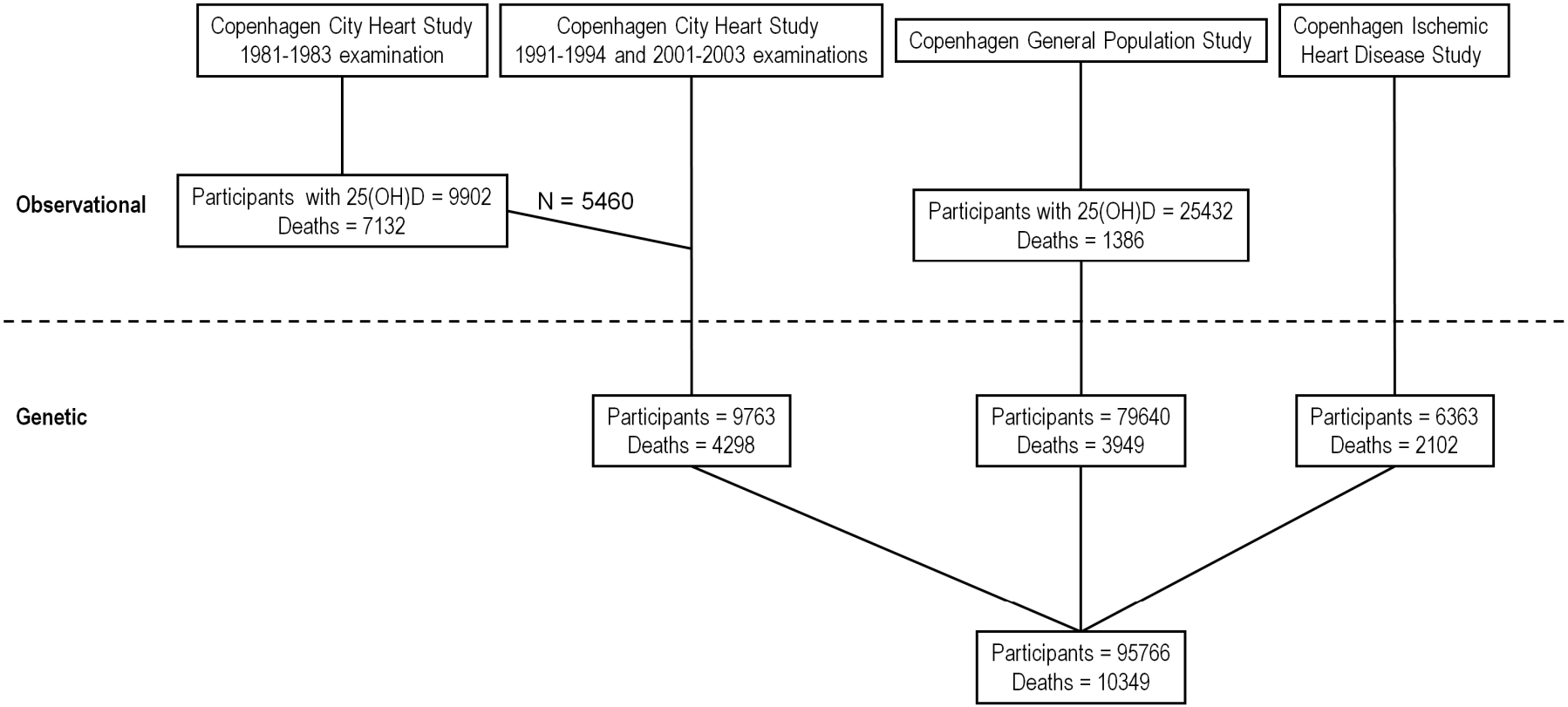
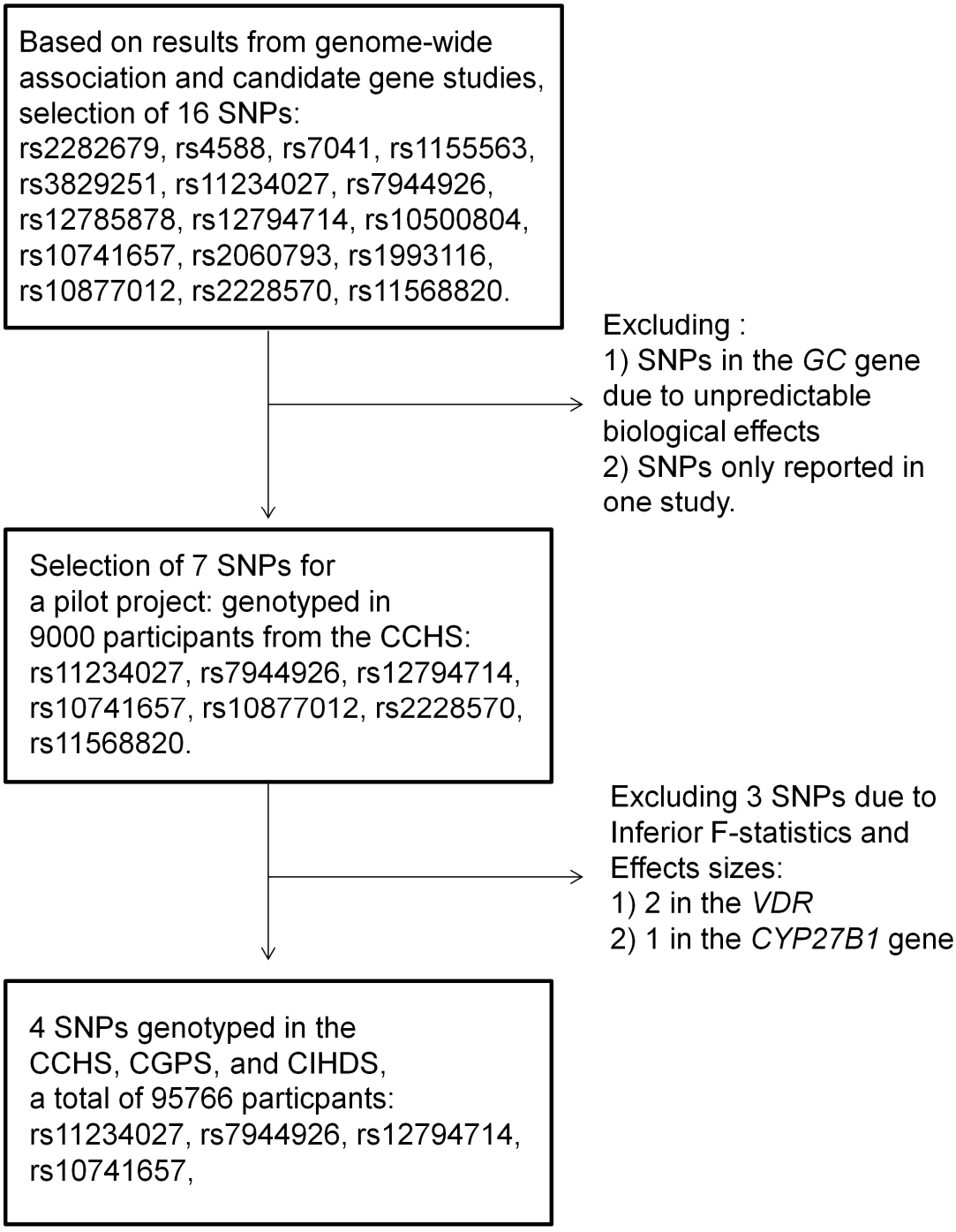
CONTENT

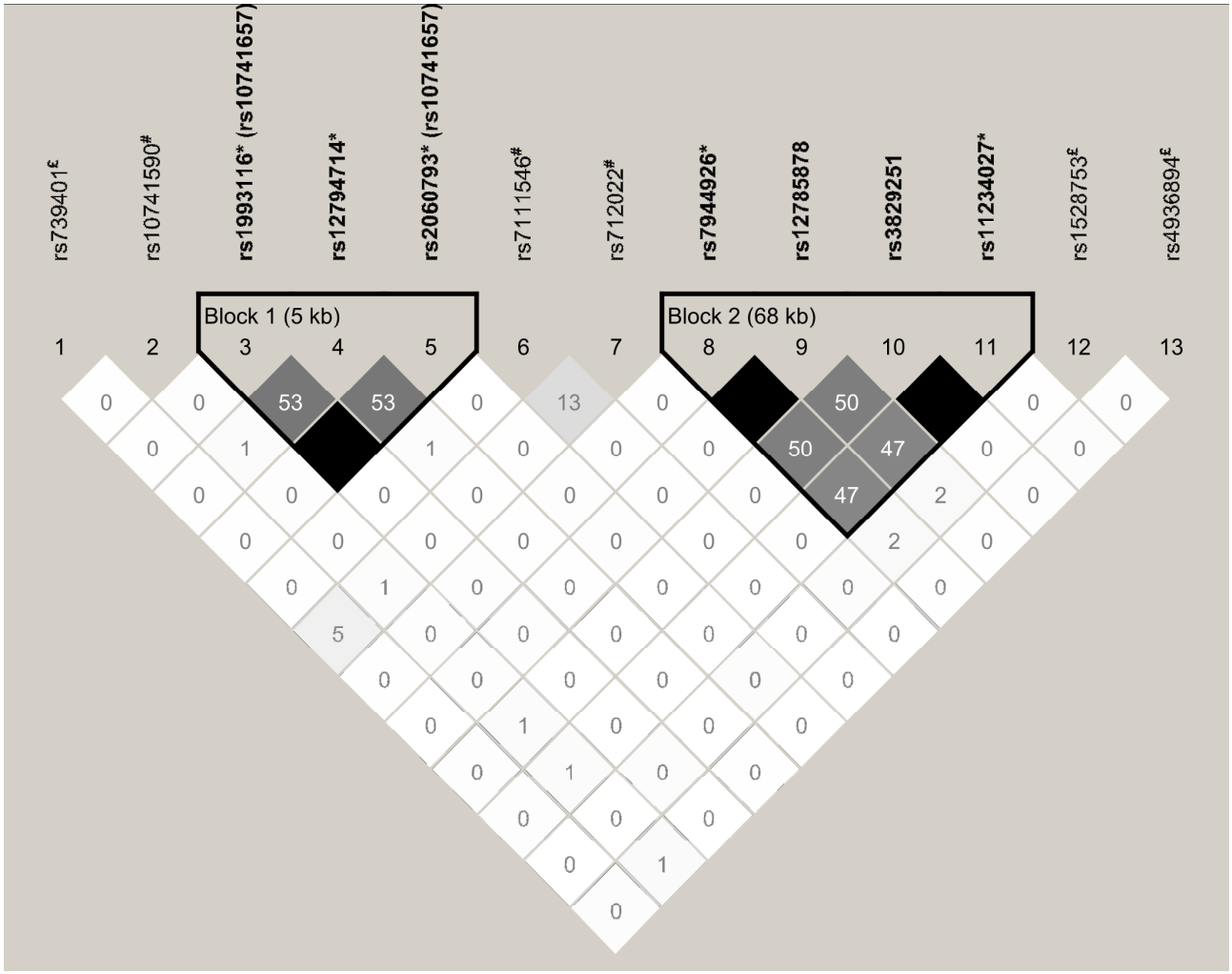
Figures



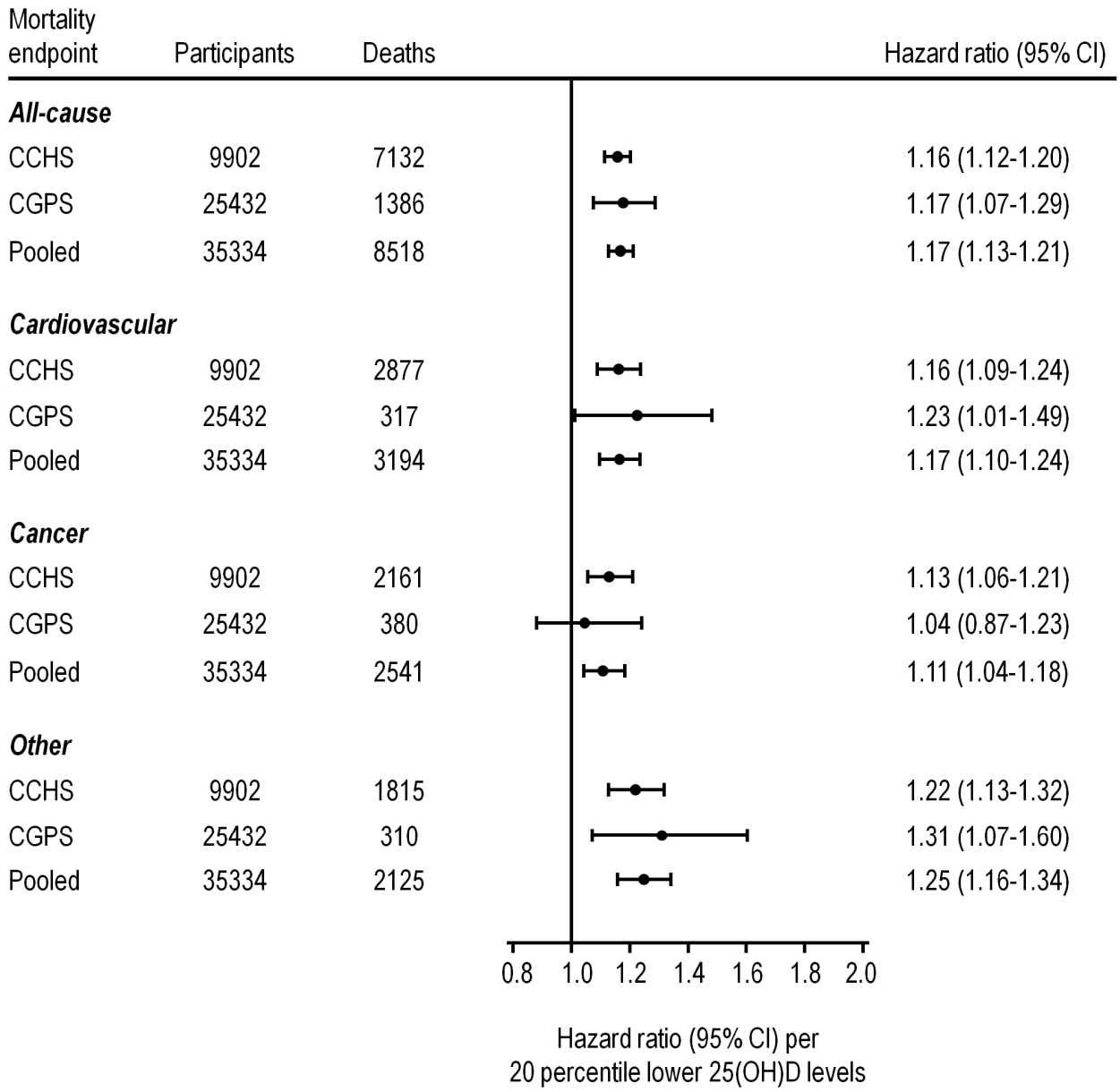
Supplementary figure A. Number of participants and events in the observational and genetic analyses.



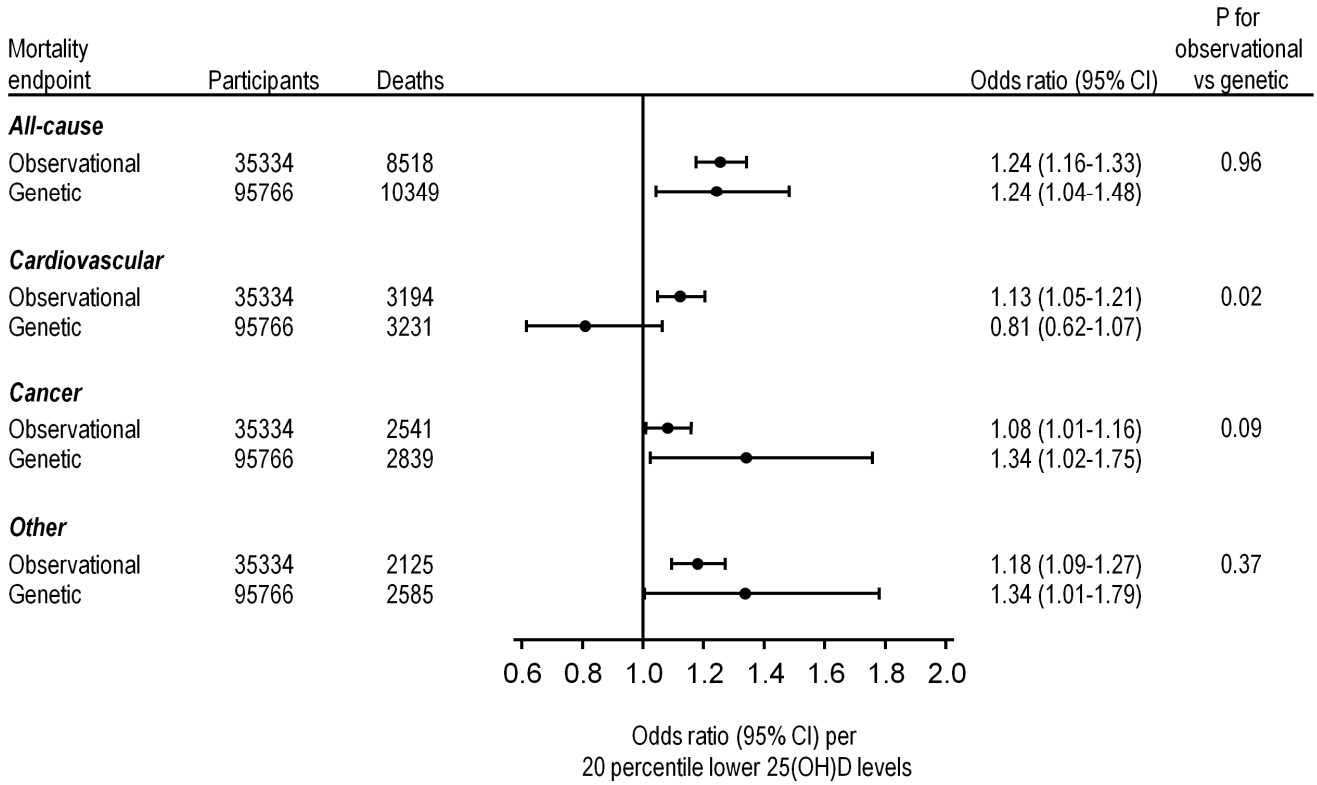
Supplementary figure B. Flowchart showing the selection process for determining which genetic instruments to use for Mendelian randomization. CCHS = Copenhagen City Heart Study. CGPS = Copenhagen General Population Study. CIHDS = Copenhagen Ischemic Heart Disease Study.



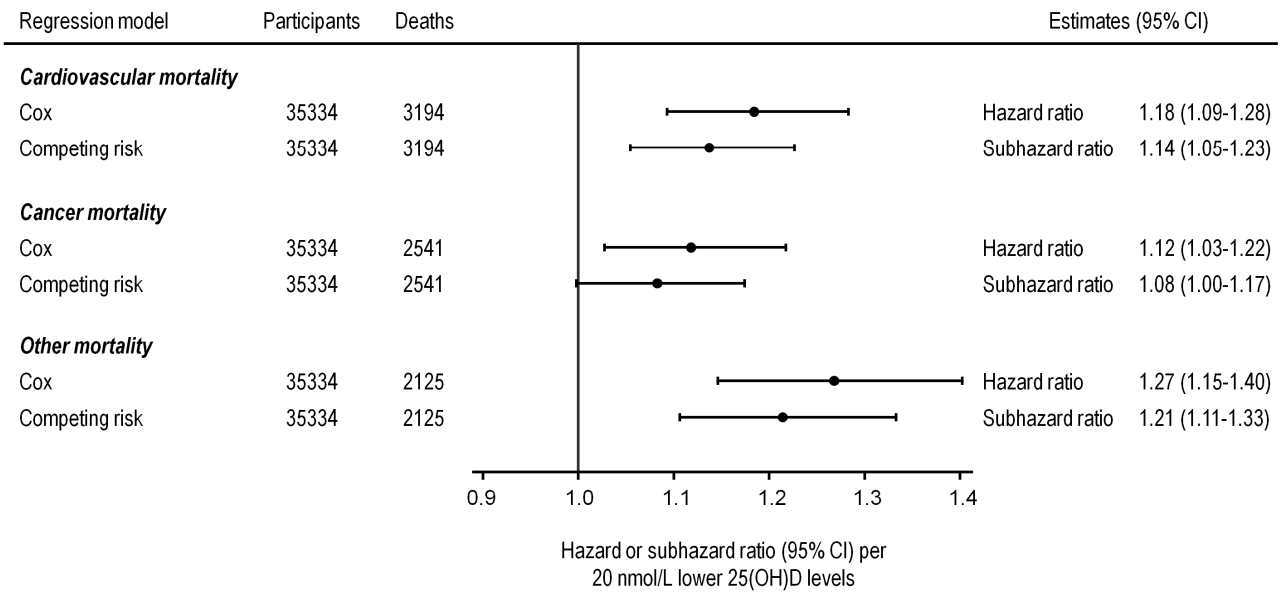
Supplementary figure C. Linkage disequilibrium plot of genetic variants on chromosome 11 found in genome-wide association studies to be associated with mortality, longevity, or 25-hydroxyvitamin D levels. The numbers in the quadrates are R^2-values in percent. The marked haplotype blocks are genetic variants associated with 25-hydroxyvitamin D in *CYP2R1* (left) and *DHCR7* (right). Based on the HapMap population. *Genotyped in the present study or tagging polymorphisms of these genotypes (R^2>0.9). #Variants possibly associated with mortality in patients with kidney or heart disease[1;2]. £Variants associated with mortality/longevity[3-5].



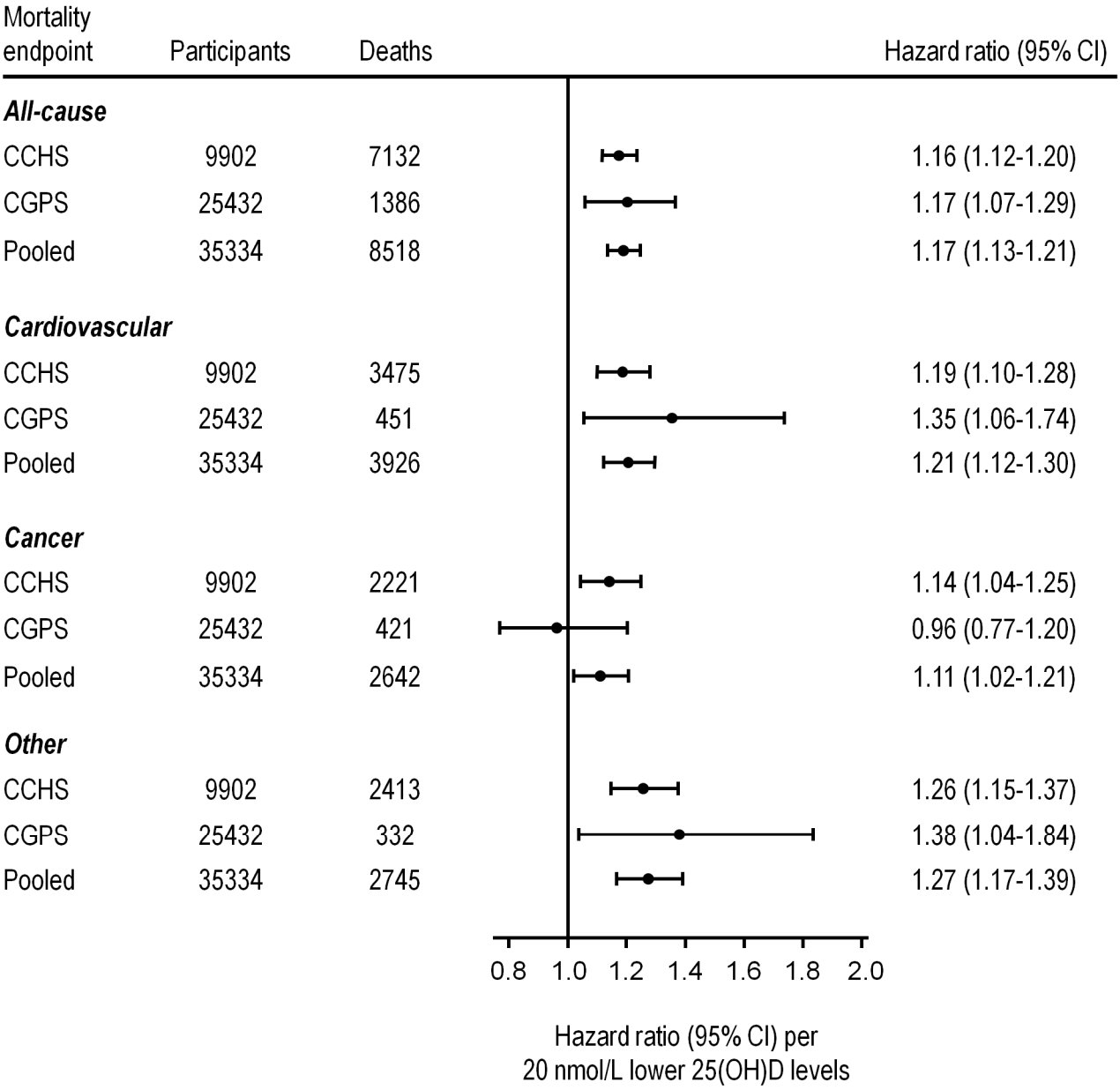
Supplementary figure D. The association of deseasonalised plasma 25-hydroxyvitamin D with cause-specific mortality in the general population. The analyses were by Cox regression adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



Supplementary figure E. Instrumental variable analysis using deseasonalised plasma 25-hydroxyvitamin D. Observational and genetic risk estimates for all-cause and cause-specific mortality for 20 percentile lower 25-hydroxyvitamin D levels. Observational estimates were by logistic regression and genetic estimates by instrumental variable analyses. Observational analyses were adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, and study. Genetic analyses were adjusted for age, year of birth, sex, and study. Observational estimates were based on participants from the Copenhagen City Heart Study and Copenhagen General Population Study combined, while genetic estimates were based on participants from the Copenhagen City Heart Study, Copenhagen General Population Study, and Copenhagen Ischemic Heart Disease Study combined. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.

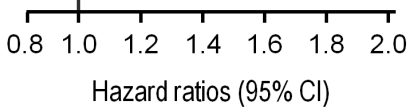


Supplementary figure F. The association of plasma 25-hydroxyvitamin D with cause-specific mortality in the general population using Cox and competing risks regression. The analyses were carried out using Cox regression or Fine and Gray's competing risk proportional subhazard models adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, season (month and year of blood sample), and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.

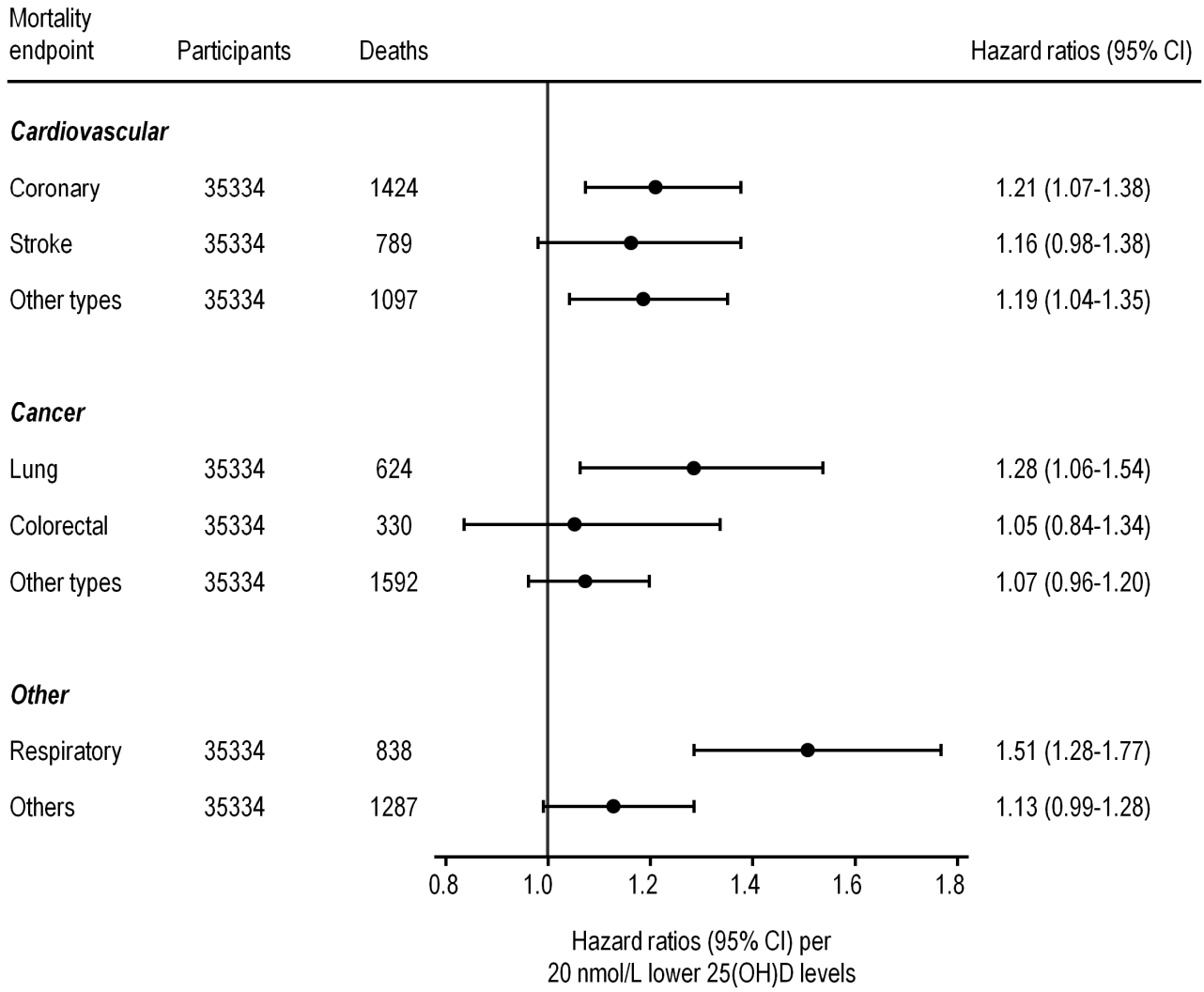


Supplementary figure G. The association of plasma 25-hydroxyvitamin D with cause-specific mortality in the general population using an alternative classification of cause-specific mortality. The regression model was identical to the main analyses; however, endpoints were classified in an overlapping manor, e.g., if there was both a cardiovascular and a cancer diagnosis on a death certificate that person would be included in analyses of both endpoints. Furthermore, all diagnoses were used and not only the three ranked diagnoses per person. There were up to 32 years of follow-up in the Copenhagen City Heart Study whereas it was up to 9.4 years in the Copenhagen General Population Study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval. CCHS = Copenhagen City Heart Study. CGPS = Copenhagen General Population Study.

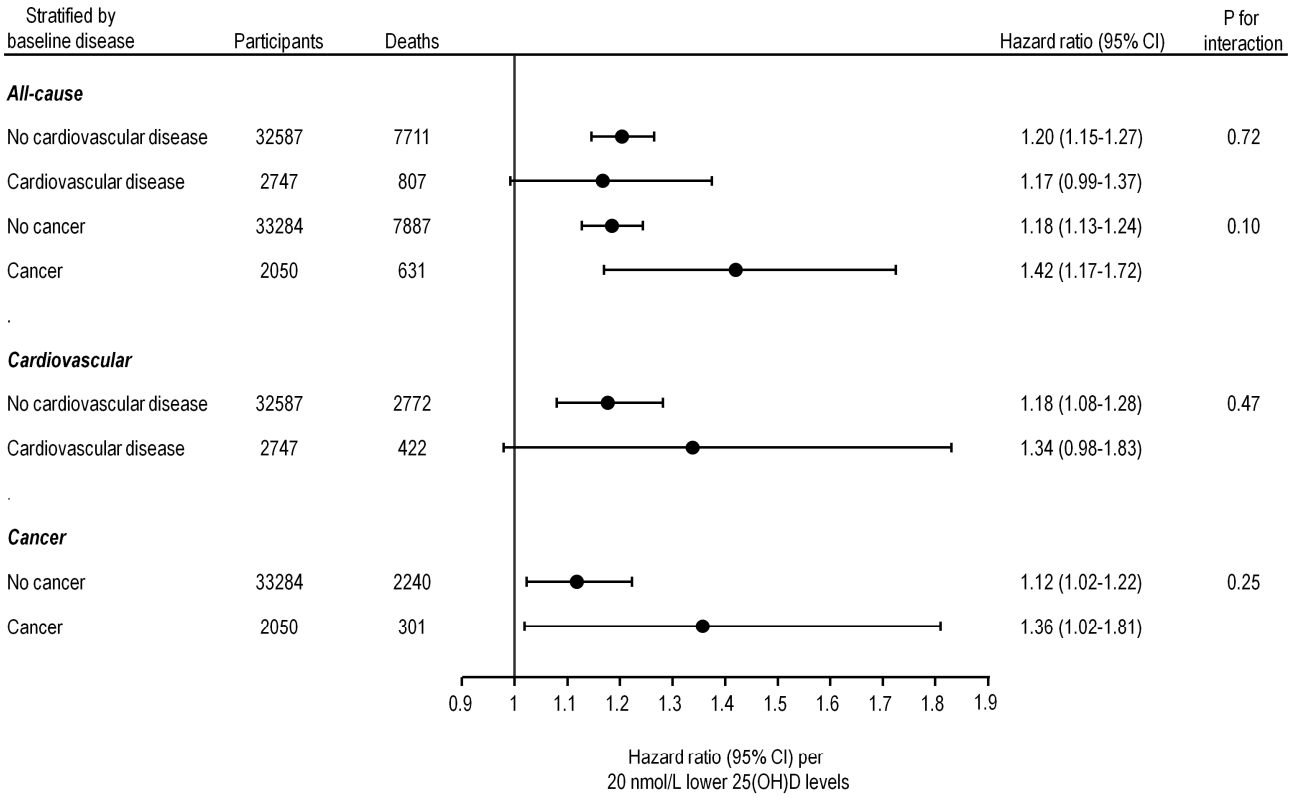
25(OH)D, nmol/L	Participants	Deaths	Hazard ratio (95% CI)	P_{trend}
All-cause				
>75	5854	767	1.00	$2 \cdot 10^{-15}$
50.1-75	11119	2118	1.04 (0.96-1.14)	
25.1-50	13095	3398	1.11 (1.02-1.21)	
≤25	5266	2235	1.36 (1.24-1.49)	
Cardiovascular				
>75	5854	269	1.00	$9 \cdot 10^{-6}$
50.1-75	11119	723	0.99 (0.86-1.15)	
25.1-50	13095	1321	1.08 (0.94-1.24)	
≤25	5266	881	1.30 (1.12-1.50)	
Cancer				
>75	5854	239	1.00	0.002
50.1-75	11119	650	1.08 (0.93-1.25)	
25.1-50	13095	1024	1.12 (0.97-1.30)	
≤25	5266	628	1.26 (1.08-1.48)	
Other				
>75	5854	187	1.00	$4 \cdot 10^{-7}$
50.1-75	11119	530	1.15 (0.96-1.37)	
25.1-50	13095	808	1.15 (0.96-1.37)	
≤25	5266	600	1.58 (1.31-1.90)	



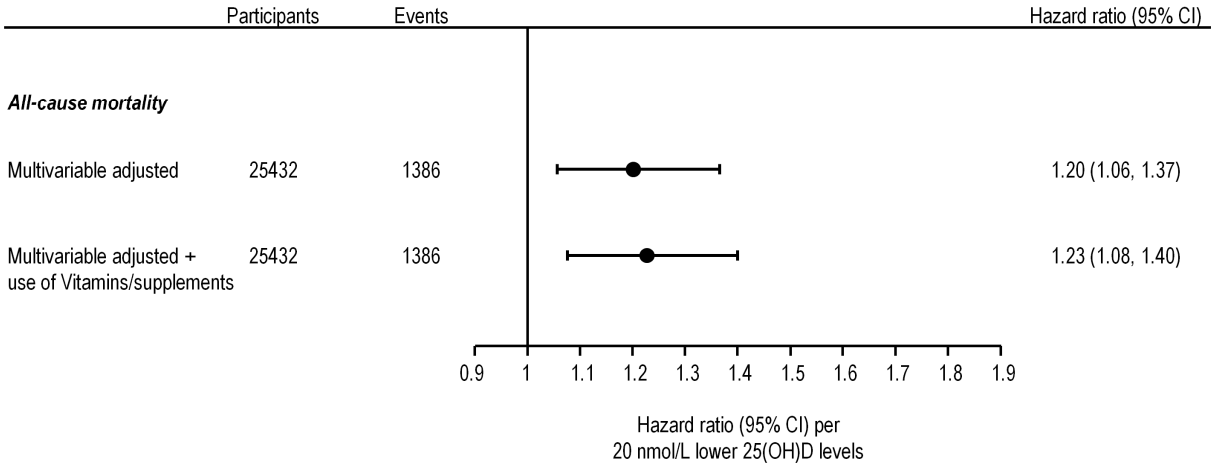
Supplementary figure H. The association of plasma 25-hydroxyvitamin D with cause-specific mortality in the general population using plasma 25-hydroxyvitamin D in clinical categories. The analyses were by Cox regression adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, season (month and year of blood sample), and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



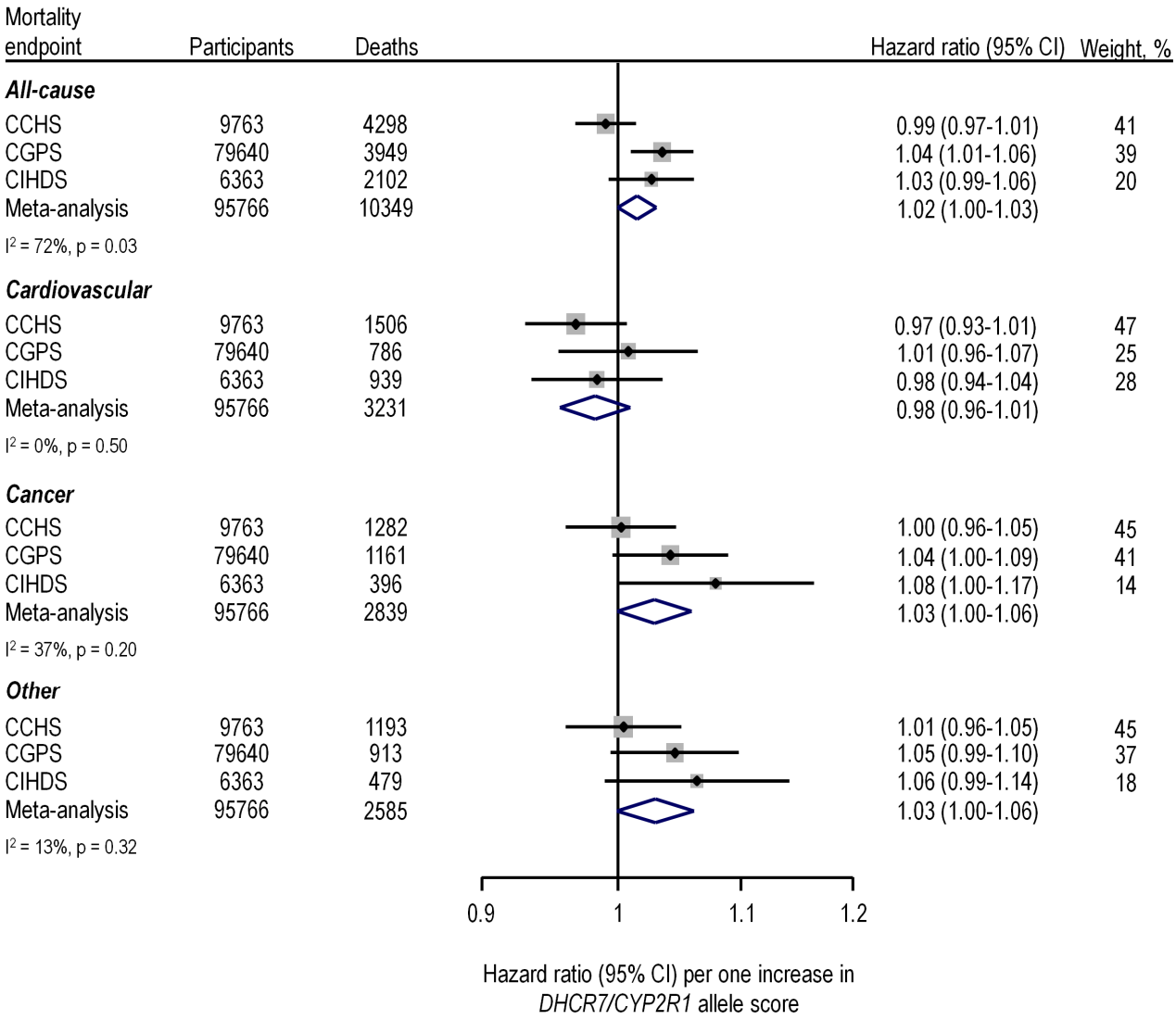
Supplementary figure I. The association of plasma 25-hydroxyvitamin D with cause-specific mortality using further subdivision of mortality endpoints. The analyses were by Cox regression adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, season (month and year of blood sample), and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval. ***Cardiovascular (ICD-8: 390-458, ICD-10:I00-I99).*** Coronary (ICD-8: 410-414, ICD-10: I20-I25). Stroke (ICD-8: 430-438, ICD-10: I60-I69). ***Cancer (ICD-8: 140-209, ICD-10:C00-C97).*** Lung (ICD-8: 162, ICD-10:C33-C34). Colorectal (ICD-8: 153-154, ICD-10:C18-C21). ***Other (remaining ICD diagnoses).*** Respiratory (ICD-8: 460-519, ICD-10: J00-J99).



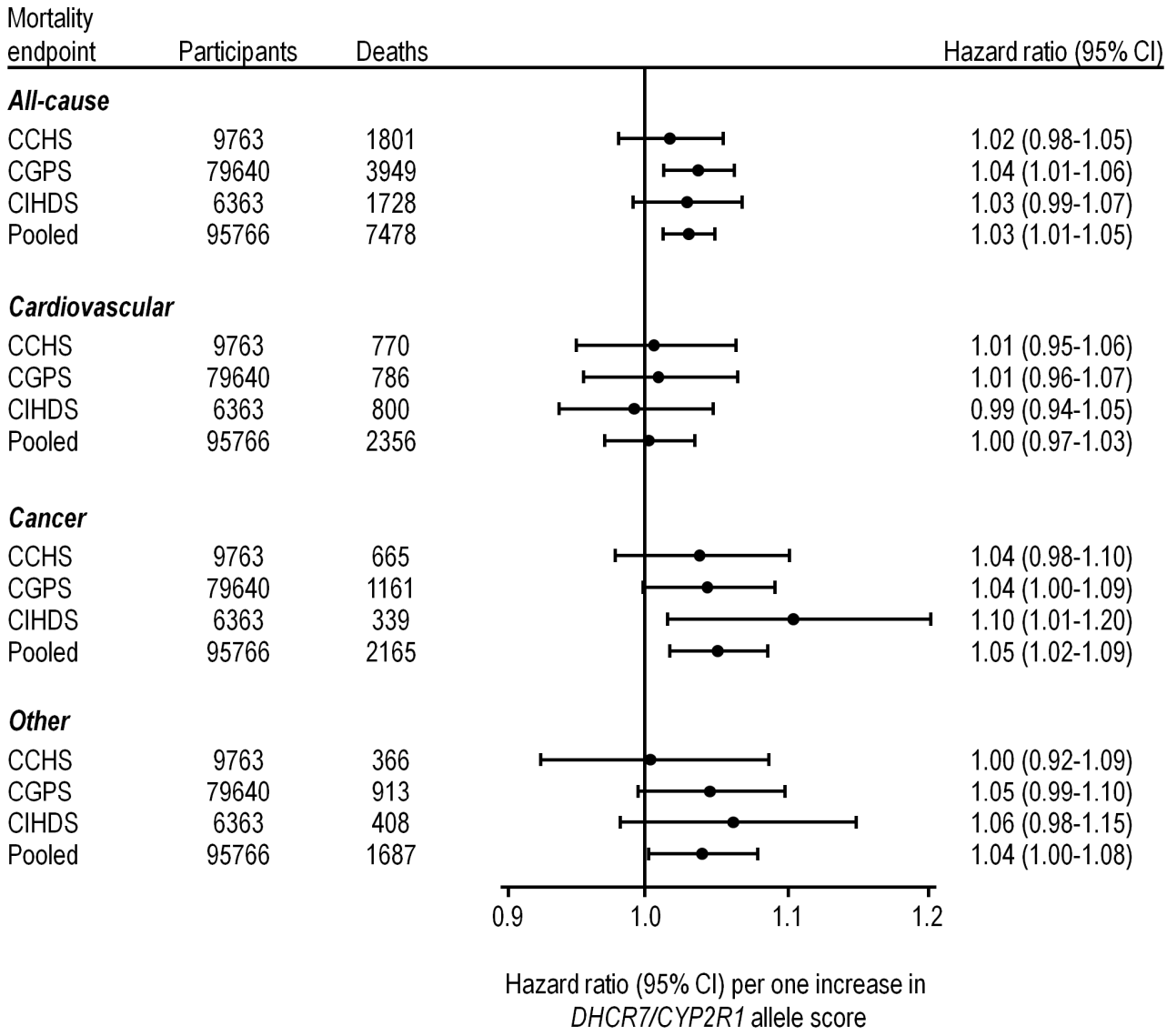
Supplementary figure J. The association of plasma 25-hydroxyvitamin D with all-cause, cardiovascular, and cancer mortality in analyses stratified according to baseline disease. The analyses were by Cox regression adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, season (month and year of blood sample), and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



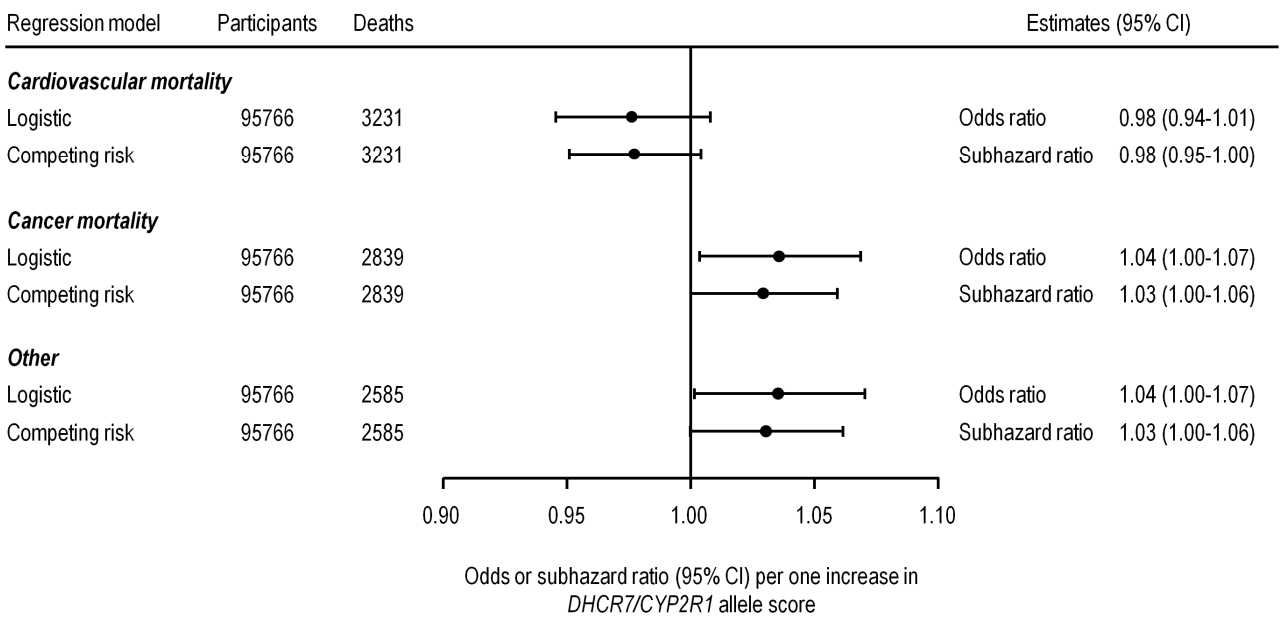
Supplementary figure K. The association of plasma 25-hydroxyvitamin D with all-cause mortality in analyses with and without adjustment for vitamin or nutritional supplement intake in CGPS. The analyses were by Cox regression adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income, diabetes, plasma cholesterol, season (month and year of blood sample), and study. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



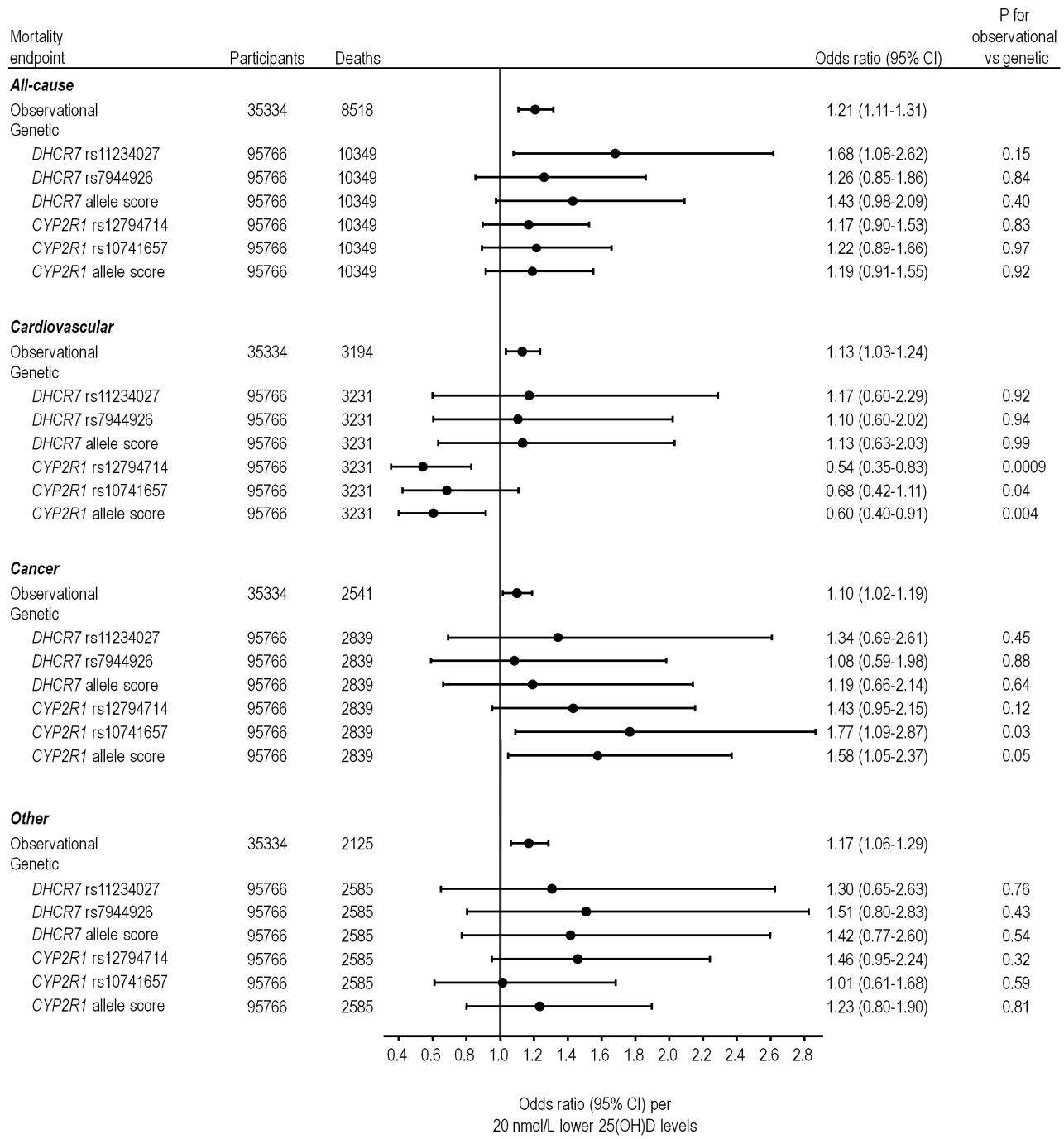
Supplementary figure L. All-cause and cause-specific mortality according to the DHCR7/CYP2R1 allele score using meta-analysis with fixed effect estimates instead of pooling of the studies. The analyses were carried out using Cox regression adjusted for age, year of birth, and sex. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval. CCHS = Copenhagen City Heart Study. CGPS = Copenhagen General Population Study. CIHDS = Copenhagen Ischemic Heart Disease Study.



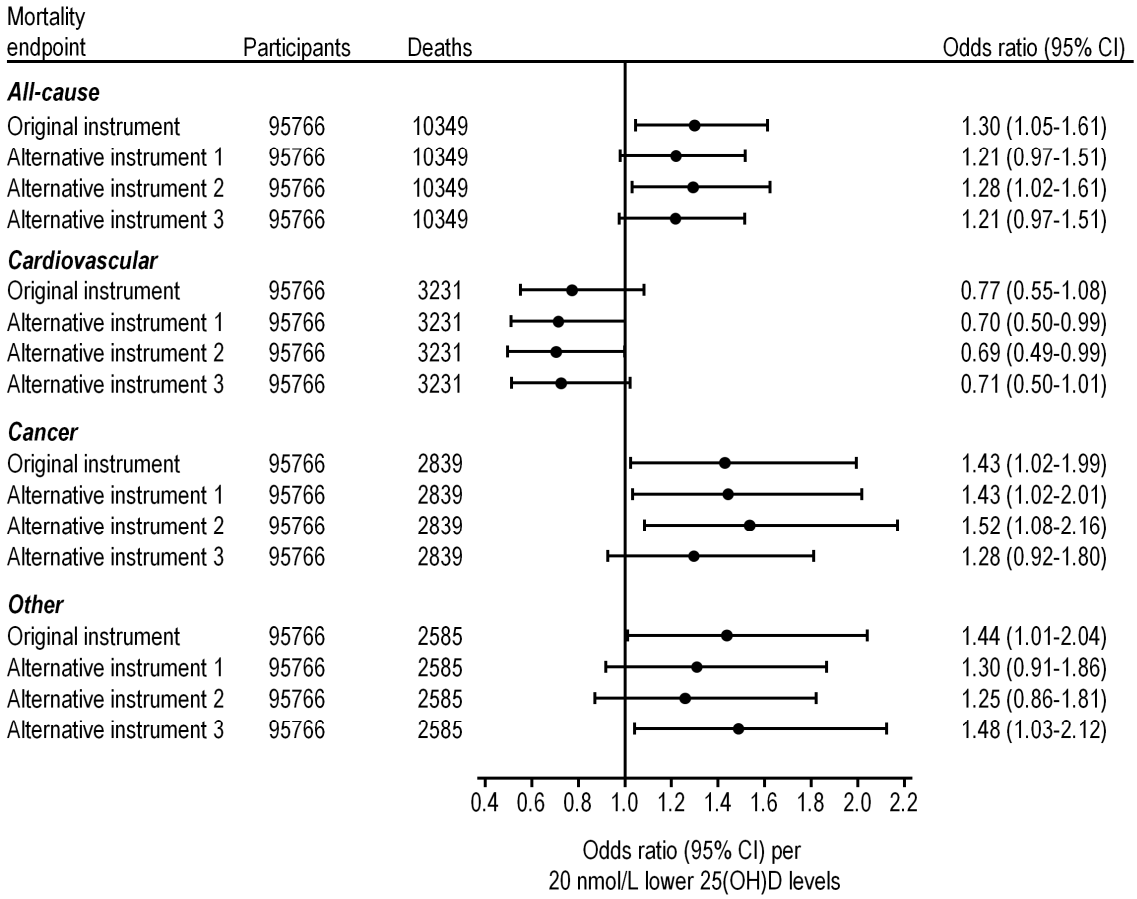
Supplementary figure M. All-cause and cause-specific mortality according to the DHCR7/CYP2R1 allele score using Cox regression restricted to 9.4 years of follow-up. The analyses were carried out using Cox regression adjusted for age, year of birth, sex, and study; the latter only in the pooled analyses. Restriction in follow-up corresponded to the longest follow-up in the Copenhagen General Population Study, which started recruiting last. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval. CCHS = Copenhagen City Heart Study. CGPS = Copenhagen General Population Study. CIHDS = Copenhagen Ischemic Heart Disease Study.



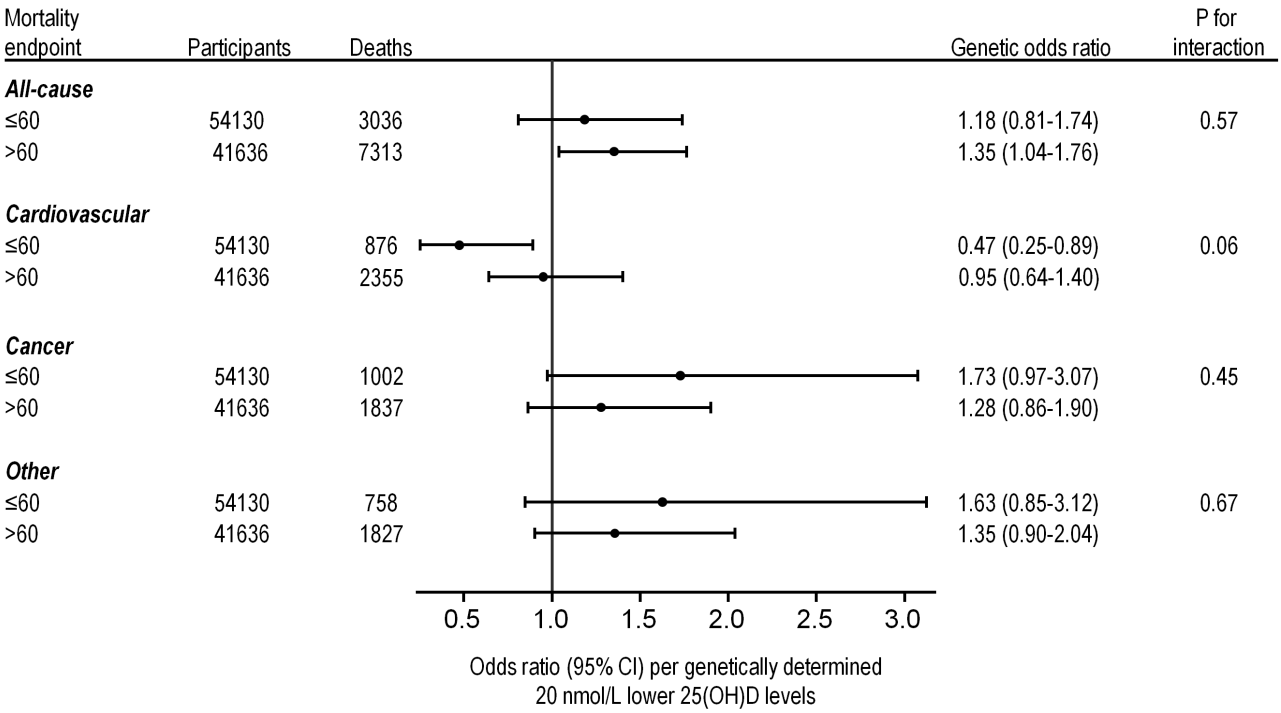
Supplementary figure N. All-cause and cause-specific mortality according to the DHCR7/CYP2R1 allele score using logistic or competing risks regression. The analyses were carried out using logistic regression or Fine and Gray's competing risk proportional subhazard models adjusted for age, year of birth, sex, and study; the latter only in the pooled analyses. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



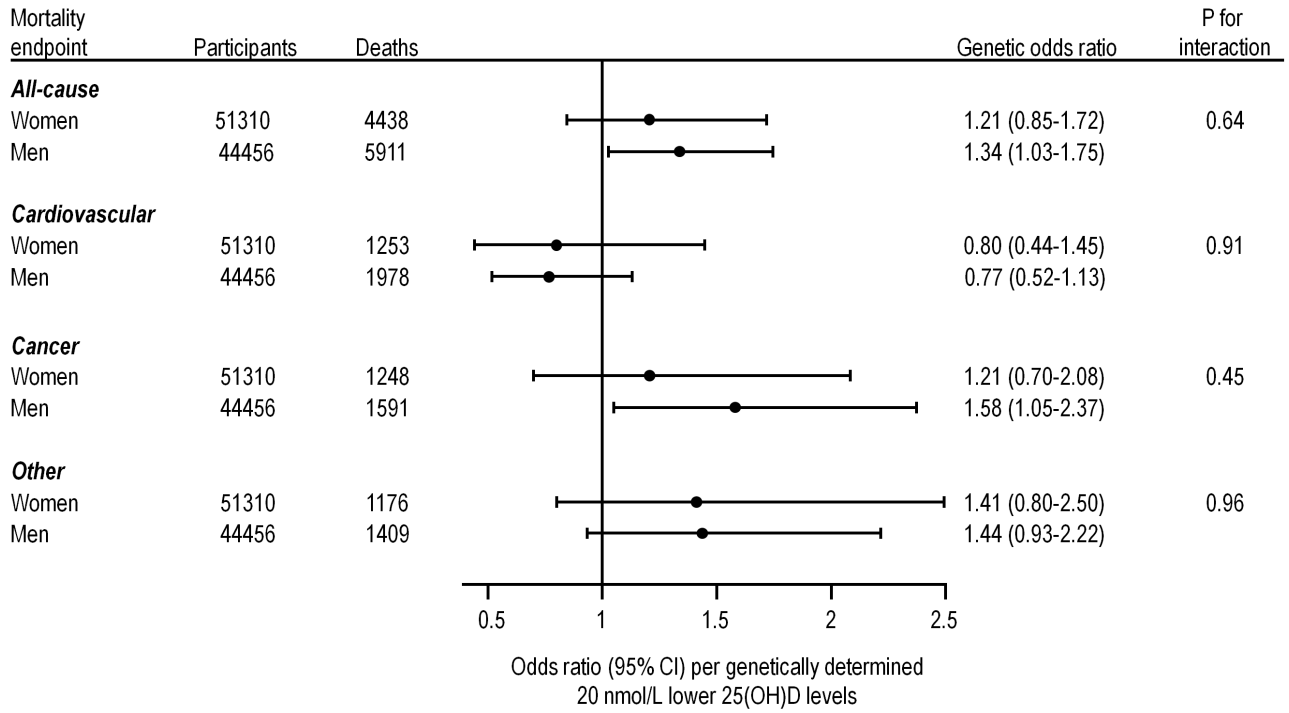
Supplementary figure O. All-cause and cause-specific mortality when using each genotype as an instrument separately. Genetic estimates were by instrumental variable analyses for 20 nmol/L lower 25-hydroxyvitamin D levels. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



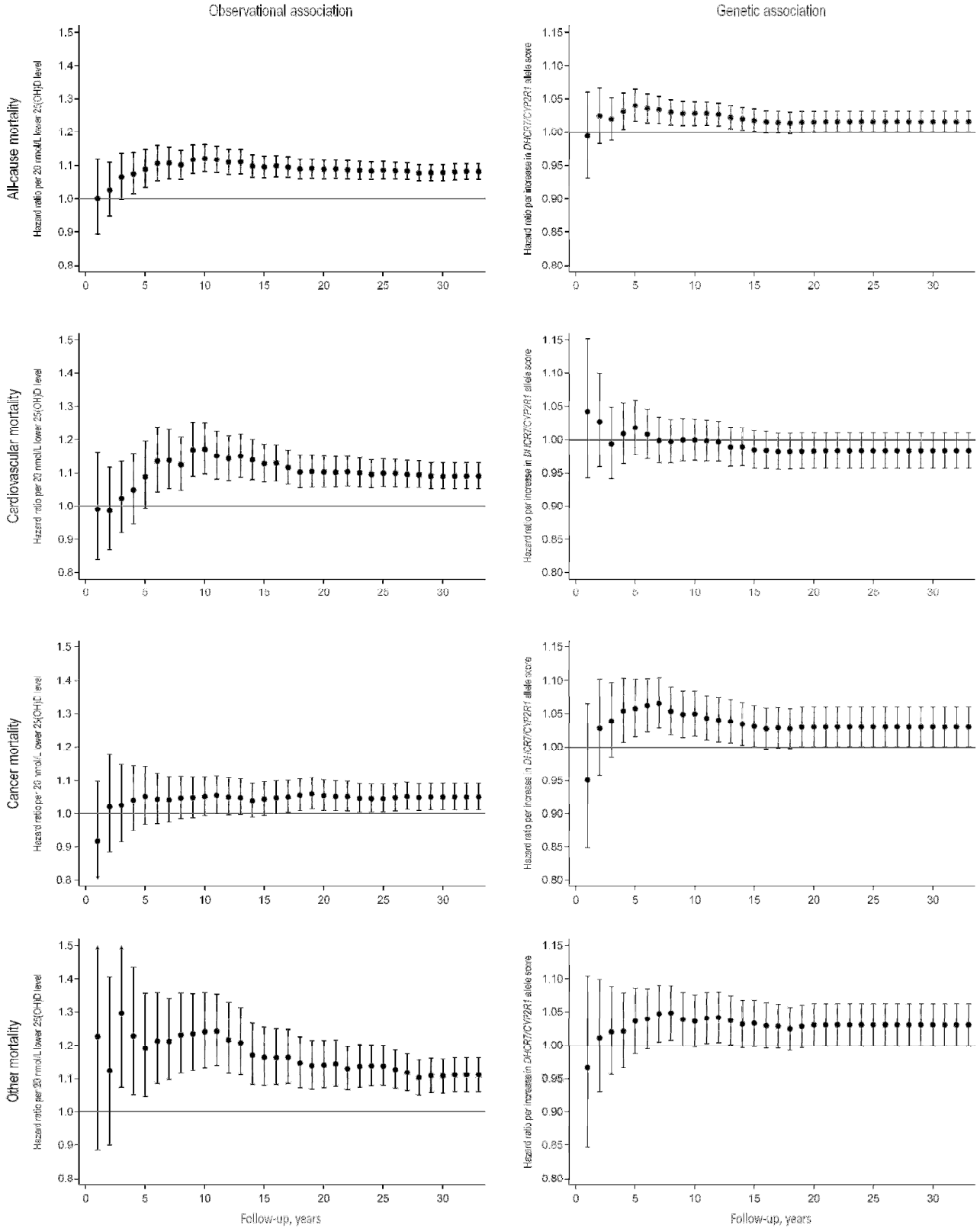
Supplementary figure P. All-cause and cause-specific mortality when using alternative definitions of the instrument. Genetic estimates were by instrumental variable analyses for 20 nmol/L lower 25-hydroxyvitamin D levels. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.
Alternative instrument 1 = allele score based on *DHCR7* rs7944926 + *CYP2R1* rs10741657 + *CYP2R1* rs12794714
Alternative instrument 2 = allele score based on *DHCR7* rs11234027 + *CYP2R1* rs10741657 + *CYP2R1* rs12794714
Alternative instrument 3 = allele score based on *DHCR7* rs7944926 + *CYP2R1* rs12794714



Supplementary figure Q. Genetic risk estimates for all-cause and cause-specific mortality according to age stratified analyses. Genetic estimates were by instrumental variable analyses for a 20 nmol/L lower 25-hydroxyvitamin D levels. Genetic estimates were based on participants from the Copenhagen City Heart Study, Copenhagen General Population Study, and Copenhagen Ischemic Heart Disease Study combined. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



Supplementary figure R. Genetic risk estimates for all-cause and cause-specific mortality according to sex stratified analyses. Genetic estimates were by instrumental variable analyses for a 20 nmol/L lower 25-hydroxyvitamin D levels. Genetic estimates were based on participants from the Copenhagen City Heart Study, Copenhagen General Population Study, and Copenhagen Ischemic Heart Disease Study combined. 25(OH)D = 25-hydroxyvitamin D. CI = confidence interval.



Supplementary figure S. Observational and genetic analyses according to restriction of follow-up from 1 to 32 years to evaluate the dependence of risk estimates on follow-up time. In observational analyses, Cox regression models were adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure time physical activity, systolic blood pressure, body mass index, income,

diabetes, plasma cholesterol, season, and study. Observational estimates were based on participants from the Copenhagen City Heart Study and Copenhagen General Population Study combined (n = 35334). The genetic risk factor was one increase in *DHCR7/CYP2R1* allele score. In genetic analyses, models were adjusted for age, year of birth, sex, and study. Genetic estimates were based on participants from the Copenhagen City Heart Study, Copenhagen General Population Study, and Copenhagen Ischemic Heart Disease Study combined (n = 95766). Filled dots represent the point estimates and the whiskers represent the 95% confidence intervals. 25(OH)D = 25-hydroxyvitamin D.

Tables

Supplementary table A. Baseline characteristics in the general population and their association with age, sex, season, and study adjusted 25-hydroxyvitamin D (25(OH)D) quintiles.

	Adjusted 25(OH)D quintiles					P values[a]
	1st (Lowest) N = 7068	2nd N = 7066	3rd N = 7068	4th N = 7066	5th (Highest) N = 7066	
Age, years	58 (49-66)	57 (48-66)	58 (49-67)	59 (49-67)	58 (49-67)	0.62
Men, %	43	46	47	45	43	0.75
Current smoker, %	40	33	29	27	26	$2 \cdot 10^{-100}$
Cumulative tobacco consumption, pack years[c]	24 (12-38)	20 (10-34)	19 (8-34)	18 (8-32)	18 (8-32)	$9 \cdot 10^{-42}$
Alcohol consumption, units/week[d]	6 (2-14)	7 (2-14)	7 (3-14)	7 (3-14)	7 (3-14)	0.12
Leisure-time physical activity <2 hours/week , %	16	10	9	7	6	$3 \cdot 10^{-85}$
Systolic blood pressure, mmHg	139 (125-153)	136 (122-150)	136 (123-150)	135 (121-150)	133 (120-148)	$8 \cdot 10^{-32}$
Body mass index, kg/m^2	26 (23-30)	26 (23-29)	25 (23-28)	25 (23-28)	24 (22-27)	$1 \cdot 10^{-223}$
Low income, %[e]	46	39	38	37	36	$4 \cdot 10^{-30}$
Diabetes, %	6	4	4	3	3	$4 \cdot 10^{-13}$
Cholesterol, mmol/L	5.8 (5.1-6.6)	5.8 (5.0-6.4)	5.7 (5.0-6.4)	5.6 (4.9-6.4)	5.4 (4.7-6.2)	$1 \cdot 10^{-119}$
Vitamin D, nmol/L	23 (16-32)	38 (28-46)	49 (39-57)	62 (52-70)	84 (73-98)	$<1 \cdot 10^{-300}$

Continuous variables are summarised as median and interquartile range.

[a]P-values were calculated using linear regression or logistic regression as appropriate.

[c]In current and former smokers only

[d]1 unit ~ 12 g alcohol

[e]Income was classified differently in the 2 cohorts: 3 groups in CCHS and 4 groups in CGPS.

Supplementary table B. Baseline characteristics in the general population and their association with the DHCR7/CYP2R1 allele score.

	DHCR7/CYP2R1 allele score					P values[a]
	0-1 N = 15560[b]	2 N = 21842	3 N = 16172	4-5 N = 27986	6-8 N = 7843	
Age, years	57 (47-66)	57 (47-66)	57 (47-66)	57 (47-66)	57 (47-66)	0.06
Men, %	45	45	45	45	45	0.61
Current smoker, %	23	23	23	23	23	0.11
Cumulative tobacco consumption, pack years[c]	24 (12-38)	20 (10-34)	19 (8-34)	18 (8-32)	18 (8-32)	0.37
Alcohol consumption, units/week[d]	8 (3-15)	8 (3-15)	8 (3-15)	7 (3-15)	8 (3-15)	0.49
Leisure-time physical activity <2 hours/week , %	7	7	7	7	7	0.80
Systolic blood pressure, mmHg	136 (123-150)	135 (122-150)	136 (122-150)	136 (122-150)	136 (122-150)	0.03^{NS}
Body mass index, kg/m^2	26 (23-28)	25 (23-28)	26 (23-28)	26 (23-28)	25 (23-28)	0.96
Low income, %[e]	36	37	36	37	37	0.25
Diabetes, %	4	4	4	4	4	0.99
Cholesterol, mmol/L	5.6 (4.9-6.3)	5.6 (4.9-6.3)	5.6 (4.9-6.3)	5.6 (4.9-6.3)	5.6 (4.9-6.3)	0.65
Vitamin D, nmol/L[f]	55 (16-32)	53 (28-46)	51 (39-57)	49 (52-70)	47 (73-98)	$4 \cdot 10^{-72}$

Continuous variables are summarised as median and interquartile range.

[a]P-values were calculated using linear regression or logistic regression as appropriate.

[b]5460 participants attended both the 1981–1983 examination with 25(OH)D measurements and the 1991–1994 and/or 2001–2003 examinations with DNA available.

[c]In current and former smokers only

[d]1 unit ~ 12 g alcohol

[e]Income was classified differently in the 2 cohorts: 3 groups in CCHS and 4 groups in CGPS.

[NS]Not significant after correcting for 11 parallel tests (required p = 0.05/11 = 0.0045)

[f]30792 participants only

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(2) Morrison AC, Felix JF, Cupples LA, Glazer NL, Loehr LR, Dehghan A et al. Genomic variation associated with mortality among adults of European and African ancestry with heart failure: the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet* 2010; 3:248-255.

(3) Walter S, Atzmon G, Demerath EW, Garcia ME, Kaplan RC, Kumari M et al. A genome-wide association study of aging. *Neurobiol Aging* 2011; 32:2109-2128.

(4) Yashin AI, Wu D, Arbeev KG, Ukraintseva SV. Joint influence of small-effect genetic variants on human longevity. *Aging (Albany NY)* 2010; 2:612-620.

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