Vitamin D, Hypertension, and Ischemic Stroke in 116 655 Individuals From the General Population

A Genetic Study

Shoaib Afzal, Børge G. Nordestgaard

See Editorial Commentary, pp 496–498

Abstract—Observational studies indicate that low concentrations of plasma 25-hydroxyvitamin D (25(OH)D) are associated with high blood pressure, hypertension, and ischemic stroke. However, whether these associations are causal remain unknown. A total of 116 655 white individuals of Danish descent from the general population were genotyped for genetic variants in *DHCR7* and *CYP2R1* affecting plasma 25(OH)D concentrations; 35 517 had plasma 25(OH)D measurements. Primary outcomes were blood pressure, hypertension, and ischemic stroke. Median follow-up for incident ischemic stroke was 9.3 years (range, 1 day–33.6 years). *DHCR7/CYP2R1* allele score was as expected associated with lower 25(OH)D concentration (F=328 and R^2=1.0%). A genetically determined 10 nmol/L lower 25(OH)D concentration was associated with a 0.68 (95% confidence interval [CI], 0.20–1.17) mm Hg higher systolic blood pressure and a 0.36 (95% CI, 0.08–0.63) mm Hg higher diastolic blood pressure with corresponding observational estimates of 0.58 (95% CI, 0.50–0.68) and 0.40 (95% CI, 0.35–0.45) mm Hg. The odds ratio for hypertension was 1.02 (95% CI, 0.97–1.08) for a genetically determined 10 nmol/L lower 25(OH)D with a corresponding observational odds ratio of 1.06 (95% CI, 1.05–1.07). The odds ratio for ischemic stroke was 0.98 (95% CI, 0.86–1.13) for a genetically determined 10 nmol/L decrease in 25(OH)D with a corresponding observational odds ratio of 1.03 (95% CI, 1.01–1.05). Genetic and observational low 25(OH)D concentrations were associated with higher blood pressure, as well as with hypertension consistent with causal relationships. Because observational but not genetic low 25(OH)D concentration was associated with ischemic stroke, and as the CIs overlapped, we can neither support nor exclude a causal relationship. **(*Hypertension*. 2017;70:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.09411.)** ● **Online Data Supplement**

Key Words: blood pressure ■ hypertension ■ odds ratio ■ stroke ■ vitamin D

Observational studies indicate that low concentrations of plasma 25-hydroxyvitamin D (25(OH)D), usually used to asses vitamin D status, are associated with higher blood pressure, hypertension, and ischemic stroke.[1–4] In addition, a Mendelian randomization study has shown an increased risk of hypertension with genetically low 25(OH)D.[3] However, randomized studies have shown minor to no effects of vitamin D supplementation on lowering of blood pressure and cardiovascular disease risk.[4–6] Furthermore, genetic studies show that some risk factors for ischemic stroke, such as obesity and an atherogenic lipid profile, may be causally associated with low concentrations of 25(OH)D.[7–9] Thus, it is unclear whether low 25(OH)D is a cause of high blood pressure, hypertension, and ischemic stroke or whether the associations are largely a result of confounding and reverse causation.

The use of genetic variants in Mendelian randomization studies allows for analyses less susceptible to confounding and free of reverse causation because the random assortment of genetic variants that occurs during gamete formation secures an equal distribution of confounding factors among different genotypes and genotypes are not affected by outcome[10,11]; thus, genetic variants in *DHCR7* and *CYP2R1* that specifically lower 25(OH)D concentrations provide an instrument for assessing the potential consequences of lifelong low 25(OH)D concentrations on blood pressure, hypertension, and ischemic stroke, largely free of confounding and free of reverse causation.

We tested the hypothesis that genetically low 25(OH)D concentrations are associated with high blood pressure, hypertension, and ischemic stroke (Figure 1). First, in observational analyses, we tested the association of 25(OH)D concentrations with blood pressure, hypertension, and ischemic stroke (Figure 1A, arrow 1); second and third, whether the selected genotypes were associated with plasma 25(OH)D

Received March 19, 2017; first decision April 11, 2017; revision accepted June 19, 2017.

From the Department of Clinical Biochemistry and the Copenhagen General Population Study, Herlev and Gentofte Hospital (S.A., B.G.N.) and the Copenhagen City Heart Study, Frederiksberg Hospital (B.G.N.), Copenhagen University Hospital, Herlev, Denmark; and Faculty of Health and Medical Sciences, University of Copenhagen, Denmark (S.A., B.G.N.).

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.117.09411/-/DC1.

Correspondence to Børge G. Nordestgaard, Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark. E-mail Boerge.Nordestgaard@regionh.dk

concentrations and with blood pressure, hypertension, and ischemic stroke (Figure 1A, arrows 2 and 3); and fourth, whether the selected genotypes were associated with blood pressure, hypertension, and ischemic stroke consistent with their effect on 25(OH)D concentrations by using instrumental variable analysis (Figure 1A, arrow 4).

Methods

Study Cohorts

The CCHS (Copenhagen City Heart Study) was initiated in 1976 to 1978 with follow-up examinations after 5 (1981–1983), 15 (1991–1994), and 25 years (2001–2003).[12] Individuals aged 20 to

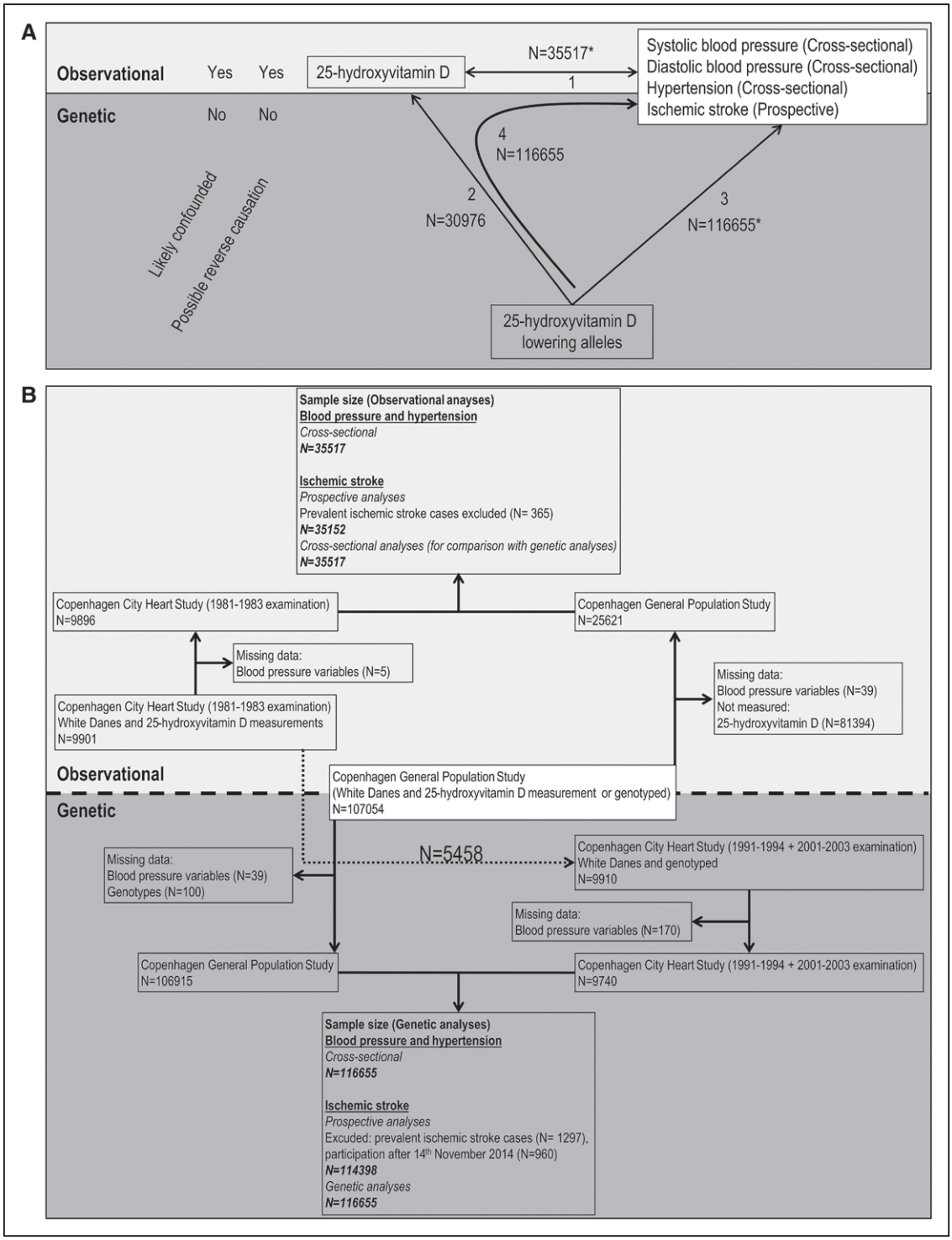


Figure 1. A, The diagram shows the 4 main analyses performed in the present study. Arrow 1, Observational association. Arrows 2 to 4, Genetic analyses. Double sided arrows show associations with undetermined direction of causality, whereas 1 sided arrows show association where a probable direction of causality can be derived. *The sample size for ischemic stroke was reduced because those with previous ischemic stroke were excluded (arrow 1, n=365 excluded; arrow 3: 2257 excluded). **B**, Flowchart of number of individuals included in each analysis.

100 years were randomly invited from the national Danish Central Person Register to reflect the Danish general population. In observational analyses, we included 9896 individuals with plasma 25(OH)D measurements from the 1981 to 1983 examination and in genetic analyses, 9740 individuals with all genotypes from the 1991 to 1994 and 2001 to 2003 examinations. Of these, 5458 individuals had both 25(OH)D measurements and genotype data available.

The CGPS (Copenhagen General Population Study) was initiated in 2003 with ongoing enrollment and with individuals recruited as for to the CCHS.[7,8] In observational analyses, we included 25 621 individuals with plasma 25(OH)D measurements and in genetic analyses, 106 915 individuals with all genotypes. Of these, 25 518 had both 25(OH)D measurements and genotype data available.

The studies were approved by institutional review boards and Danish ethical committees, and individuals provided written informed consent. No individuals appeared in >1 study, and all were white of Danish descent. A flowchart depicting how we arrived at our sample sizes is presented in Figure 1B.

Plasma 25(OH)D Measurements

We used the DiaSorin Liaison 25(OH)D TOTAL assay blinded to outcome and genotype data. CGPS plasma samples were collected in 2004 to 2005 (n=12 501; stored at −80°C for ≈5 years) and in 2009 to 2011 (n=13 120; measured on fresh samples) while CCHS plasma samples were collected in 1981 to 1983 (n=9896; stored at −20°C for ≈26 years); all samples were collected on the day of examination. Assay precision was tested daily while assay accuracy was tested using an external quality control program (DEQAS). The interassay coefficient of variance was 10% for a low level control (≈40 nmol/L) and 8% for a high level control (≈135 nmol/L). Samples for measurement were consecutive individuals for the time periods mentioned for CGPS and all available plasma samples from the CCHS 1981 to 1983 examination.

Genotypes

Genotyping using TaqMan assays was conducted blinded to 25(OH)D concentration and outcome data. Genotypes were selected among those having the strongest, largest association with 25(OH)D concentration in genome-wide association studies[13,14]; genetic variants around *DHCR7* (rs7944926 and rs11234027) and *CYP2R1* (rs10741657 and rs12794714) were specifically chosen because they are expected to influence 25(OH)D concentration through synthesis of 25(OH)D. We deliberately did not include polymorphisms in the vitamin D–binding protein because these do not associate predictably with 25(OH)D's biological activity.[15] Genotypes were verified by sequencing of 32 randomly selected samples in the 2 cohorts. Call rates for the genotypes were >99% after 2 reruns. For *DHCR7*, *CYP2R1*, and *DHCR7/CYP2R1* allele scores, weighted allele scores were constructed by multiplying each variant allele with its effect on plasma 25(OH)D concentration adjusted for the effect of the other variant in each gene, for example, the effect of *DHCR7* rs7944926 on plasma 25(OH)D was adjusted for *DHCR7* rs11234027, because these 2 genotypes are correlated. Weighted allele score were used for all allele score and instrumental variable analyses. Furthermore, for sensitivity analyses, allele scores were created by simply counting all alleles across the 4 genotypes instead of the more complex weighted scores.[16]

Potential Confounders

Confounders were chosen based on the known important risk factors for ischemic stroke and their possible association with plasma 25(OH)D.[1] Individuals reported on smoking status, daily tobacco consumption, alcohol consumption, intensity of leisure time physical activity, income, diabetes mellitus, and occurrence of stroke in parents, and all information was reviewed together with an investigator on the day of attendance. Cumulative tobacco consumption was calculated in pack-years, where 1 pack-year was 20 cigarettes or equivalent smoked daily for 1 year. Body mass index was measured weight (kg) divided by measured height (m) squared on the day of examination. Furthermore, baseline atrial fibrillation was determined by registry diagnoses. Standard hospital assays were used to measure total and high-density lipoprotein (HDL) cholesterol; non-HDL cholesterol was total minus HDL cholesterol. Last, we adjusted for kidney function using estimated glomerular filtration rate (CKD-EPI equation [Chronic Kidney Disease Epidemiology Collaboration])[17] because kidney function affects both blood pressure and 25(OH)D levels.

Outcomes

Brachial systolic and diastolic blood pressures on the left arm (mm Hg) were measured on the day of examination by trained technicians either using a London School of Hygiene sphygmomanometer or an automatic Digital Blood Pressure Monitor (Kivex) as a single measurement on the left arm after 5 minutes of rest and with the subject in the sitting position.[18] Hypertension was defined as self-reported use of antihypertensive medication as systolic blood pressure ≥140 mm Hg or as diastolic blood pressure ≥90 mm Hg. Severe hypertension was defined as self-reported use of antihypertensive medication as systolic blood pressure ≥160 mm Hg or as diastolic blood pressure ≥100 mm Hg. Blood pressure was adjusted for use of antihypertensives by adding 10 and 5 mm Hg to systolic and diastolic blood pressures, respectively.[19]

Diagnosis of cerebrovascular disease, including ischemic and hemorrhagic strokes, was collected from 1976 until November 2014 by reviewing hospital admissions with diagnoses entered in the national Danish Patient Registry and causes of death entered in the national Danish Causes of Death Registry.[2,12,20] For individuals with registered cerebrovascular disease, records from general practitioners and hospital were requested, and the diagnosis of ischemic stroke was validated by 2 independent doctors with special interest in stroke according to World Health Organization criteria and classified into subgroups using clinical description, computed tomography or MRI scan, spinal fluid examination, autopsy, or surgical description.[20] Median follow-up for incident ischemic stroke was 9.3 years (range, 1 day–33.6 years). Case-fatality rate, defined as death within 30 days of an ischemic stroke event, was 5.8%.

Statistical Analyses

We used Stata/S.E. 13.1. χ^2 tests evaluated Hardy–Weinberg equilibrium. More than 99% of observations were present for the included variables. We imputed missing data by using multivariable chained imputation (mi impute chained) with fully conditional specification; however, results were similar without using imputation. All analyses were adjusted for age, sex, and study as a minimum; in addition, observational analyses were adjusted for smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, body mass index, income, diabetes mellitus, ratio of non-HDL to HDL cholesterol, stroke in parents, atrial fibrillation, and month and year of blood sample. Hypertension was not included in the ischemic stroke model because it could be a mediator from low 25(OH)D to ischemic stroke. All analyses with blood pressure and hypertension as outcome were cross-sectional, whereas the analyses with ischemic stroke were prospective when using measured plasma 25(OH)D and the allele scores as exposures. To maximize power the instrumental variable estimates for ischemic stroke, all registered cases were used.

First, we tested whether plasma 25(OH)D concentrations were observationally associated with blood pressure, hypertension, and ischemic stroke (Figure 1A, arrow 1). For these analyses, we used multiple linear regression, logistic regression, and Cox regression models with age as time scale, respectively. The 25(OH)D concentrations were categorized as deficient (<25 nmol/L), insufficient (25–49 nmol/L), and sufficient (≥50 nmol/L)[21] and were also modeled using nonlinear terms in the regression models. Specifically, we used restricted cubic splines with 3 knots for nonlinear associations.[22]

Second, we used Cuzick nonparametric trend test to assess trend across genotypes and allele scores of 25(OH)D concentrations. We assessed the strengths of genotypes and allele scores as instruments by using the F statistic and R^2 as a measure of variation explained by genotypes and allele scores (Figure 1A, arrow 2).[11]

Third, we examined associations of *DHCR7/CYP2R1* allele score with blood pressure, hypertension, and ischemic stroke using the

same models as in observational analyses; however, these analyses were only adjusted for age, sex, month and year of blood sample, and study because allele scores were randomly distributed across potential confounders (Figure 1A, arrow 3).

Fourth, we calculated instrumental variable estimates per 10 nmol/L lower 25(OH)D by using the Wald-type ratio estimator, which involves taking the ratio of the outcome allele score coefficient to the exposure allele score coefficient and for odds ratios exponentiating to express the estimate as an odds ratio (Figure 1A, arrow 4).[11,23] We used the delta method to derive SEs of Wald-type instrumental variable log odds ratios.[24] For comparison, we derived observational estimates per 10 nmol/L lower 25(OH)D by using multiple linear regression for blood pressure and logistic regression models for hypertension and ischemic stroke adjusted for age, sex, and study. Additional details can be found in the online-only Data Supplement.

Results

Plasma 25(OH)D concentration was associated with all major risk factors for ischemic stroke except for occurrence of stroke in parents (Table). In contrast, the *DHCR7*/*CYP2R1* allele score was not associated with these potential confounders (Table S1 in the online-only Data Supplement), illustrating that the allele score can be used as a largely unconfounded instrument to assess the association of genetically low 25(OH)D with blood pressure, hypertension, and ischemic stroke. *DHCR7* and *CYP2R1* genotypes were not in linkage disequilibrium (R^2=0%), implying that genetic variants in the 2 genes were completely unrelated. Within each gene, the variants each explained 49% of the variation in the other. However, there were no linkage disequilibrium with other genetic variants associated with hypertension and stroke on chromosome 11 identified through genome-wide association studies (Figure S1). The characteristics of individuals included in the observational and genetic studies were comparable (Table S2).

25(OH)D, Blood Pressure, Hypertension, and Ischemic Stroke: Observational Estimates

We tested the association of 25(OH)D concentrations with blood pressure, hypertension, and incident ischemic stroke using in cubic spline models that indicated an almost linear increase in blood pressure and risk of hypertension and ischemic stroke with decreasing 25(OH)D concentrations <50 nmol/L (Figure 2).

Systolic blood pressures was 2.56 (95% confidence interval [CI], 1.85–3.27) mm Hg higher for individuals with 25(OH)D of <25 versus ≥50 nmol/L; the corresponding difference in diastolic blood pressure was 1.88 (95% CI, 1.47–2.29) mm Hg (Figure S2). The multivariable-adjusted odds ratio for hypertension was 1.28 (95% CI, 1.18–1.38) for individuals with 25(OH)D of <25 versus ≥50 nmol/L. The multivariable-adjusted hazard ratio for incident ischemic stroke was 1.23 (95% CI, 1.06–1.42) for individuals with 25(OH)D of <25 versus ≥50 nmol/L.

Genotypes and Plasma 25(OH)D

The combined unweighted *DHCR7*/*CYP2R1* allele score was associated with 8.4 (95% CI, 7.4–9.5) nmol/L lower 25(OH)D concentration for 6 to 8 versus 0 to 1 variant alleles; the F-value was 328 and R^2 was 1.0% (Figure 3). For each variant allele, 25(OH)D was lowered by 1.9 (95% CI, 1.7–2.1) nmol/L for the unweighted allele score. Each increase in

Table. Baseline Characteristics According to Plasma 25-Hydroxyvitamin D Concentration in the General Population

	Plasma 25-Hydroxyvitamin D, nmol/L			
	≥50	25–49	<25	*P* Value*
Characteristics	n=17 630	n=12 984	n=4903	n=35 517
Age, y	59 (49–68)	58 (48–66)	57 (49–65)	1×10^{-20}
Men, %	42	48	49	3×10^{-33}
Current smokers, %	24	33	51	1×10^{-260}
Cumulative tobacco consumption, pack-years†	18 (8–31)	20 (10–35)	26 (15–40)	1×10^{-106}
Alcohol consumption, g/wk	84 (36–168)	84 (24–168)	72 (0–180)	5×10^{-23}
Leisure time physical activity <2 h/wk, %	6	10	19	7×10^{-153}
Body mass index, kg/m^2	24.8 (22.7–27.5)	25.7 (23.3–28.7)	26.2 (23.3–29.4)	8×10^{-108}
Low income, %	17	21	30	4×10^{-67}
Diabetes mellitus, %	4	5	5	5×10^{-11}
Ratio of non-HDL to HDL cholesterol	2.6 (1.9–3.7)	3.2 (2.2–4.5)	3.7 (2.6–5.1)	$<1\times10^{-300}$
Stroke in parents, %	22	22	22	0.6
Atrial fibrillation, %	3	2	1	3×10^{-11}
Use of antihypertensive medication, %	19	17	14	9×10^{-19}
eGFR, mL/min per 1.73 m^2	78 (66–90)	76 (64–88)	74 (61–87)	1×10^{-43}

Continuous variables are summarized as median and interquartile range.
eGFR indicates estimated glomerular filtration rate; and HDL, high-density lipoprotein.
**P* values were calculated using Cuzick nonparametric trend test.
†In former and current smokers only.

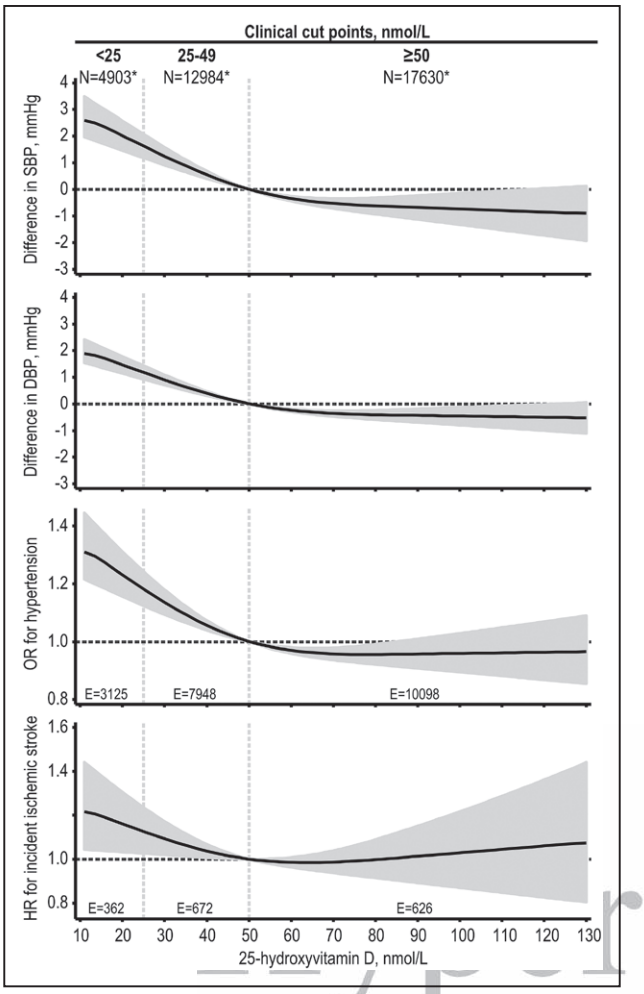


Figure 2. Association of plasma 25-hydroxyvitamin D concentration with blood pressure, hypertension, and incident ischemic stroke in the general population using a spline model. Multivariable-adjusted regression models included age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, body mass index, income, diabetes mellitus, ratio of non–high-density lipoprotein (HDL) to HDL cholesterol, stroke in parents, atrial fibrillation, estimated glomerular filtration rate, antihypertensive medication, month and year of blood sample, and study. The analyses for blood pressure and hypertension were cross-sectional because 25-hydroxyvitamin D, blood pressure, and use of antihypertensive medication were assessed on baseline measurements, whereas analysis of ischemic stroke was prospective. Differences, odds ratios (ORs), and hazard ratios (HRs) are given with 95% confidence intervals. *The sample size for ischemic stroke was reduced because those with previous ischemic stroke were excluded (n=365). E indicates number of events; DBP, diastolic blood pressure; and SBP, systolic blood pressure.

weighted allele score corresponded to a 1 nmol/L lowering of 25(OH)D concentration. The association of individual genotypes with 25(OH)D showed similar results though with less power (Figure S3).

Allele Score and Blood Pressure, Hypertension, and Ischemic Stroke: Genetic Estimates

Systolic blood pressure was 0.07 (95% CI, 0.02–0.12) mm Hg higher per 1-unit increase in the weighted *DHCR7*/*CYP2R1* allele score; the corresponding estimate for diastolic blood pressure was 0.04 (95% CI, 0.01–0.06) mm Hg (Figure 4). The odds ratio for hypertension was 1.002 (95% CI, 0.997–1.007) per one increase in the weighted *DHCR7*/*CYP2R1* allele score. The corresponding hazard ratio for incident ischemic stroke was 0.997 (95% CI, 0.980–1.013).

25(OH)D, Blood Pressure, Hypertension, and Ischemic Stroke: Instrumental Variable and Observational Estimates

A genetically determined 10 nmol/L lower 25(OH)D concentration was associated with a 0.68 (95% CI, 0.20–1.17) mm Hg higher systolic blood pressure and a 0.36 (95% CI, 0.08–0.63) mm Hg higher diastolic blood pressure (Figure 5). Corresponding observational estimates were 0.59 (95% CI, 0.50–0.68) and 0.40 (95% CI, 0.35–0.45) mm Hg, respectively. The odds ratio for hypertension was 1.02 (95% CI, 0.97–1.08) for genetically determined 10 nmol/L lower 25(OH)D concentration. The corresponding observational odds ratio was 1.06 (95% CI, 1.05–1.07). The odds ratio for ischemic stroke was 0.98 (95% CI, 0.86–1.13) for genetically determined 10 nmol/L lower 25(OH)D concentration. The corresponding observational odds ratio was 1.03 (95% CI, 1.01–1.05).

Sensitivity Analyses

Using individual genotypes or unweighted alleles scores, the results were similar to those using the weighted allele score for all outcomes (Figures S4–S6). Because the 2 genotypes within *DHCR7* and *CYP2R1* were correlated, supplementary instrumental variable analyses using only one genotype from each gene were performed, and results were similar to the main analyses for associations with 25(OH)D and for blood pressure, hypertension, and ischemic stroke (Figures S7–S9). Furthermore, the instrumental variable for systolic and diastolic blood after exclusion of those on antihypertensive medication showed similar results to those presented in Figure 5 albeit with reduced power (Figure S10). Finally, the genetic analyses were restricted to those with a 25(OH)D measurement, and the results were statistically comparable to results presented in Figure 5 albeit with reduced power (ie, broad CIs; Figure S11).

In addition, we compared the estimates for difference in blood pressure with each genetic variant in our study with publically available genome-wide association data (International Consortium for Blood Pressure[25,26]); for all 4 genetic variants, the estimates were comparable with the estimates from International Consortium for Blood Pressure (Figure S12). Likewise, we compared estimates from our study with a previous Mendelian randomization Study on 25(OH)D concentrations and blood pressure and hypertension[3]; the results were again similar (Figure S13).

Also, we tested the association of 25(OH)D concentration with severe hypertension defined as systolic/diastolic blood pressure >160/100 mm Hg or use antihypertensive medication (Figure S14). The odds ratio for severe hypertension was 1.10 (95% CI, 1.04–1.17) for a genetically determined 10 nmol/L lower 25(OH)D concentration. The corresponding observational odds ratio was 1.04 (95% CI, 1.02–1.05). Last, we investigated our blood pressure measurements for last digit

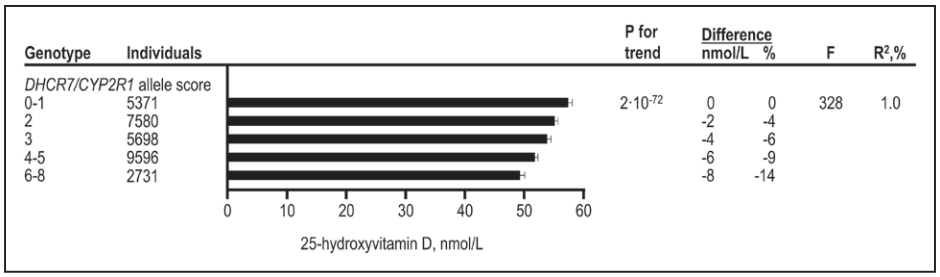


Figure 3. Concentrations of 25-hydroxyvitamin D adjusted for age, sex, month and year of blood sample, and study according to the allele score used as instrumental variables in genetic analyses. Columns show mean concentrations with 95% confidence intervals, F test is for statistical strength of instrument, and R^2 is a measure of explained variation. The 25-hydroxyvitamin D analyses are based on 30 976 individuals from the general population (Copenhagen City Heart Study and Copenhagen General Population Study combined), where both genotypes and 25-hydroxyvitamin D were measured. Analyses were cross-sectional.

preference; there was a tendency for rounding to 0 and to a lesser degree 5 (Figure S15).

Discussion

Using a Mendelian randomization approach in 116 655 individuals from the general population, we found that genetic and observational low 25(OH)D concentrations were associated with high blood pressure and hypertension compatible with causal relationships. Because observational but not genetic low 25(OH)D concentration was associated with ischemic stroke, and as the CIs overlapped, we can neither support nor exclude a causal relationship.

Biological mechanisms proposed to link low vitamin D concentrations with blood pressure include effects on the renin–angiotensin system and arterial wall thickness or stiffness. Some animal and human studies suggest that the active form of vitamin D, 1,25-dihydroxyvitamin D, may suppress renin secretion,[27,28] whereas other studies show little effect of 1,25-dihydroxyvitamin D on renin secretion.[29] Likewise, results from randomized intervention trials investigating the effects of vitamin D supplementation on arterial stiffness have been conflicting, some supporting a reduction in arterial stiffness while other studies show no effect.[30] Also, blood pressure is affected by plasma concentrations of parathyroid hormone and calcium that are suppressed and increased, respectively, by vitamin D supplementation, indicating that vitamin D may have indirect effects on blood pressure through other molecules.[31] Thus, plausible mechanisms have been suggested that link low 25(OH)D with increased blood pressure and hypertension, but the evidence supporting these mechanisms is conflicting.

The results from the present study are at odds with recent randomized intervention trials showing no effects of vitamin D supplementation on blood pressure in normotensive and hypertensive individuals.[5] This could be explained by the nonlinear association of 25(OH)D concentration with blood pressure observed in the present study, where the inverse association was primarily present for 25(OH)D <50 nmol/L with an average

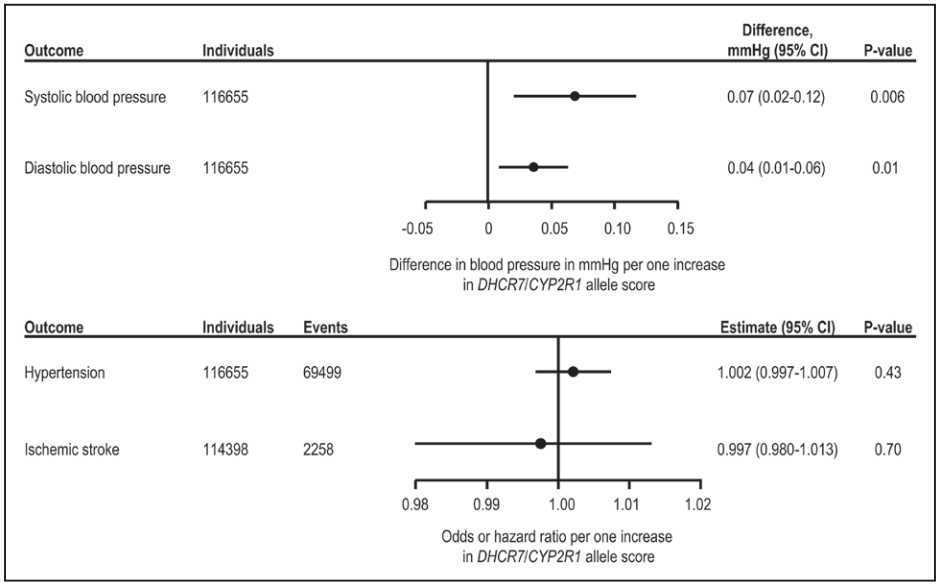


Figure 4. Blood pressure, hypertension, and incident ischemic stroke according to *DHCR7/CYP2R1* allele score. Analyses were adjusted for age, sex, and study. The analyses for blood pressure and hypertension were cross-sectional because 25-hydroxyvitamin D, blood pressure, and use of antihypertensive medication were assessed on baseline measurements, whereas analysis of ischemic stroke was prospective. Blood pressure was analyzed using multiple regression, hypertension using logistic regression, and ischemic stroke using Cox regression. We used a weighted allele score meaning that a unit increase in the allele score corresponded to approximately a 1 nmol/L higher 25-hydroxyvitamin D concentration. The sample size for ischemic stroke was reduced because those with previous and those recruited after end of follow-up for ischemic stroke were excluded (n=2257). CI indicates confidence interval.

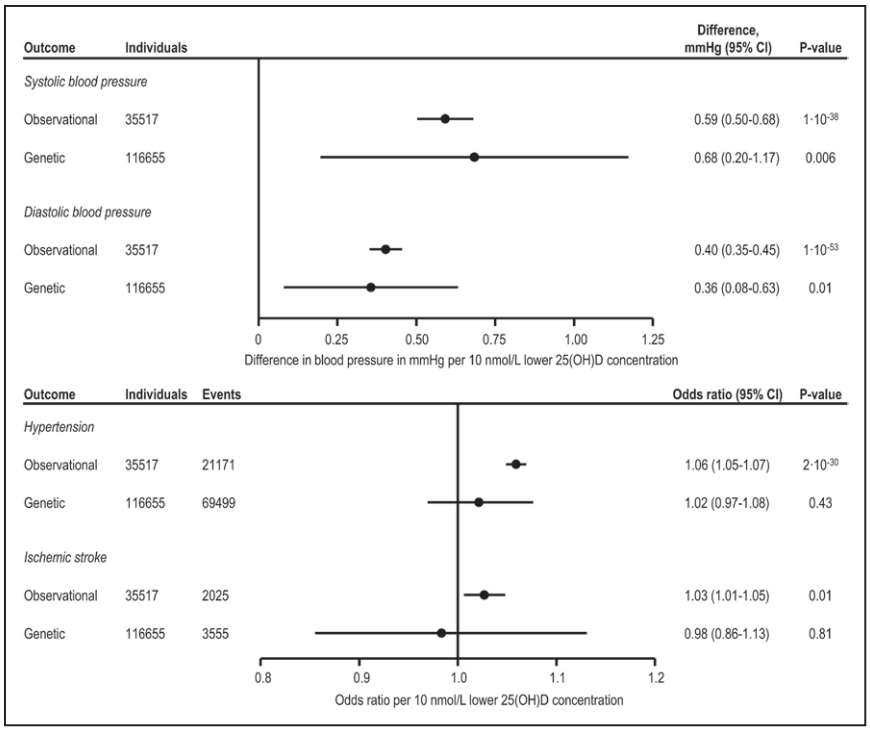


Figure 5. Observational and genetic risk estimates for blood pressure, hypertension, and all registered ischemic stroke cases in the general population for a 20 nmol/L lower 25-hydroxyvitamin D concentration. Observational estimates were by linear or logistic regression and genetic estimates by instrumental variable analyses. Analyses were cross-sectional and adjusted for age, sex, and study. 25(OH)D indicates 25-hydroxyvitamin D; and CI, confidence interval.

estimated effect of ≈0.5 mm Hg increase in blood pressure per 10 nmol/L decrease in 25(OH)D. Thus, little effect is expected in individuals with 25(OH)D >50 nmol/L, and the sample size required to show a 1-mm Hg change in systolic blood pressure with 80% power in a randomized intervention trials would be ≈3000 individuals (based on data from the DAYLIGHT trial [The Vitamin D Therapy in Individuals at High Risk of Hypertension Trial][32]). Furthermore, although the implication of a positive finding in a Mendelian randomization study is the presence of causality, it should be remembered that the setting is different from a randomized intervention trial, that is, we investigated lifelong exposure to low 25(OH)D and not short-term intervention with vitamin D. Nonetheless, a previous Mendelian randomization study has also shown an effect of genetically low 25(OH)D concentration on high blood pressure and hypertension, indicating that these findings are robust.[3]

Although a modest genetic effect on blood pressure could be shown, a clear genetic effect of low vitamin D could not be seen on risk of ischemic stroke in the present study. Given the small effect size on blood pressure and failure to demonstrate causal associations of low 25(OH)D with cardiovascular risk factors and outcomes in previous studies,[7–9,33] this is perhaps not surprising. In principle, this could be a power issue because the present study had 80% power to show odds ratios of ≥1.5; however, given the present results and the risk of adverse events,[4] supplementation with vitamin D for prevention of ischemic stroke does not seem like a viable option for general clinical practice.

Strengths of our study include that we had enough statistical power to detect an association of 25(OH)D lowering genotypes with blood pressure. Furthermore, we did not detect any violations of the assumptions underlying Mendelian randomization as far as they could be tested, and our instruments used for instrumental variable analyses were strong with an F-value of 328. In addition, because individuals were included at random and consecutively from the general population, both for genetic and 25(OH)D analyses, the potential for selection bias is minimal.

The Mendelian randomization approach assumes absence of genetic pleiotropy and linkage disequilibrium with other genetic variants associated with the outcome for the genetic variants used as instruments. However, as shown previously, there is no evidence of genetic pleiotropy,[7,34] and the variants affecting 25(OH)D concentration are not in linkage disequilibrium with other genetic variants associated with blood pressure, atrial fibrillation, or ischemic stroke in genome-wide association studies. Furthermore, 25(OH)D concentrations are known to vary with sun exposure and skin color, and we only studied white Danes, thus potentially limiting the generalizability of the results to other geographical regions. However, population homogeneity does eliminate population admixture as a potential confounder in our study. Ideally, we would have preferred to have 25(OH)D measurements in all included individuals; however, although our study is one of the largest cohorts with plasma 25(OH)D measurements, the cost is too high for measurement in all participants with genotypes. Furthermore, Mendelian randomization approaches are not dependent on complete measurement of phenotype; rather, several approaches advocate use of subsets or independent samples with phenotype or genotype measurements, respectively, to reduce bias, save cost, and maximize power instead of restricting to analyses to samples with complete measurements, which could introduce bias.[35–38] Other potential limitations are that we only measured blood pressure once and that we adjusted for use of antihypertensive medication by adding 10 and 5 mm Hg to systolic and diastolic blood pressures. This adds measurement noise to our data decreasing power as the first read in a blood pressure measurement may yield higher

blood pressures on average and as the correction may be more or less precise for each individual. However, our sensitivity analyses indicate that results were similar when excluding those using antihypertensive medication and using a higher cut off for defining hypertension yielded stronger evidence for a causal effect. Thus, these potential biases are unlikely to explain any positive findings. Last, given the small effects sizes and somewhat limited power for ischemic stroke, we can neither support nor exclude that low 25(OH)D leads to ischemic stroke.

Perspectives

In summary, genetic and observational low 25(OH)D concentrations were associated with higher blood pressure, as well as with hypertension consistent with causal relationships. Because observational but not genetic low 25(OH)D concentration was associated ischemic stroke, and as the CIs overlapped, we can neither support nor exclude a causal relationship. Thus, our study indicates the need for larger genetic studies and larger randomized intervention trials investigating the effects of vitamin D on ischemic stroke. However, given the modest effects of vitamin D status on blood pressure, the data do not support a clear-cut recommendation for vitamin D supplementation for reducing blood pressure to prevent cardiovascular complications, such as ischemic stroke.

Acknowledgment

We thank the staff and participants of the Copenhagen General Population Study for their important contributions to our study.

Sources of Funding

This work was supported by Herlev and Gentofte Hospital, Copenhagen University Hospital. The funding source had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Disclosures

None.

References

1. O'Donnell MJ, Xavier D, Liu L, et al; INTERSTROKE Investigators. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *Lancet*. 2010;376:112–123. doi: 10.1016/S0140-6736(10)60834-3.
2. Brøndum-Jacobsen P, Nordestgaard BG, Schnohr P, Benn M. 25-hydroxyvitamin D and symptomatic ischemic stroke: an original study and meta-analysis. *Ann Neurol*. 2013;73:38–47. doi: 10.1002/ana.23738.
3. Vimaleswaran KS, Cavadino A, Berry DJ, et al; LifeLines Cohort Study Investigators; International Consortium for Blood Pressure (ICBP); Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium; Global Blood Pressure Genetics (Global BPGen) Consortium; Caroline Hayward. Association of vitamin D status with arterial blood pressure and hypertension risk: a Mendelian randomisation study. *Lancet Diabetes Endocrinol*. 2014;2:719–729. doi: 10.1016/S2213-8587(14)70113-5.
4. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ*. 2014;348:g2035.
5. Beveridge LA, Struthers AD, Khan F, et al; D-PRESSURE Collaboration. Effect of vitamin D supplementation on blood pressure: a systematic review and meta-analysis incorporating individual patient data. *JAMA Intern Med*. 2015;175:745–754. doi: 10.1001/jamainternmed.2015.0237.
6. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH, Montori VM. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2011;96:1931–1942. doi: 10.1210/jc.2011-0398.
7. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Vitamin D concentration, obesity, and risk of diabetes: a Mendelian randomisation study. *Lancet Diabetes Endocrinol*. 2014;2:298–306. doi: 10.1016/S2213-8587(13)70200-6.
8. Ooi EM, Afzal S, Nordestgaard BG. Elevated remnant cholesterol in 25-hydroxyvitamin D deficiency in the general population: Mendelian randomization study. *Circ Cardiovasc Genet*. 2014;7:650–658. doi: 10.1161/CIRCGENETICS.113.000416.
9. Vimaleswaran KS, Berry DJ, Lu C, et al; Genetic Investigation of Anthropometric Traits-GIANT Consortium. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*. 2013;10:e1001383. doi: 10.1371/journal.pmed.1001383.
10. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*. 2004;33:30–42. doi: 10.1093/ije/dyh132.
11. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–1163. doi: 10.1002/sim.3034.
12. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA*. 2008;300:2142–2152. doi: 10.1001/jama.2008.621.
13. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;376:180–188. doi: 10.1016/S0140-6736(10)60588-0.
14. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet*. 2010;19:2739–2745. doi: 10.1093/hmg/ddq155.
15. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med*. 2013;369:1991–2000. doi: 10.1056/NEJMoa1306357.
16. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. *Int J Epidemiol*. 2013;42:1134–1144. doi: 10.1093/ije/dyt093.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
18. Timpson NJ, Harbord R, Davey Smith G, Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. Does greater adiposity increase blood pressure and hypertension risk?: Mendelian randomization using the FTO/MC4R genotype. *Hypertension*. 2009;54:84–90. doi: 10.1161/HYPERTENSIONAHA.109.130005.
19. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med*. 2005;24:2911–2935. doi: 10.1002/sim.2165.
20. Truelsen T, Grønbaek M, Schnohr P, Boysen G. Stroke case fatality in Denmark from 1977 to 1992: the Copenhagen City Heart Study. *Neuroepidemiology*. 2002;21:22–27.
21. Cranney A, Horsley T, O'Donnell S et al. Effectiveness and safety of vitamin D in relation to bone health. *Evid Rep Technol Assess (Full Rep)*. 2007;158:1–235.
22. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8:551–561.
23. Didelez V, Meng S, Sheehan NA. Assumptions of IV methods for observational epidemiology. *Statist Sci*. 2010;25:22–40.
24. Thomas DC, Lawlor DA, Thompson JR. Re: Estimation of bias in nongenetic observational studies using "Mendelian triangulation" by Bautista *et al*. *Ann Epidemiol*. 2007;17:511–513. doi: 10.1016/j.annepidem.2006.12.005.
25. Ehret GB, Munroe PB, Rice KM et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.
26. Wain LV, Verwoert GC, O'Reilly PF, et al; LifeLines Cohort Study; EchoGen consortium; AortaGen Consortium; CHARGE Consortium Heart Failure Working Group; KidneyGen consortium; CKDGen consortium; Cardiogenics Consortium; CardioGram. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. *Nat Genet*. 2011;43:1005–1011. doi: 10.1038/ng.922.

27. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002;110:229–238. doi: 10.1172/JCI15219.
28. Schroten NF, Ruifrok WP, Kleijn L, Dokter MM, Silljé HH, Lambers Heerspink HJ, Bakker SJ, Kema IP, van Gilst WH, van Veldhuisen DJ, Hillege HL, de Boer RA. Short-term vitamin D3 supplementation lowers plasma renin activity in patients with stable chronic heart failure: an open-label, blinded end point, randomized prospective trial (VitD-CHF trial). *Am Heart J*. 2013;166:357–364.e2. doi: 10.1016/j.ahj.2013.05.009.
29. Tiosano D, Schwartz Y, Braver Y, Hadash A, Gepstein V, Weisman Y, Lorber A. The renin-angiotensin system, blood pressure, and heart structure in patients with hereditary vitamin D-resistance rickets (HVDRR). *J Bone Miner Res*. 2011;26:2252–2260. doi: 10.1002/jbmr.431.
30. Rodríguez AJ, Scott D, Srikanth V, Ebeling P. Effect of vitamin D supplementation on measures of arterial stiffness: a systematic review and meta-analysis of randomized controlled trials. *Clin Endocrinol (Oxf)*. 2016;84:645–657. doi: 10.1111/cen.13031.
31. Vaidya A, Brown JM, Williams JS. The renin-angiotensin-aldosterone system and calcium-regulatory hormones. *J Hum Hypertens*. 2015;29:515–521. doi: 10.1038/jhh.2014.125.
32. Arora P, Song Y, Dusek J, et al. Vitamin D therapy in individuals with prehypertension or hypertension: the DAYLIGHT trial. *Circulation*. 2015;131:254–262. doi: 10.1161/CIRCULATIONAHA.114.011732.
33. Brøndum-Jacobsen P, Benn M, Afzal S, Nordestgaard BG. No evidence that genetically reduced 25-hydroxyvitamin D is associated with increased risk of ischaemic heart disease or myocardial infarction: a Mendelian randomization study. *Int J Epidemiol*. 2015;44:651–661. doi: 10.1093/ije/dyv078.
34. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. *BMJ*. 2014;349:g6330.
35. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *Int J Epidemiol*. 2016;45:1717–1726. doi: 10.1093/ije/dyx028.
36. Thompson JR, Minelli C, Abrams KR, Tobin MD, Riley RD. Meta-analysis of genetic studies using Mendelian randomization–a multivariate approach. *Stat Med*. 2005;24:2241–2254. doi: 10.1002/sim.2100.
37. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol*. 2013;178:1177–1184. doi: 10.1093/aje/kwt084.
38. Minelli C, Thompson JR, Tobin MD, Abrams KR. An integrated approach to the meta-analysis of genetic association studies using Mendelian randomization. *Am J Epidemiol*. 2004;160:445–452. doi: 10.1093/aje/kwh228.

Novelty and Significance

What Is New?

- We used a Mendelian randomization design to test whether the association of low 25-hydroxyvitamin D with high blood pressure, hypertension, and ischemic stroke is causal or the result of confounding and reverse causation. One previous large scale Mendelian randomization has investigated the association with high blood pressure and hypertension while the genetic association with ischemic stroke has not been investigated in this setting before.

What Is Relevant?

- Vitamin D supplementation is easily administered, and if low 25-hydroxyvitamin D is causally associated with high blood pressure, hypertension, or ischemic stroke, it could have wide implications for public health strategies.

Summary

Given the present results with a causal but modest effect of low vitamin D on high blood pressure, a clear-cut recommendation for supplementation with vitamin D for prevention of hypertension cannot be given. Further studies are required to investigate potential minor effects if any on risk of ischemic stroke.

Hypertension
Journal of the American Heart Association

Vitamin D, Hypertension, and Ischemic Stroke in 116 655 Individuals From the General Population: A Genetic Study

Shoaib Afzal and Børge G. Nordestgaard

Hypertension. published online July 31, 2017;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563

VITAMIN D, HYPERTENSION, AND ISCHEMIC STROKE IN 116655 INDIVIDUALS FROM THE GENERAL POPULATION: A GENETIC STUDY

Shoaib Afzal, MD, PhD[1,2] and Børge G. Nordestgaard, MD, DMSc[1,2,3].

[1]The Department of Clinical Biochemistry and The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark

[2]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[3]The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Frederiksberg, Denmark

Correspondence:

Børge G. Nordestgaard, Professor, Chief Physician, MD, DMSc

Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital

Herlev Ringvej 75, DK-2730 Herlev, Denmark

e-mail: Boerge.Nordestgaard@regionh.dk

Tel. no.: +45 38683297, Fax: +45 38683311

Supplementary Methods

The Mendelian randomization approach

Several reviews of the Mendelian randomization approach have been published both with regard to concepts and methods.[1-6] Here we summarize the general assumptions and some aspects of use in in with multiple samples. The Mendelian randomization approach is a variant of instrumental variable analysis using genetic variants as instruments. Instrumental variable analysis is different from other observational designs in that it tries to eliminate confounding without measuring confounders. This can only be achieved if the three core assumptions of instrumental variable analysis hold:

1. *The instruments (genetic variants) are associated with the exposure.*
2. *The instruments (genetic variants) are associated with the outcome only through the exposure.*
3. *The instruments (genetic variants) are independent of the confounders of the exposure and outcome association.*

Of these assumptions only the first is directly testable. Assumptions 2 and 3 cannot be directly tested, but one can try to estimate whether the assumptions are reasonable, e.g. by testing whether the measured confounders are associated with the instruments, studying whether the instruments are associated with other unrelated phenotypes, or there may be confounding due to the genetic architecture around the chosen genetic variant. If assumptions hold, the instrumental variable estimates are considered to be largely free of confounding and reverse causation, since disease cannot affect the genotypes you are born with and genotypes are acquired randomly at conception independently of confounders measured later in life.

Measurement of phenotypes and genotypes is not required to be carried out in the same populations as genotype-outcome and genotype-phenotype can be combined across studies if the populations are considered to be comparable. Furthermore, if the analyses for the genotype-phenotype are carried out in a subsample, those results can be extended to whole population without worrying about comparability. The combing of results across samples or extending results from a subsample to the complete sample have been recommended for economic reasons, statistical reasons (may reduce bias away from null), and increasing power.[7-10] Thus, methods combining multiple data sources should be used to avoid spurious results due to low power or bias and this approach has become standard practice in major Mendelian randomization studies.[11-18]

References

1. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1-22.
2. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*. 2004;33:30-42.
3. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. *Stat Methods Med Res*. 2007;16:309-330.
4. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133-1163.
5. Didelez V, Meng S, Sheehan NA. Assumptions of IV Methods for Observational Epidemiology. *Statist Sci*. 2010;22-40.
6. Smith GD, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Human Molecular Genetics*. 2014;23:R89-R98.
7. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *International Journal of Epidemiology*. 2016;45:1717-1726.
8. Thompson JR, Minelli C, Abrams KR, Tobin MD, Riley RD. Meta-analysis of genetic studies using Mendelian randomization--a multivariate approach. *Stat Med*. 2005;24:2241-2254.
9. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol*. 2013;178:1177-1184.
10. Minelli C, Thompson JR, Tobin MD, Abrams KR. An integrated approach to the meta-analysis of genetic association studies using Mendelian randomization. *Am J Epidemiol*. 2004;160:445-452.

11. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. *BMJ*. 2014;349:g6330.
12. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Vitamin D concentration, obesity, and risk of diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol*. 2014;2:298-306.
13. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, Voros S, Giugliano RP, Davey Smith G, Fazio S, Sabatine MS. Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. *N Engl J Med*. 2016;375:2144-2153.
14. Nelson CP, Hamby SE, Saleheen D et al. Genetically Determined Height and Coronary Artery Disease. *N Engl J Med*. 2015;372:1608-1618.
15. Vimaleswaran KS, Berry DJ, Lu C et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*. 2013;10:e1001383.
16. Vimaleswaran KS, Cavadino A, Berry DJ et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. *Lancet Diabetes Endocrinol*. 2014;2:719-729.
17. Voight BF, Peloso GM, Orho-Melander M et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *The Lancet*.380:572-580.
18. White J, Sofat R, Hemani G et al. Plasma urate concentration and risk of coronary heart disease: a Mendelian randomisation analysis. *The Lancet Diabetes & Endocrinology*.4:327-336.

Tables

Supplementary Table S1. Baseline characteristics according to *DHCR7/CYP2R1* allele score in the general population.

	***DHCR7/CYP2R1* allele score**					
Characteristics	**0-1 N=20387**	**2 N=28454**	**3 N=21036**	**4-5 N=36539**	**6-8 N=10239**	**P* N=116655**
Age, years	58 (48-68)	58 (48-68)	58 (48-67)	58 (48-68)	58 (48-66)	0.1
Men, %	45	45	45	45	45	0.2
Current smokers, %	19	19	19	20	20	0.1
Cumulative tobacco consumption, pack-years†	17 (7-31)	17 (6-31)	17 (7-31)	17 (7-32)	17 (6-30)	0.6
Alcohol consumption, g/week	96 (36-180)	96 (36-180)	96 (36-180)	96 (36-180)	96 (36-168)	0.3
Leisure time physical activity <2 hours/week, %	6	7	7	7	6	0.5
Body mass index, kg/m²	25.6 (23.2-28.4)	25.5 (23.1-28.3)	25.5 (23.1-28.3)	25.5 (23.2-28.4)	25.5 (23.1-28.4)	0.6
Low income, %	13	13	12	13	13	0.2
Diabetes, %	4	4	4	4	4	0.6
Ratio of non-HDL to HDL cholesterol	2.5 (1.8-3.6)	2.5 (1.8-3.6)	2.5 (1.8-3.6)	2.5 (1.8-3.6)	2.5 (1.8-3.6)	0.01‡
Stroke in parents, %	21	23	22	22	22	0.6
Atrial fibriallation, %	3	3	3	3	3	0.2
Use of antihypertensive medication, %	19	19	19	19	19	0.9
eGFR, mL/min per 1.73 m²	80 (69-90)	80 (69-91)	80 (69-91)	80 (69-91)	80 (69-91)	0.3

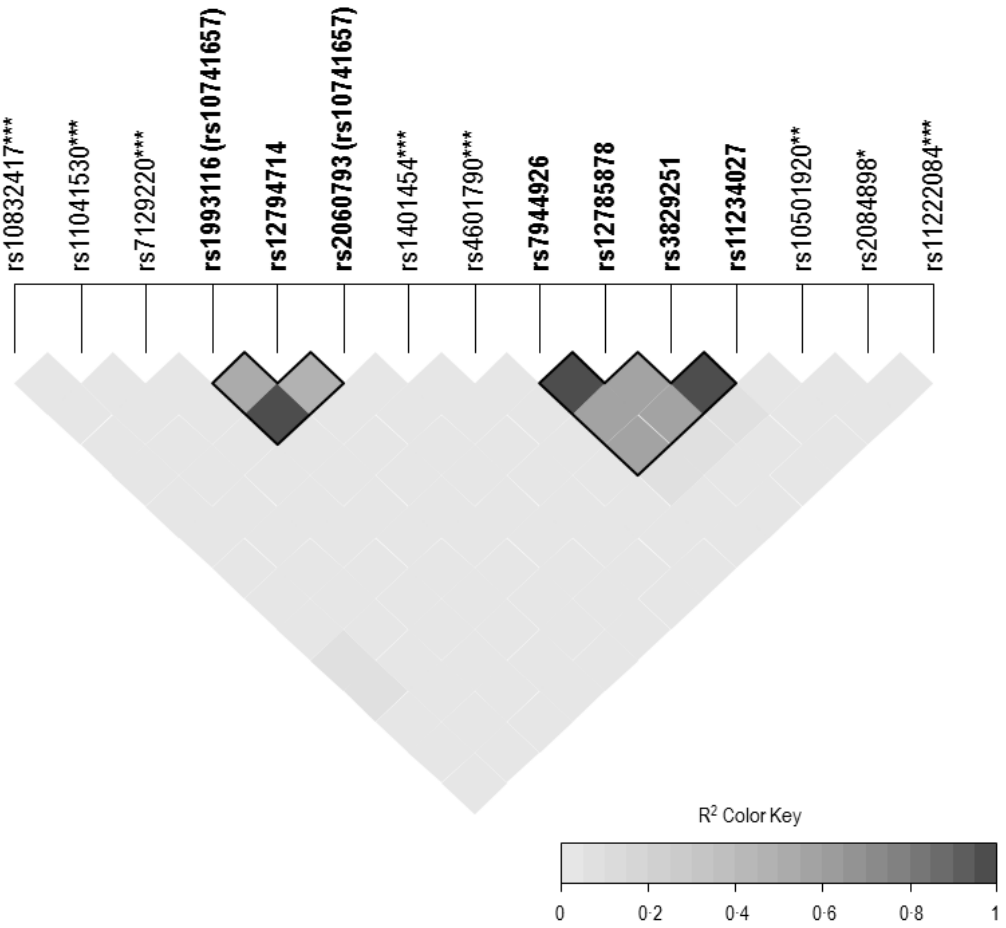
Continuous variables are summarised as median and interquartile range.
HDL = high-density lipoprotein.
*P-values were calculated using Cuzick's nonparametric trend test.
†In former and current smokers only.
‡Not significant after correction for 11 parallel tests (required P=0·05/12=0·004)

Supplementary Table S2. Baseline characteristics for cohorts used for observational and genetic analyses. Cohorts were partially overlapping as indicated in Supplementary Figure S1.

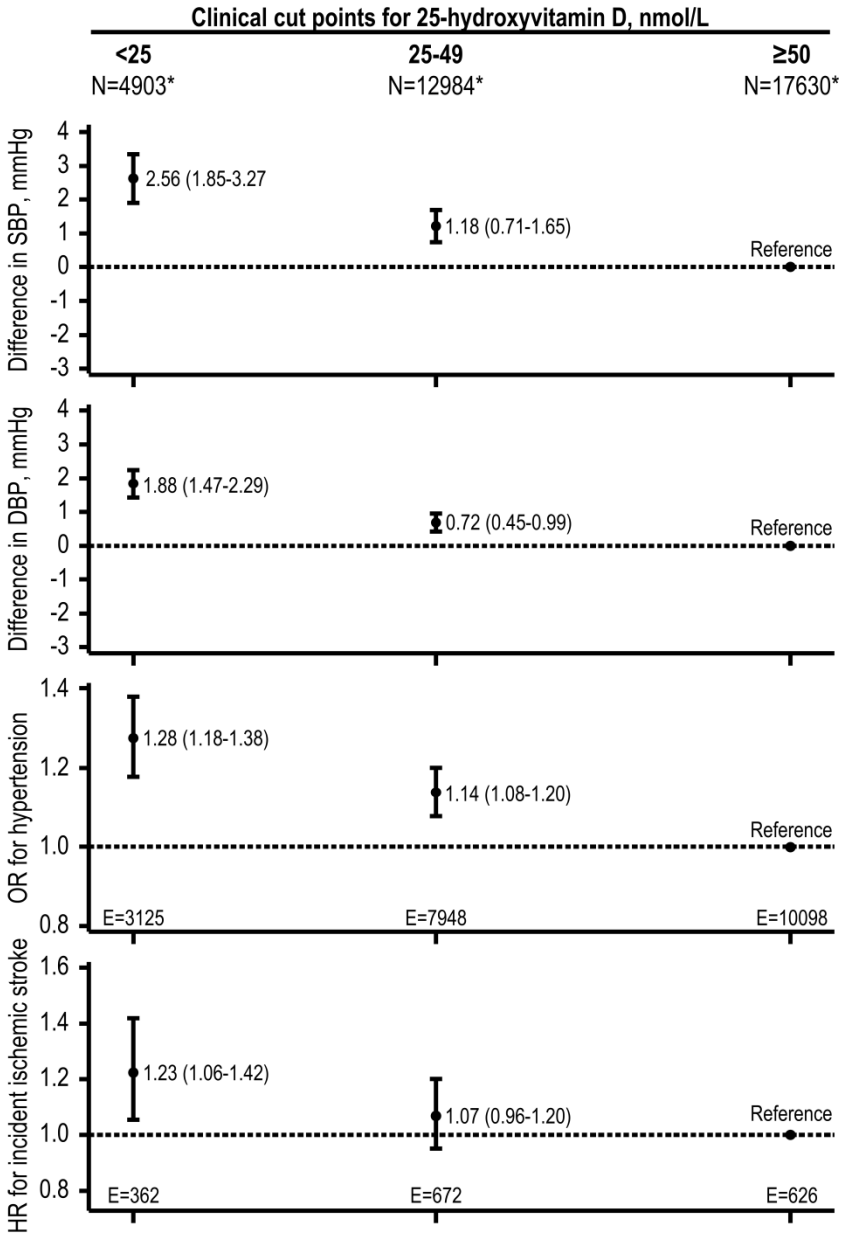
	Cohort	
Characteristics	**Observational N=35517**	**Genetic N=116655**
Age, years	58 (49-67)	58 (48-68)
Men, %	45	45
Current smokers, %	31	20
Cumulative tobacco consumption, pack-years†	20 (9-34)	17 (7-31)
Alcohol consumption, g/week	84 (24-168)	96 (36-180)
Leisure time physical activity <2 hours/week, %	10	7
Body mass index, kg/m^2	25.4 (23.0-28.2)	25.5 (23.1-28.4)
Low income, %	21	13
Diabetes, %	4	4
Ratio of non-HDL to HDL cholesterol	2.9 (2.0-4.2)	2.5 (1.8-3.5)
Stroke in parents, %	22	22
Atrial fibriallation, %	2	3
Use of antihypertensive medication, %	18	19
eGFR, mL/min per 1.73 m^2	77 (65-89)	80 (69-91)

Continuous variables are summarised as median and interquartile range.
HDL = high-density lipoprotein.
†In former and current smokers only.

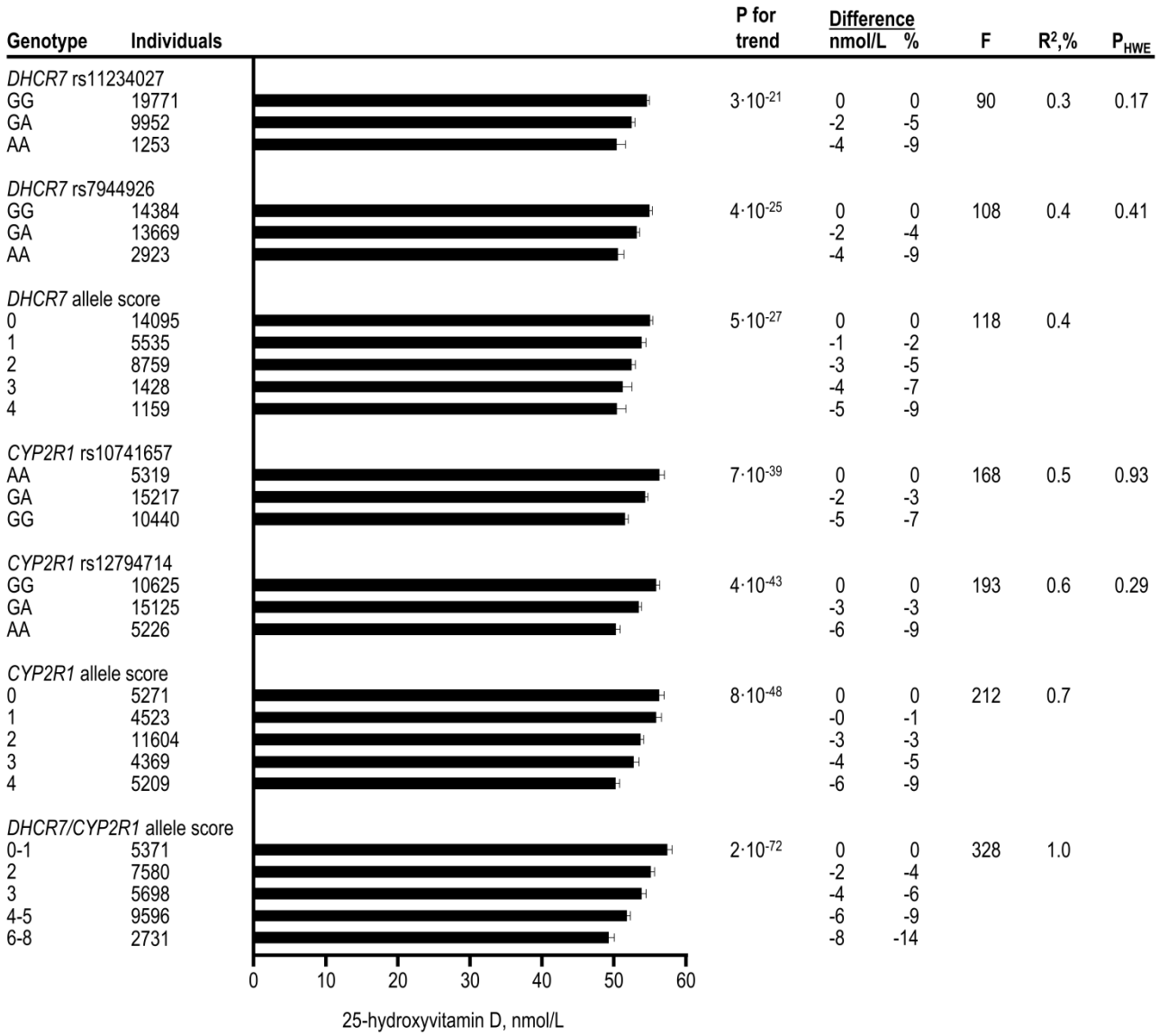
Figures



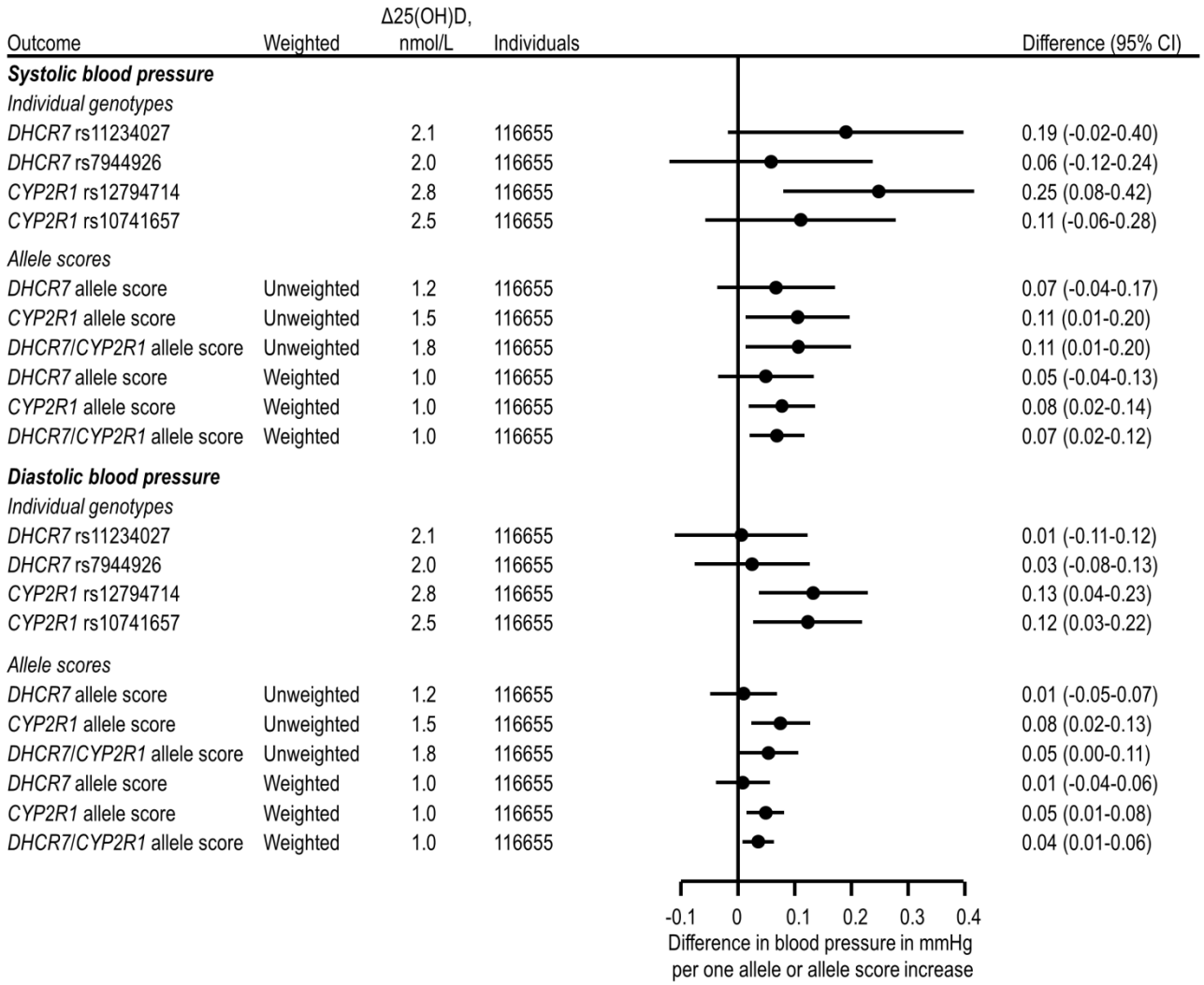
Supplementary Figure S1. Linkage disequilibrium plot of available genetic variants on chromosome 11 found in genome-wide association studies to be associated with blood pressure, atrial fibrillation, stroke, or 25-hydroxyvitamin D concentration. Plot made from data on the HapMap population. The marked haplotype blocks are genetic variants associated with 25-hydroxyvitamin D in *CYP2R1* (left) and *DHCR7* (right). These or tagging polymorphisms (R^2>0.9) were genotyped in the present study. *Association with stroke in pediatric patients. **Association with atrial fibrillation. ***Association with blood pressure.



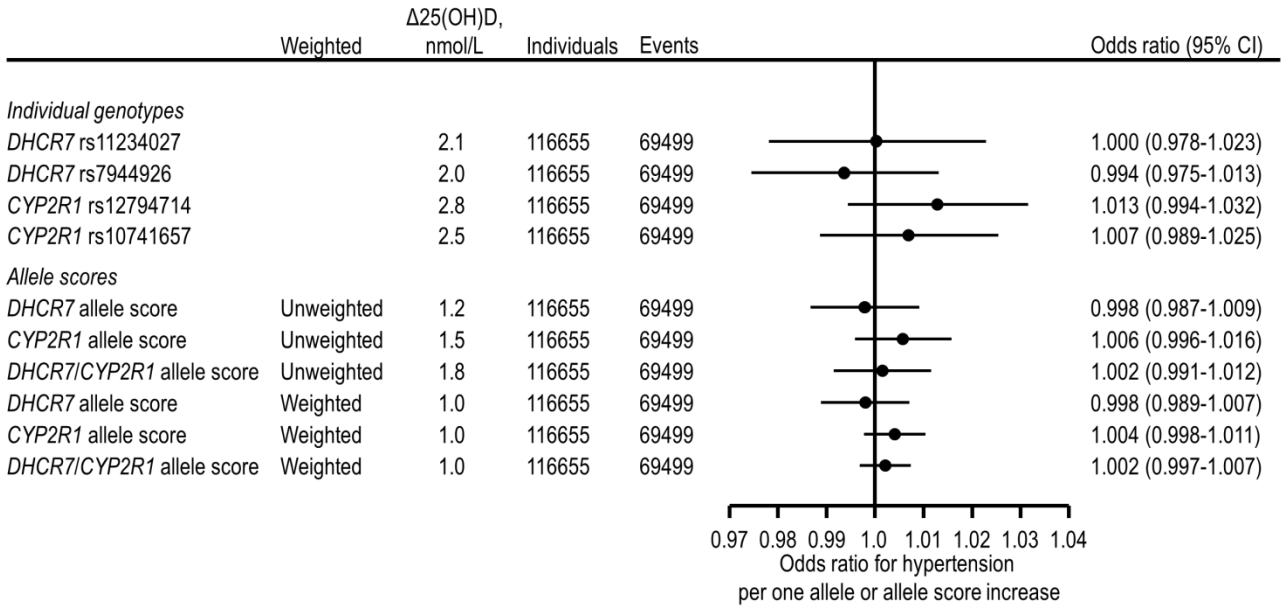
Supplementary Figure S2. Association of plasma 25-hydroxyvitamin D concentration with blood pressure, hypertension, and ischemic stroke in the general population in clinical categories. Multivariable adjusted regression models included age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, body mass index, income, diabetes, ratio of non-HDL to HDL cholesterol, stroke in parents, atrial fibrillation, eGFR, antihypertensive medication, month and year of blood sample and study. The analyses for blood pressure and hypertension were cross-sectional as 25-hydroxyvitamin D, blood pressure, and use of anti-hypertensive medication were assessed on baseline measurements, while analysis of incident ischemic stroke was prospective. Differences, odds ratios, and hazard ratios are given with 95% confidence intervals. *The sample size for ischemic stroke was reduced, since those with previous ischemic stroke were excluded (N=365). SBP = systolic blood pressure. DBP = diastolic blood pressure. OR = odds ratio. HR = hazard ratio. N = number of individuals. E = number of events.



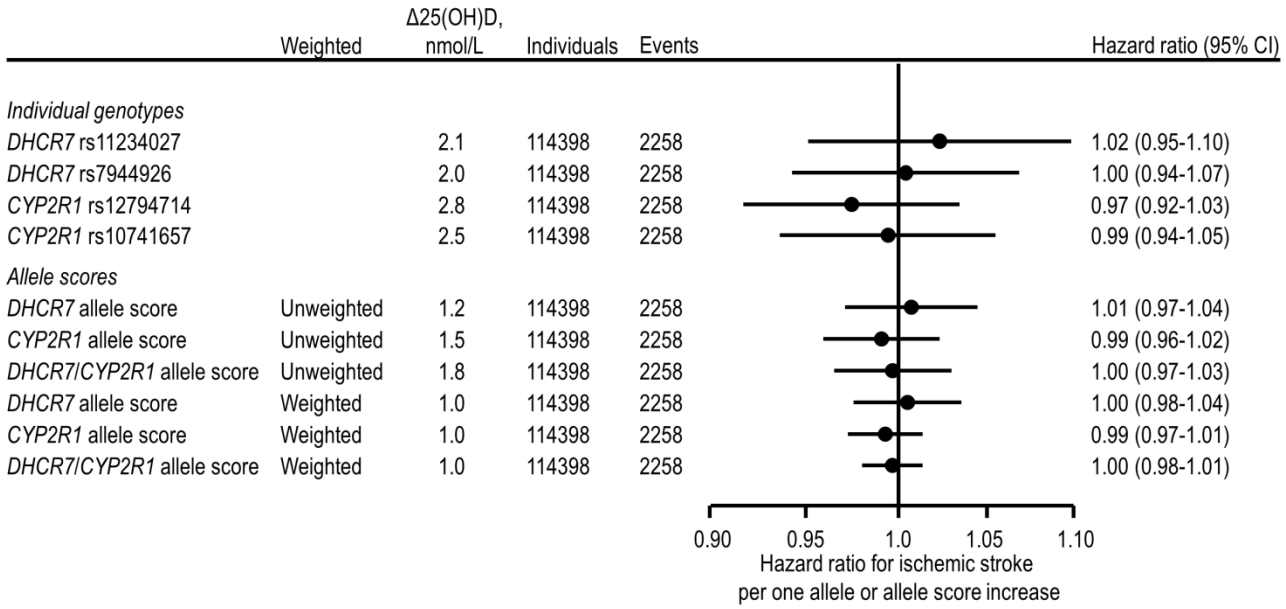
Supplementary Figure S3. Concentrations of 25-hydroxyvitamin D adjusted for age, sex, month and year of blood sample, and study according to genotypes and allele scores used as instrumental variables in genetic analyses. Columns show mean concentrations with 95% confidence intervals, F test is for statistical strength of instrument, and R^2 is a measure of explained variation. 25-hydroxyvitamin D analyses are based on 30976 individuals from the general population (Copenhagen City Heart Study and Copenhagen General Population Study combined), where both genotypes and 25-hydroxyvitamin D were measured. These analyses were cross-sectional. P_{HWE} = P for Hardy-Weinberg equilibrium.



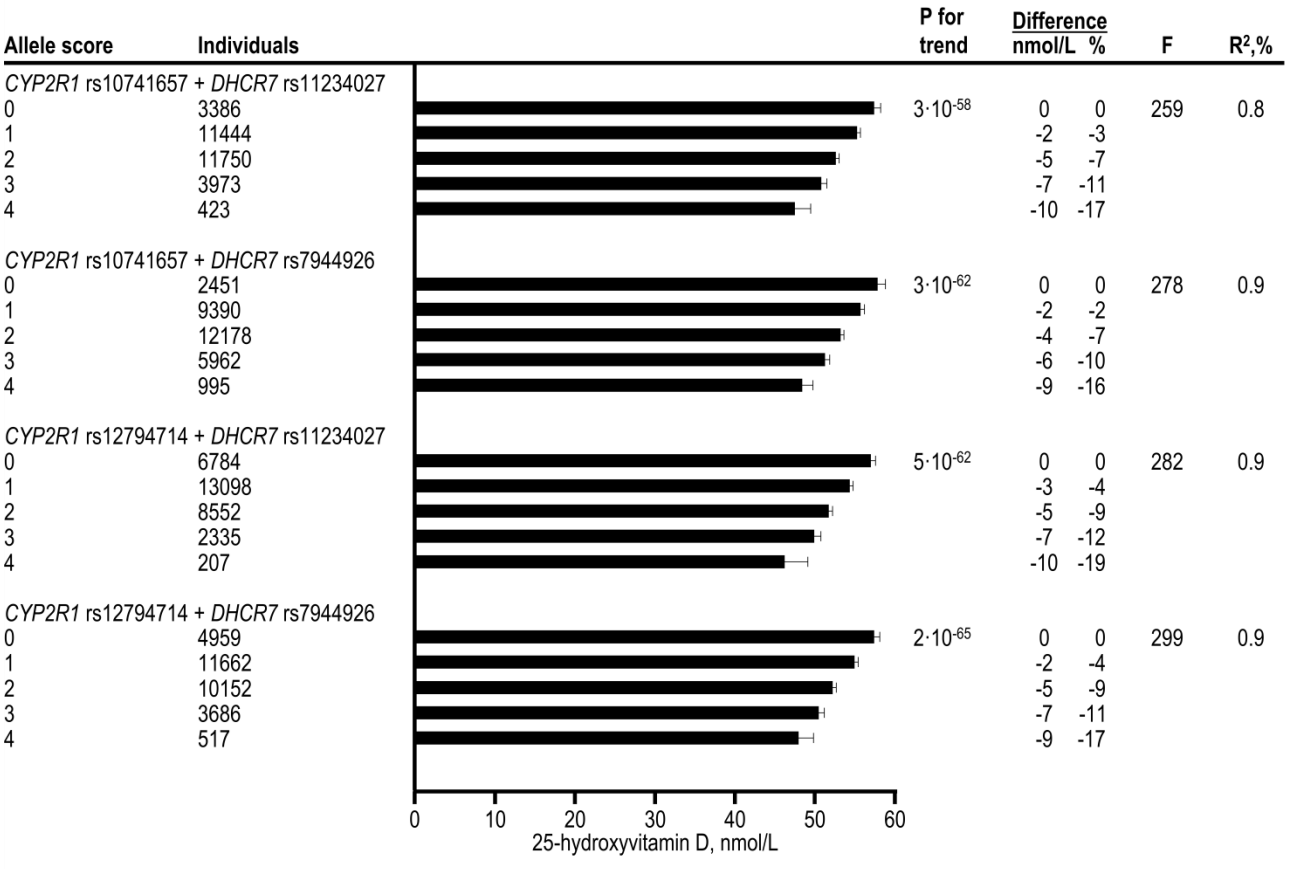
Supplementary Figure S4. Blood pressure according to individual genotypes and unweighted and weighted allele scores. Analyses were adjusted for age, sex, and study. Blood pressure was analysed using multiple regression. These analyses were cross-sectional. Δ25(OH)D=difference per allele. CI = confidence interval.



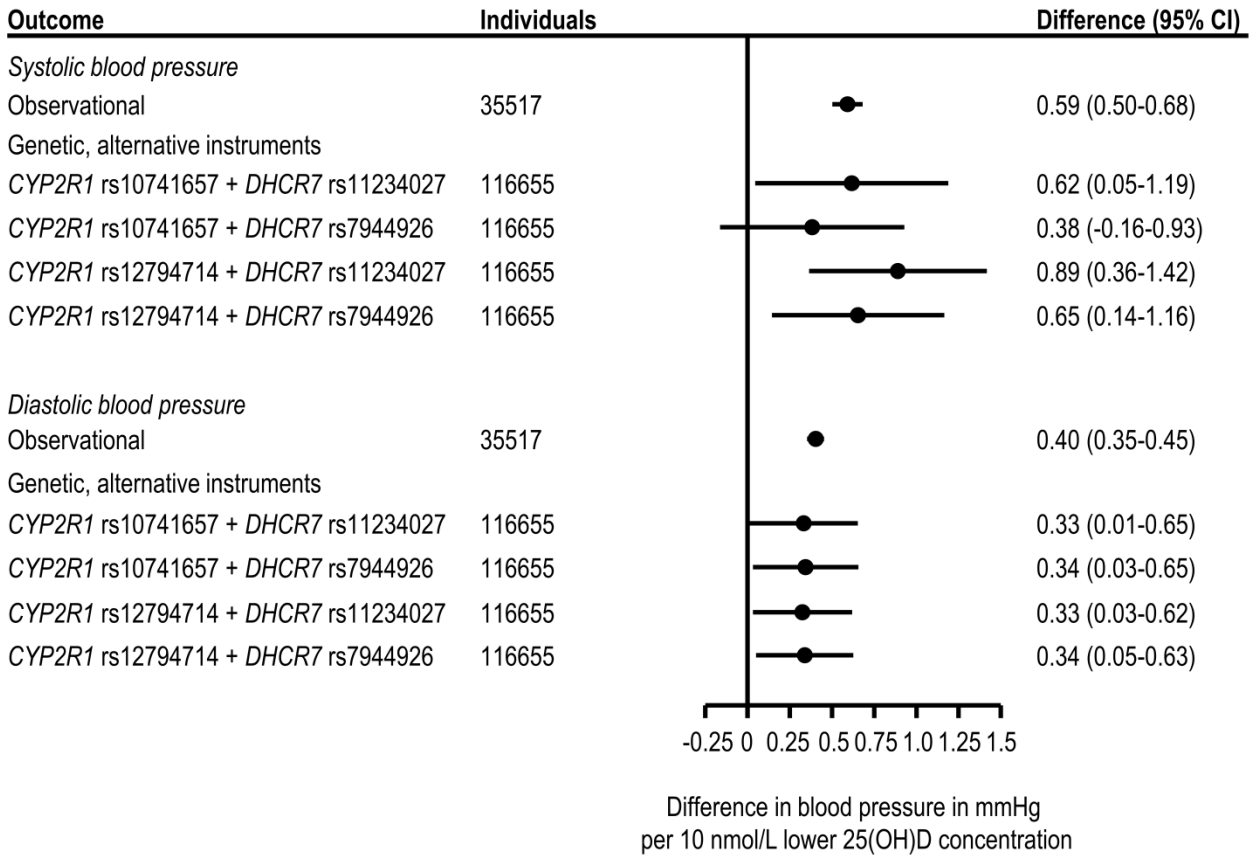
Supplementary Figure S5. Risk of hypertension according to individual genotypes and unweighted and weighted allele scores. Analyses were adjusted for age, sex, and study. Hypertension was analysed using logistic regression. These analyses were cross-sectional. Δ25(OH)D=difference per allele. CI = confidence interval.



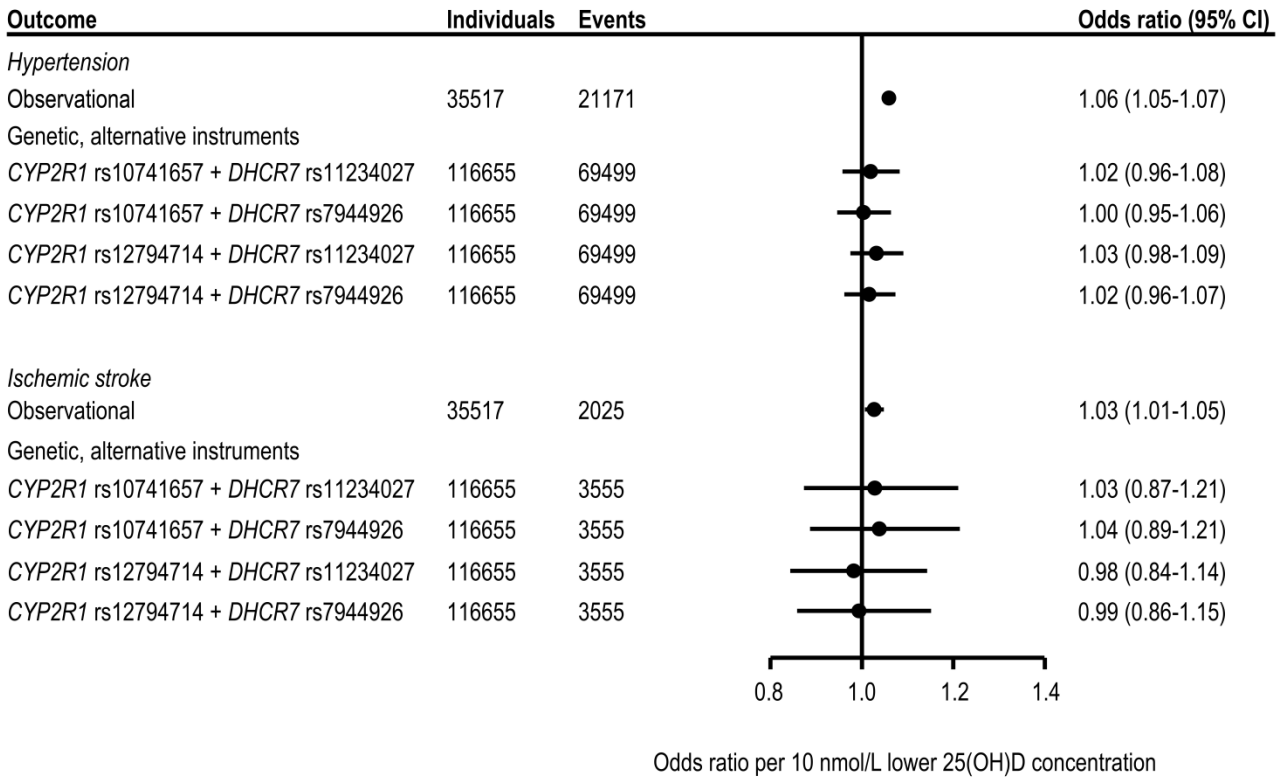
Supplementary Figure S6. Risk of ischemic stroke according to individual genotypes and unweighted and weighted allele scores. Analyses were adjusted for age, sex, and study ischemic stroke was analysed using cox regression. These analyses were prospective, thus, the sample size for ischemic stroke was reduced, since those with previous and those recruited after end of follow-up for ischemic stroke were excluded (N=2257, see Supplementary Figure S1). Δ25(OH)D=difference per allele. CI = confidence interval.



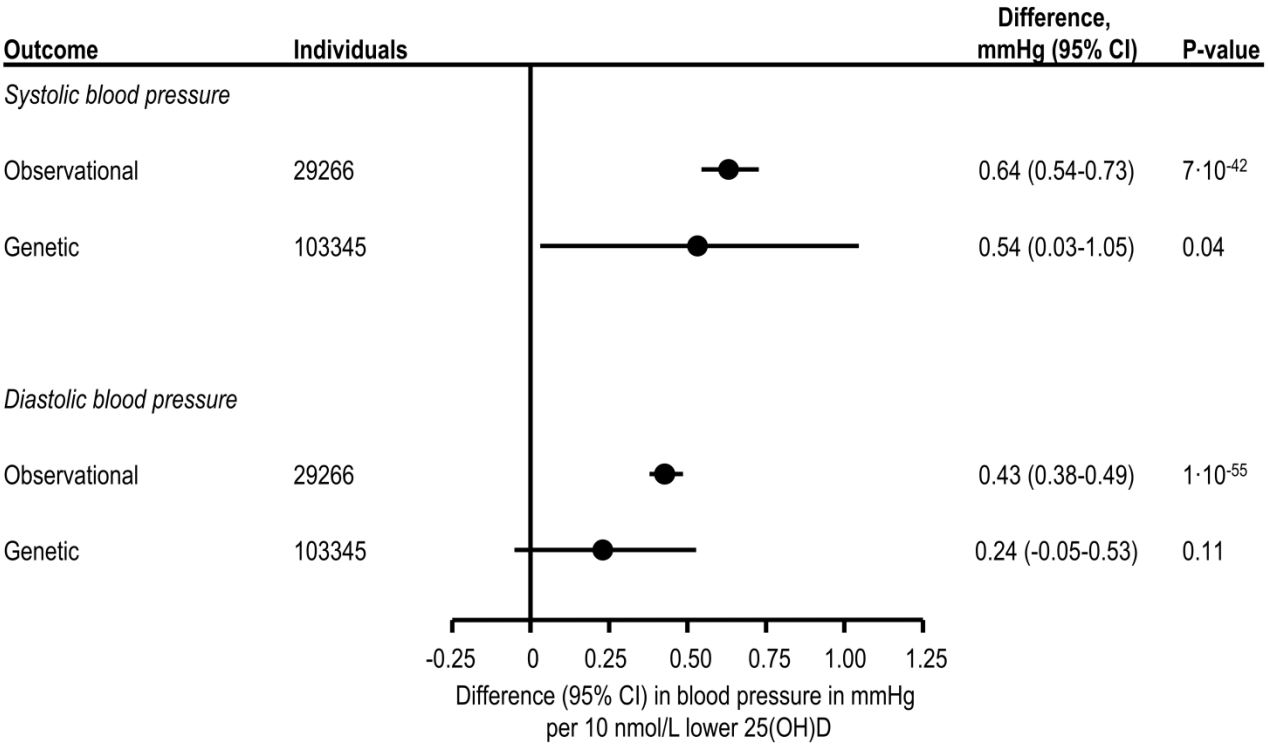
Supplementary Figure S7. Concentrations of 25-hydroxyvitamin D adjusted for age, sex, month and year of blood sample, and study according to allele scores constructed from only two genotypes used as alternative instrumental variables in genetic analyses. Columns show mean concentrations with 95% confidence intervals, F test is for statistical strength of instrument, and R^2 is a measure of explained variation. 25-hydroxyvitamin D analyses are based on 30976 individuals from the general population (Copenhagen City Heart Study and Copenhagen General Population Study combined), where both genotypes and 25-hydroxyvitamin D were measured. These analyses were cross-sectional.



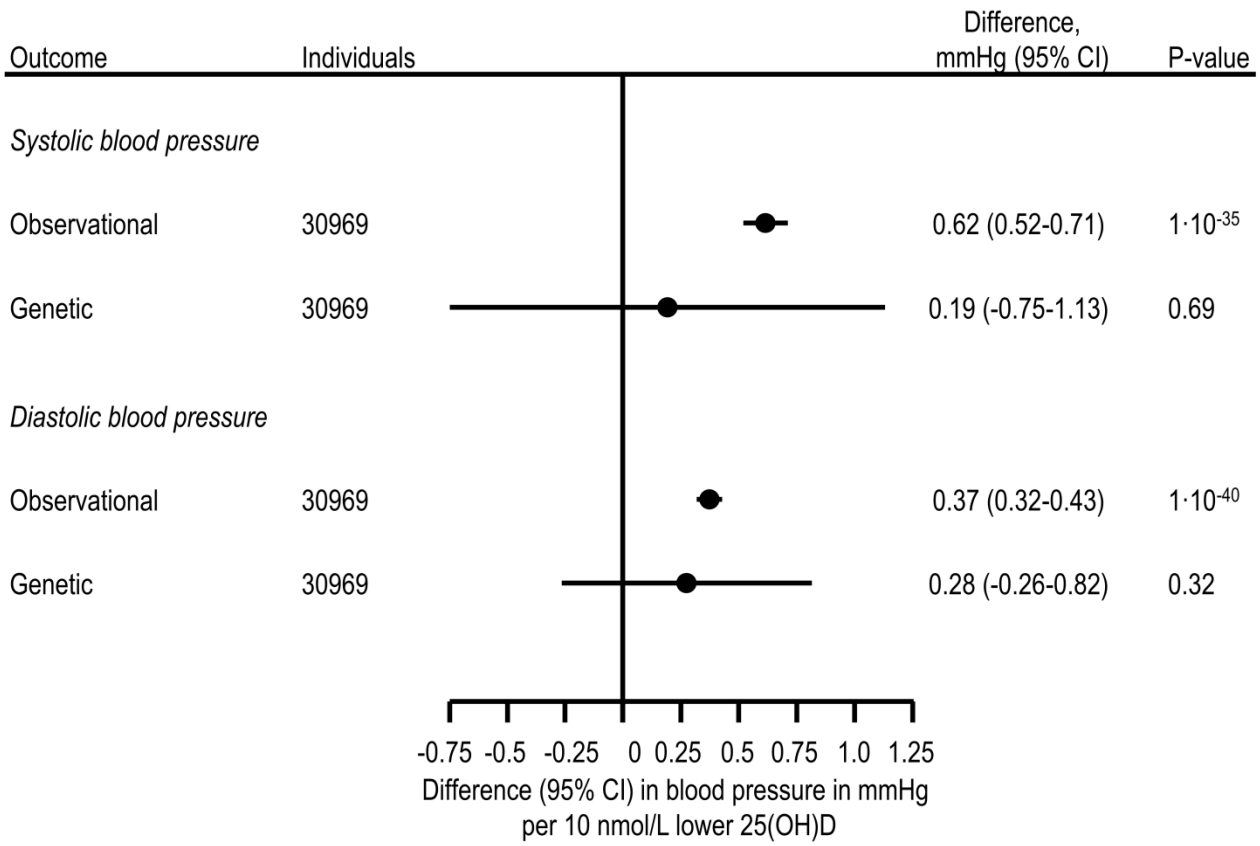
Supplementary Figure S8. Instrumental variable analysis in the general population for difference in blood pressure per 10 nmol/L lower 25-hydroxyvitamin D using combinations of only two uncorrelated genotypes as instruments. Observational estimates were by linear regression and genetic estimates by instrumental variable analyses. Analyses were adjusted for age, sex, and study. These analyses were cross-sectional. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



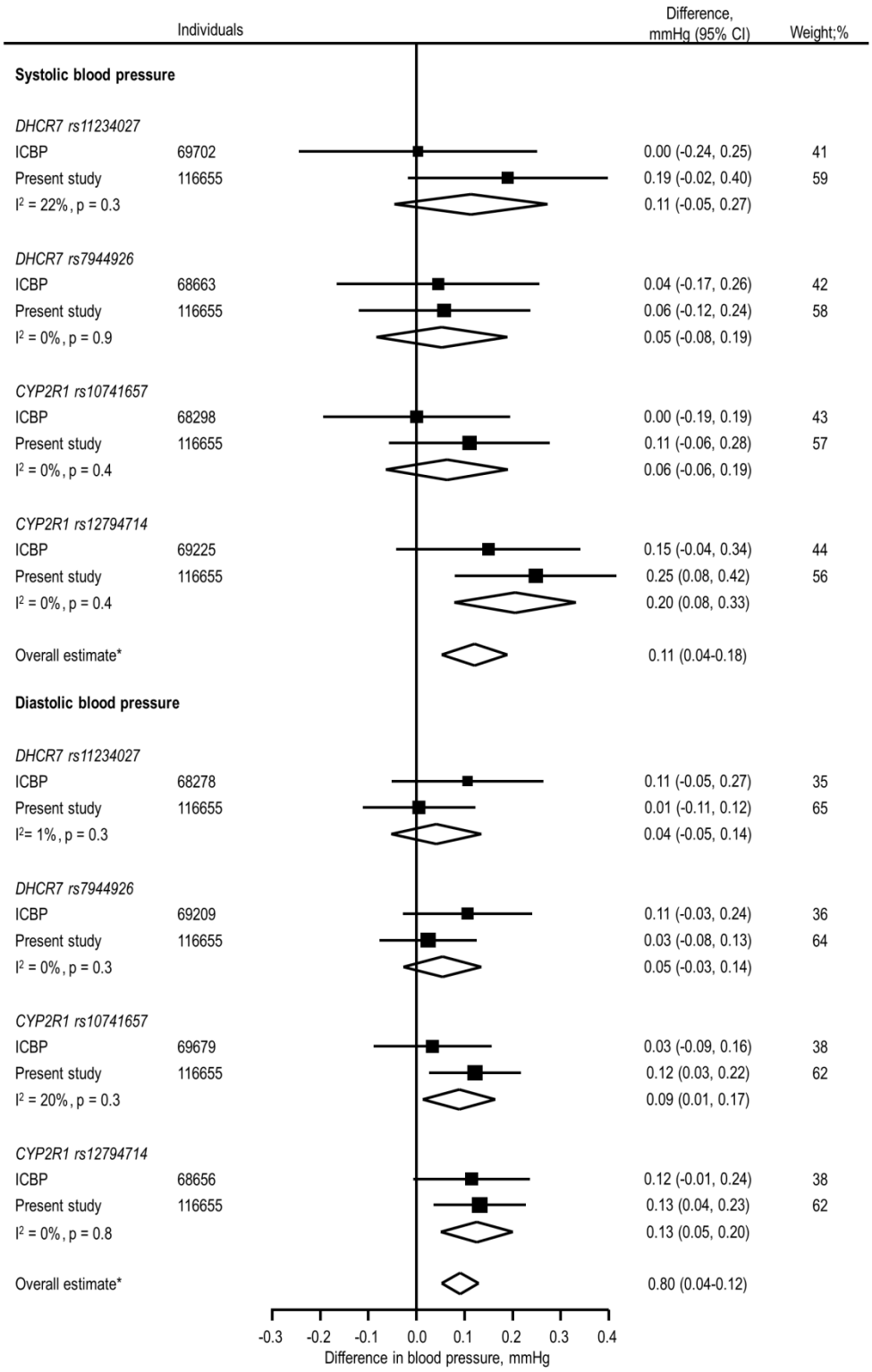
Supplementary Figure S9. Instrumental variable analysis in the general population for risk of hypertension and ischemic stroke per 10 nmol/L lower 25-hydroxyvitamin D using combinations of only two uncorrelated genotypes as instruments. Observational estimates were by logistic regression and genetic estimates by instrumental variable analyses. Analyses were adjusted for age, sex, and study. These analyses were cross-sectional. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



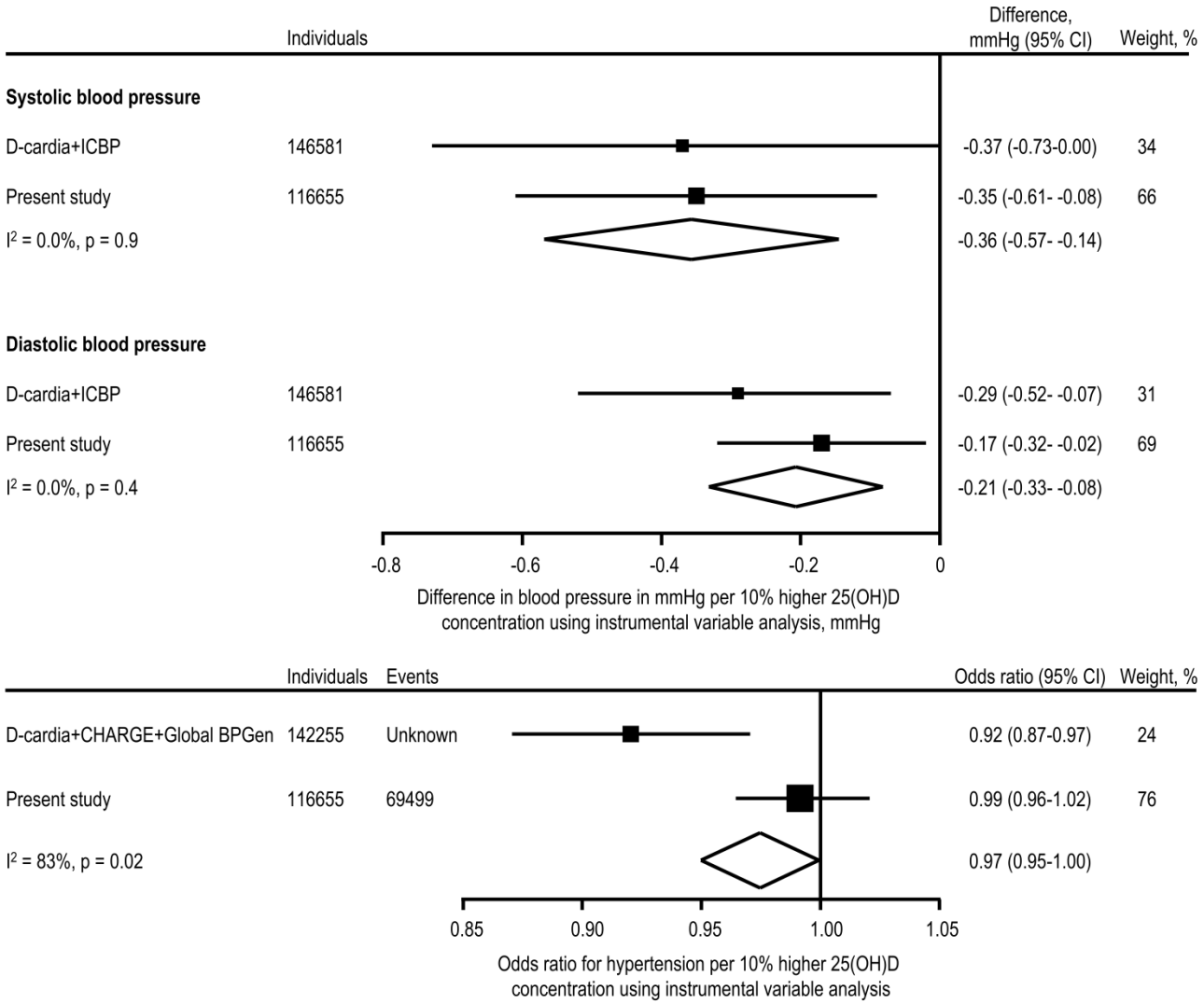
Supplementary Figure S10. Instrumental variable analysis in the general population for difference in blood pressure per 10 nmol/L lower 25-hydroxyvitamin D after exclusion of those on antihypertensive medication. Observational estimates were by linear regression and genetic estimates by instrumental variable analyses. Analyses were adjusted for age, sex, and study. These analyses were cross-sectional. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



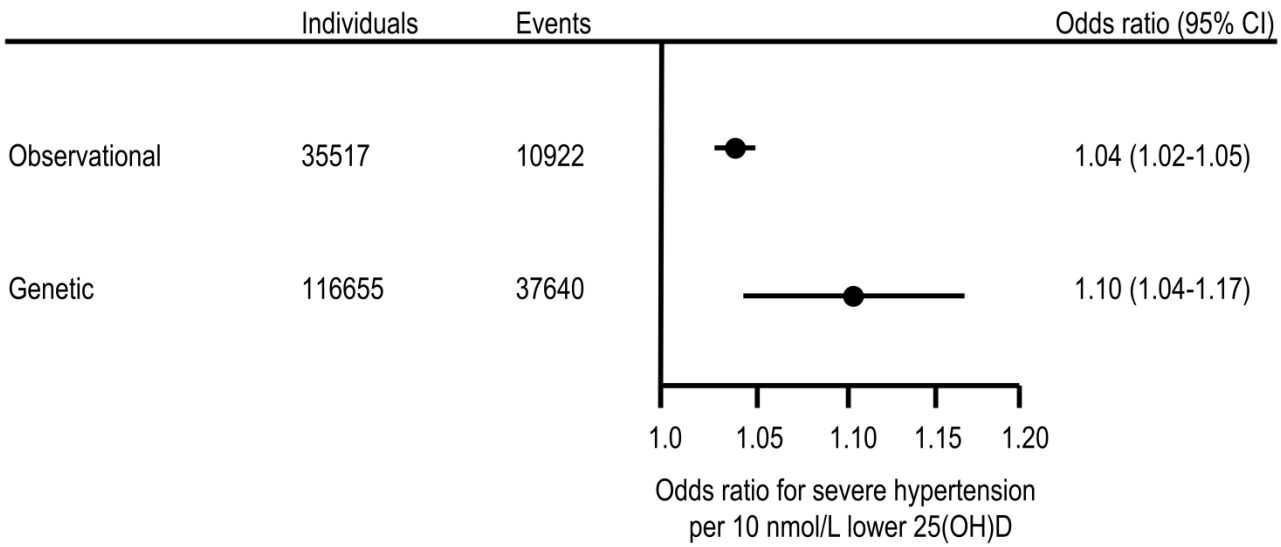
Supplementary Figure S11. Instrumental variable analysis in the general population for difference in blood pressure per 10 nmol/L lower 25-hydroxyvitamin D in the reduced sample of those with both 25(OH)D measurement and genotypes. Observational estimates were by linear regression and genetic estimates by instrumental variable analyses. Analyses were adjusted for age, sex, and study. These analyses were cross-sectional. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



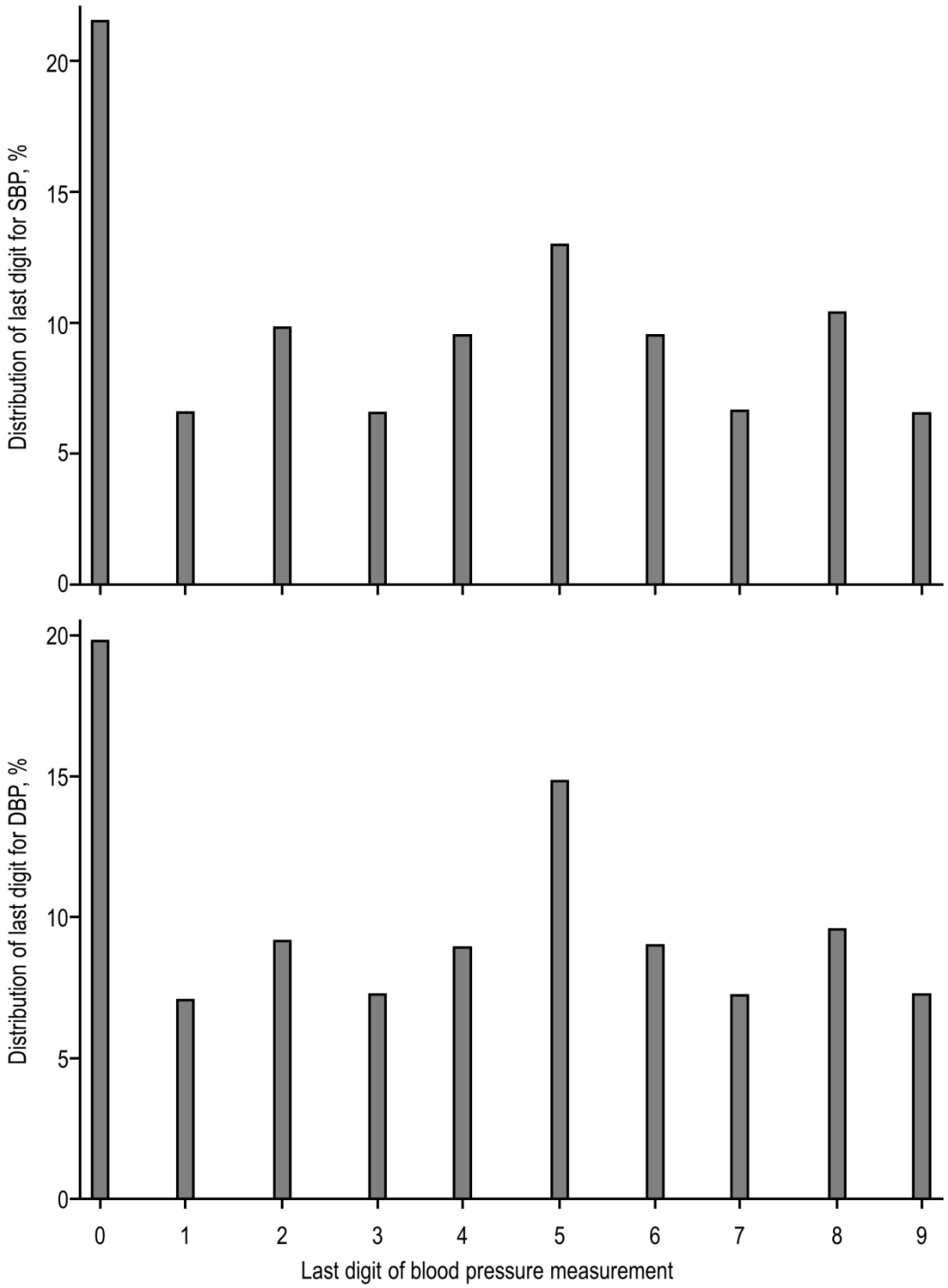
Supplementary Figure S12. Meta-analysis and comparison of difference in blood pressure with our genetic variants using ICBP and present study. Fixed effects estimates were used for the meta-analysis and I^2 was used to estimate heterogeneity between the estimates. These analyses were cross-sectional. CI = confidence interval. ICBP = International Consortium for Blood Pressure[19,20]. *Overall estimates assume independence of the individual estimates; however, the genotypes within the same gene, e.g. *DHCR7*, are correlated, but the error should be marginal.



Supplementary Figure S13. Meta-analysis and comparison of difference in blood pressure and risk of hypertension using instrumental variable analysis in D-cardia, ICBP, CHARGE, Global BPGen, and present study. Fixed effects estimates were used for the meta-analysis and I^2 was used to estimate heterogeneity between the estimates. CI = confidence interval. D-cardia = Vitamin D and the risk of cardiovascular disease. ICBP = International Consortium for Blood Pressure.[19,20] CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology.[21] Global BPGen=Global Blood Pressure Genetics.[22]



Supplementary Figure S14. Observational and genetic risk estimates for severe hypertension in the general population for a 20 nmol/L lower 25-hydroxyvitamin D concentration. Severe hypertension was defined as systolic/diastolic blood pressure > 160/100 mmHg or use of antihypertensive medication. Observational estimates were by logistic regression and genetic estimates by instrumental variable analyses. Analyses were adjusted for age, sex, month and year of blood sample, and study. These analyses were cross-sectional. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



Supplementary Figure S15. Last digit preference in blood pressure measurements. These analyses were cross-sectional. SBP = systolic blood pressure. DBP = diastolic blood pressure.

References for Figures

19. Ehret GB, Munroe PB, Rice KM et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103-109.
20. Wain LV, Verwoert GC, O'Reilly PF et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. *Nat Genet*. 2011;43:1005-1011.
21. Levy D, Ehret GB, Rice K et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet*. 2009;41:677-687.
22. Newton-Cheh C, Johnson T, Gateva V et al. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet*. 2009;41:666-676.