	β=-0.22 p=0.53	β=0.36 p=0.49	β=0.37 p=0.36	OR=1.05, CI=0.87-1.28, p=0.60	OR= 0.87, CI=0.61-1.24, p=0.45	OR= 0.77, CI=0.51-1.17, p=0.22

SNP=Single Nucleotide Polymorphism, BP=Blood Pressure, β=effect estimate (in mmHg) per a 1-sd change in the GRS or per risk allele, SE=standard error, †=effect sign equals original GWA study, ‡=effect sign differs from original GWA study, OR=Odds Ratio, CI=95% Confidence Interval. A substitution method was used for subjects under antihypertensive treatment in 2001 (N=58) or 2007 (N=143) by adding 15 mmHg to the systolic and 10 mmHg to the diastolic BP measured in 2001 or 2007.

Table S3. Pairwise Pearson's correlation results between rs11191548 and the CYP17A1 SNPs.

SNP	Pearson's correlation coefficient with rs11191548
rs17115100	0.686
rs4919686	0.017
rs1004467	0.686
rs4919687	0.0050
rs3781287	0.085
rs284847	0.0070
rs10786712	0.087
rs6163	0.086
rs743572	0.087
rs2486758	0.025
rs17115149	0.0
rs11191548	1.0

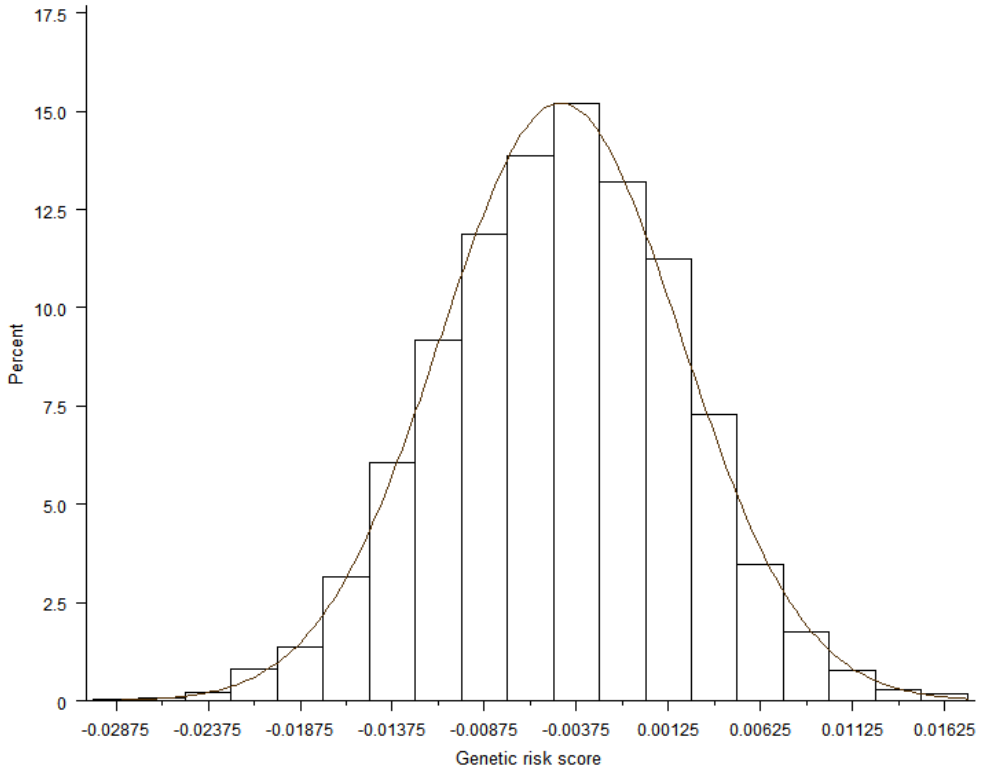

Figure S1. Distribution of the weighted combination GRS (N=2.357, mean=-0.0045847, SD=0.00656028). The GRS were normally distributed (Kolmogorov-Smirnov test p>0.06).

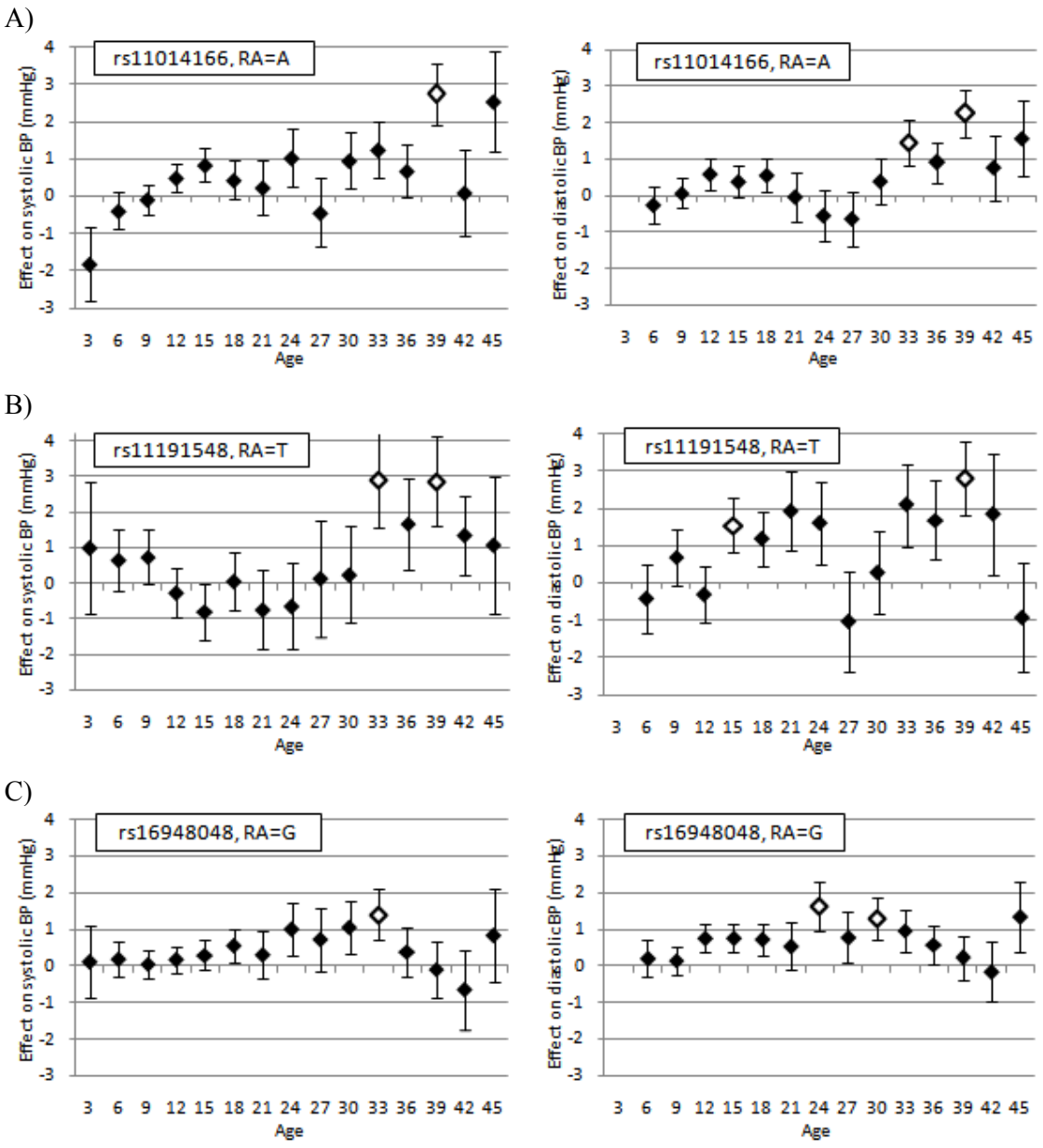

Figure S2. Effects of the most significant SNPs on blood pressure traits (in mmHg per risk allele) in all age groups: A) rs11014166 on systolic BP, B) rs16948048 on systolic BP, C) rs11191548 on systolic BP, D) rs11014166 on diastolic BP, E) rs16948048 on diastolic BP, F) rs11191548 on diastolic BP. Open symbols denote linear regression $p<0.05$. Error bars denote standard error. RA=reference allele. Diastolic blood pressure was measured from age 6 onward.

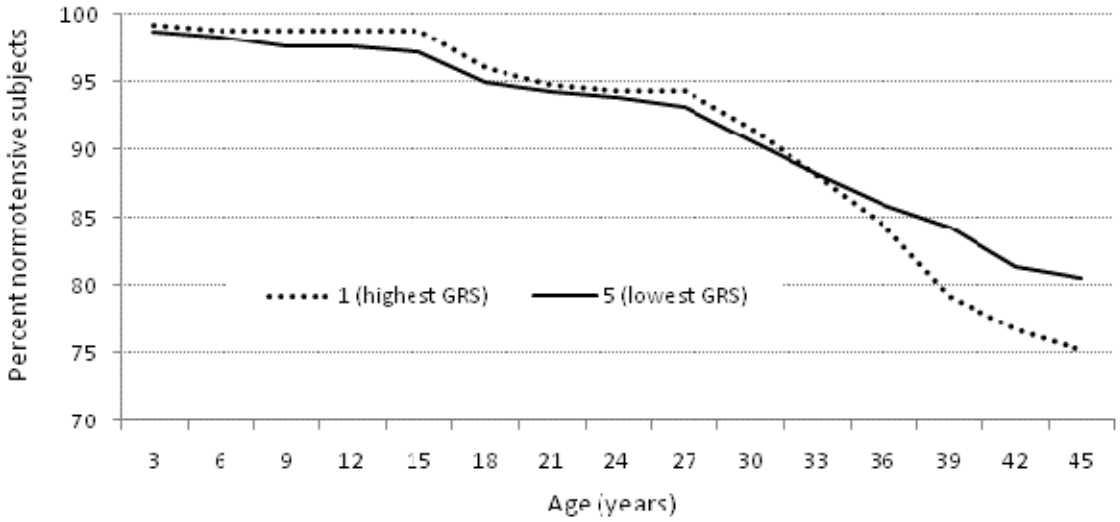

Figure S3. Proportions of normotensive subjects in the highest and lowest quintiles of the combination GRS from childhood and adolescence through adulthood.

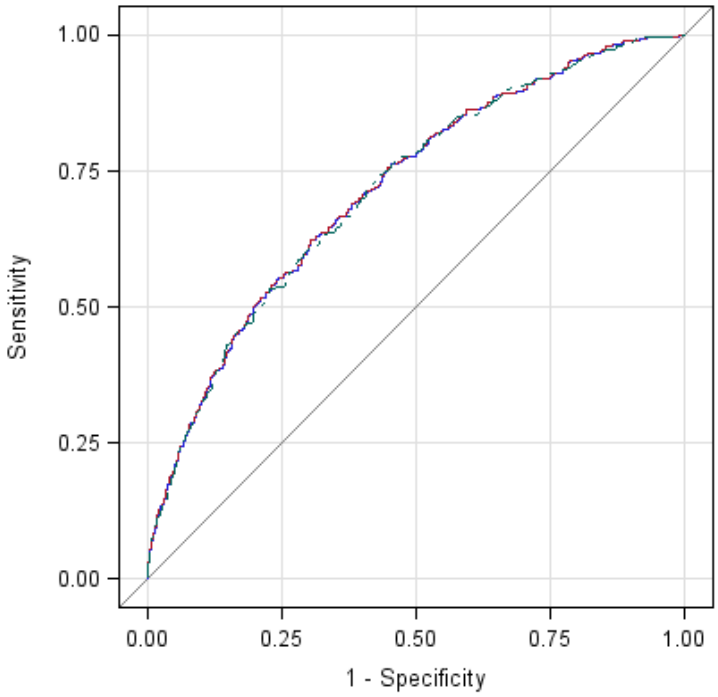

Figure S4. Comparison of models for prediction of adult hypertension. Red line=Model 1 included age, sex and BMI as predictors (AUC=0.71). Blue dashed line=Model 2 included also the combination GRS (AUC=0.72). The increase in AUC after the inclusion of combination GRS was non-significant (P=0.33).

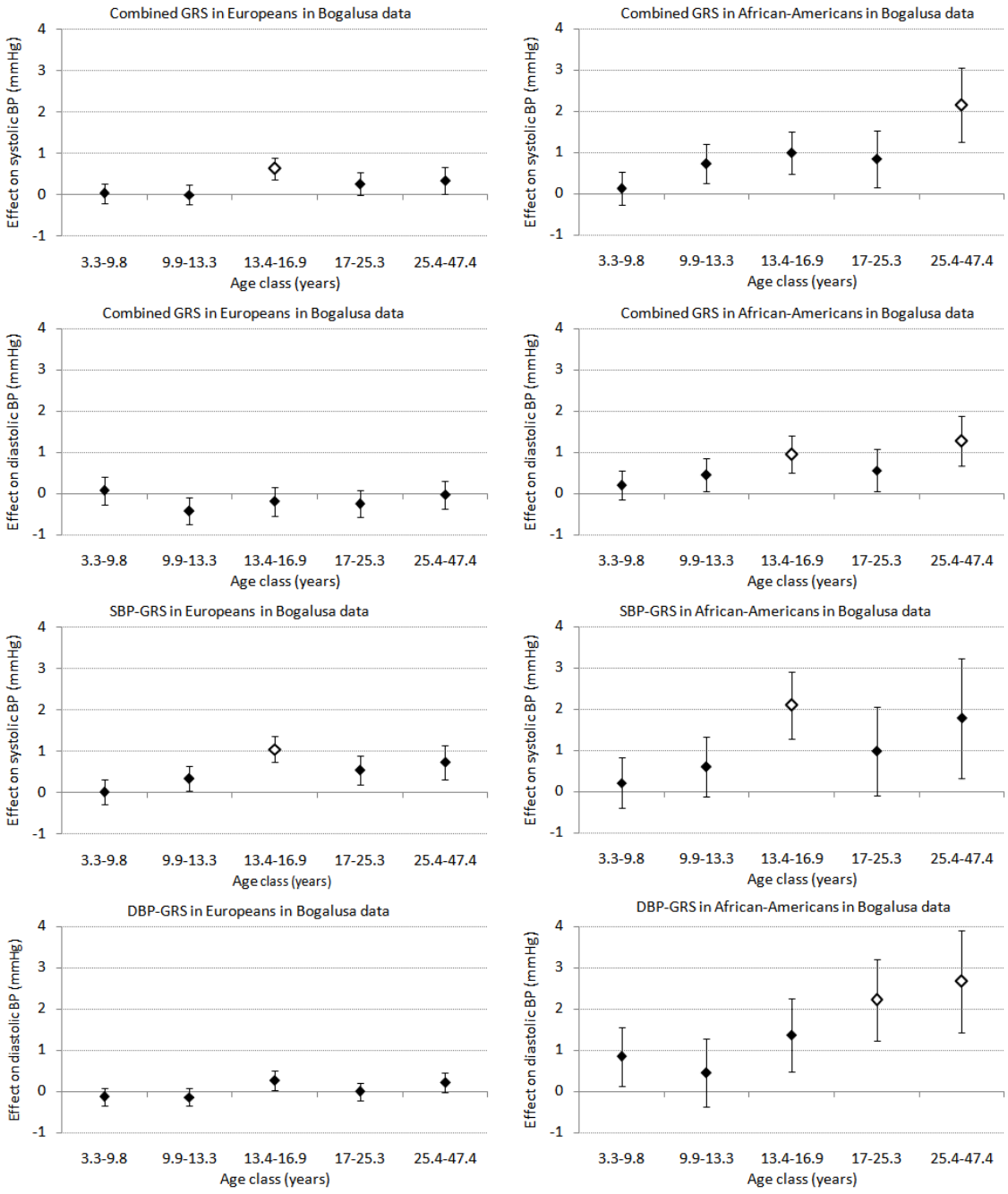

Figure S5. Replication of the age-dependent effects of the GRSs in the populations of European and African-American ancestry in the Bogalusa study.