

## Supplementary Online Content

Levin GP, Robinson-Cohen C, de Boer IH, Houston DK, Lohman K, Liu Y, et al. Genetic variants and associations of 25-hydroxyvitamin D concentrations with major clinical outcomes. *JAMA*. 2012;308(18):doi:10.1001/jama.2012.17304.

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### eReferences

This supplementary material has been provided by the authors to give readers additional information about their work.

**eTable 1.** Detailed methods for measurement of 25(OH)D concentrations and genotyping in the Cardiovascular Health Study<sup>1</sup>, Health ABC<sup>2</sup>, InCHIANTI<sup>3</sup>, and ULSAM<sup>4</sup> cohorts

	CHS	Health ABC	InCHIANTI	ULSAM
25(OH)D Concentration				
Measurement	Mass spectrometry (Waters Quattro Micro)	Radioimmunoassay (DiaSorin RIA)	Radioimmunoassay (DiaSorin RIA)	Liquid chromatography-mass spectrometry (HP 1100)
Coefficient of Variation	< 3.4%	< 6.8%	< 10.2%	< 7.6%
Genotyping				
Array type(s)	Illumina 370 CNV BeadChip	Illumina Human1M-Duo BeadChip	Illumina 550K	Illumina Golden Gate, Illumina Infinum II
Genotype Calling	Illumina BeadStudio	Illumina BeadStudio	Illumina BeadStudio	Illumina GenomeStudio

**eTable 2.** Incident events in the Cardiovascular Health Study, Health ABC, InCHIANTI, and ULSAM cohorts

	CHS (N = 1,514)	Health ABC (N = 922)	InCHIANTI (N = 835)	ULSAM (N = 970)
Median (maximum) follow-up, in years	11 (15)	7 (8)	6 (8)	13 (19)
Numbers of events				
Composite outcome	948	317	184	715
Hip fracture	129	28	15	63
Myocardial infarction	179	45	58	132
Cancer	304	137	12	258
Death	336	107	99	262
Person-years of follow-up	11,424	5,554	8,128	10,612
Incidence rates <sup>a</sup> of composite outcome (95% CI)				
Overall	6.8 (6.3, 7.2)	5.7 (5.1, 6.4)	3.9 (3.5, 4.3)	6.7 (6.3, 7.2)
Normal 25(OH)D	6.4 (6.0, 6.9)	5.5 (4.8, 6.2)	3.2 (2.9, 3.8)	6.7 (6.2, 7.2)
Low 25(OH)D	8.2 (7.2, 9.3)	6.6 (5.2, 8.3)	6.2 (5.1, 7.5)	7.0 (5.9, 8.2)
Difference <sup>b</sup>	1.8 (0.6, 2.9)	1.1 (-0.6, 2.8)	2.9 (1.6, 4.2)	0.3 (-1.0, 1.5)
Association <sup>c</sup> of low 25(OH)D <sup>d</sup> with risk of the composite outcome (HR (95% CI); <i>p</i> -value)	1.32 (1.13, 1.54); <i>p</i> = 0.001	1.33 (1.02, 1.73); <i>p</i> = 0.04	1.20 (0.85, 1.70); <i>p</i> = 0.30	1.05 (0.88, 1.26); <i>p</i> = 0.60

Abbreviations: HR = hazard ratio, CI = confidence interval

<sup>a</sup> Unadjusted rates are reported as the number of events per 100 person-years, with Poisson-based confidence intervals

<sup>b</sup> Difference computed as incidence rate in participants with low 25(OH)D minus rate in those with normal 25(OH)D concentrations

<sup>c</sup> Adjusted for age and sex

<sup>d</sup> Normal/Low 25(OH)D defined by above/below the season-specific 20th percentile of 25(OH)D concentrations

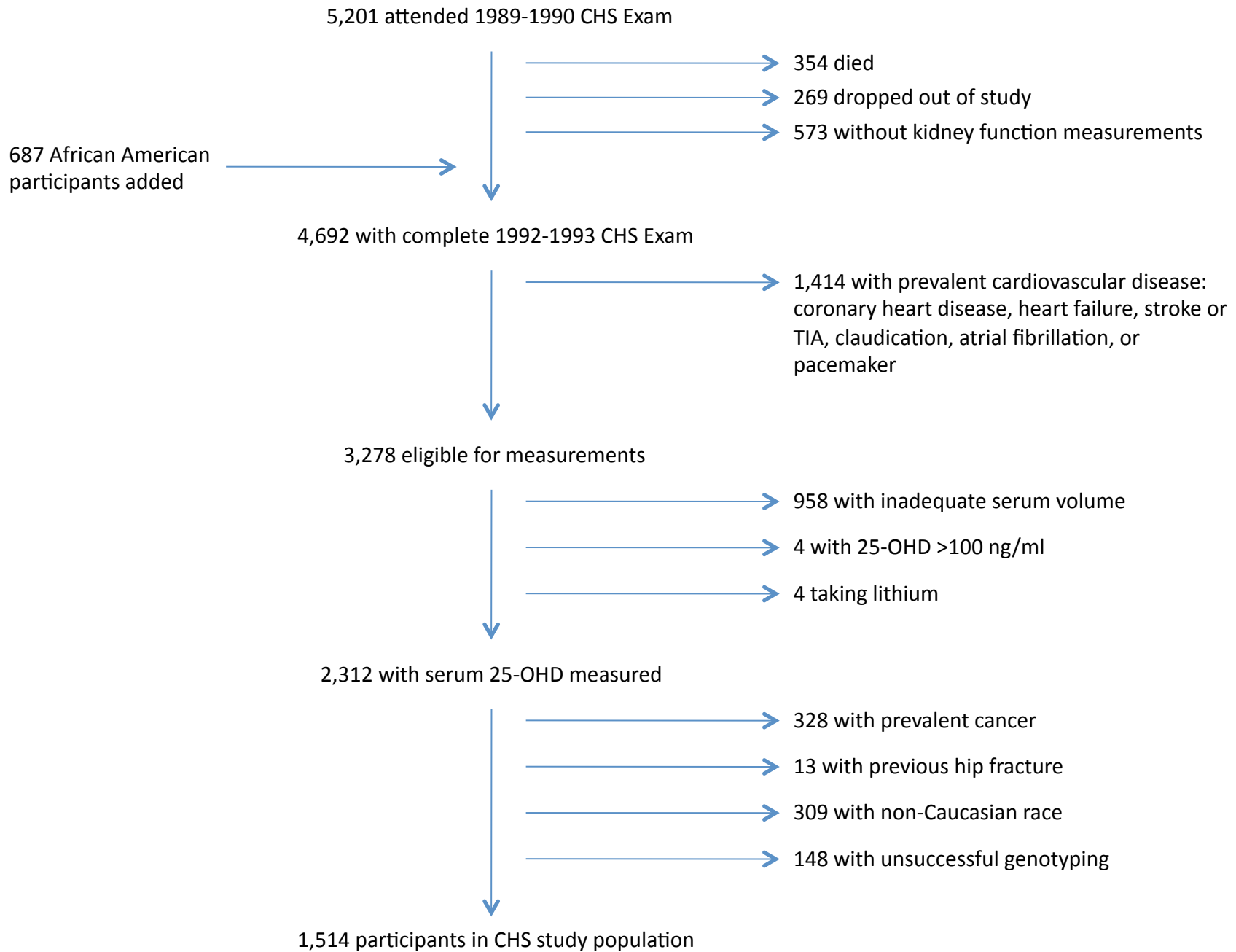
**eTable 3.** Detailed results in the Cardiovascular Health Study, Health ABC, InCHIANTI, and ULSAM cohorts for interactions between low vitamin D and VDR SNPs rs7968585 and rs2239179 on risk of the composite outcome

SNP	Endpoint	CHS	Health ABC	InCHIANTI	ULSAM	Meta-analysis
		HRR <sup>a</sup> (95% CI)	HRR (95% CI)	HRR (95% CI)	HRR (95% CI)	HRR (95% CI)
rs7968585	Hip fracture	1.25 (0.80, 1.95)	1.50 (0.96, 2.33)	1.49 (0.24, 9.35)	0.73 (0.34, 1.53)	1.25 (0.94, 1.66)
	Myocardial Infarction	1.25 (0.81, 1.92)	1.27 (0.92, 1.81)	3.55 (0.74, 17.04)	1.31 (0.72, 2.37)	1.30 (1.02, 1.66)
	Cancer	1.51 (1.06, 2.16)	1.03 (0.80, 1.32)	2.09 (0.70, 6.21)	1.15 (0.77, 1.72)	1.18 (0.99, 1.42)
	Death	1.31 (1.04, 1.64)	1.06 (0.87, 1.29)	1.32 (0.70, 2.50)	1.02 (0.78, 1.32)	1.13 (1.00, 1.28)
	Composite	1.40 (1.13, 1.73)	1.14 (0.97, 1.34)	1.59 (1.01, 2.51)	1.12 (0.88, 1.42)	1.22 (1.09, 1.36)
rs2239179	Hip fracture	0.92 (0.59, 1.46)	0.68 (0.44, 1.04)	2.60 (0.45, 15.12)	NA	0.82 (0.60, 1.11)
	Myocardial Infarction	0.73 (0.47, 1.15)	0.90 (0.62, 1.29)	0.77 (0.14, 4.04)	NA	0.83 (0.62, 1.09)
	Cancer	0.61 (0.43, 0.87)	0.90 (0.71, 1.15)	0.54 (0.15, 1.85)	NA	0.79 (0.65, 0.96)
	Death	0.83 (0.66, 1.04)	1.09 (0.90, 1.33)	0.56 (0.29, 1.05)	NA	0.95 (0.82, 1.09)
	Composite	0.74 (0.60, 0.92)	0.95 (0.81, 1.10)	0.66 (0.41, 1.05)	NA	0.85 (0.76, 0.97)

Abbreviations: CI = confidence interval, HRR = hazard ratio ratio (adjusted for age and sex), SNP = single nucleotide polymorphism

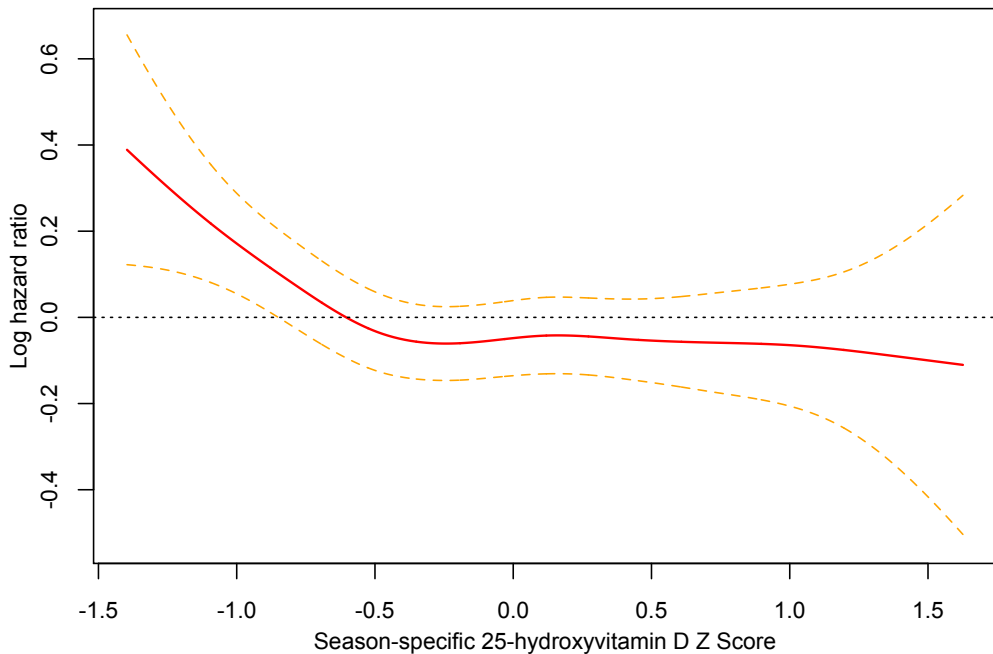
<sup>a</sup> The hazard ratio ratio (HRR) is the ratio, for each additional minor allele, of the hazard ratio describing the association between low 25-hydroxyvitamin D and disease risk. Normal/Low 25(OH)D defined by above/below the season-specific 20th percentile of 25(OH)D concentrations.

**eFigure 1. Flow chart defining the Cardiovascular Health Study discovery cohort**



**eFigure 2.** Penalized spline describing the association between season-specific 25-hydroxyvitamin D Z score and risk of the composite outcome, adjusted for age and sex, in the Cardiovascular Health Study

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Winter

10

20

30

Summer

20

30

40

25-hydroxyvitamin D Concentration (ng/ml)



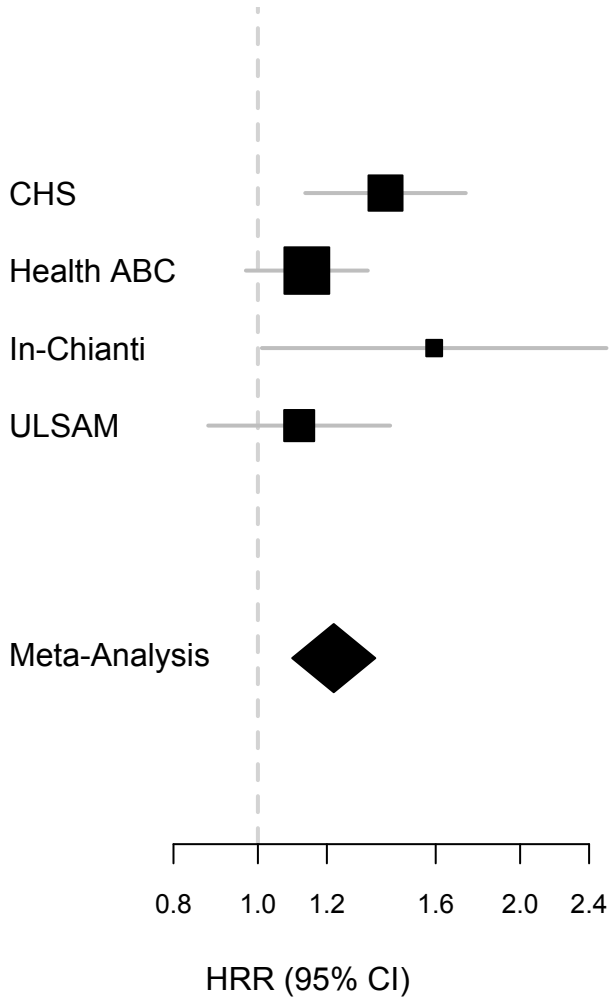
X-axes representing the corresponding 25(OH)D concentrations in the winter and summer seasons are also displayed. The spline was estimated using a proportional hazards model. The dashed lines represent pointwise 95% confidence intervals for the estimated log hazard ratios.

The dotted line indicates a log hazard ratio of 0 (hazard ratio of 1).

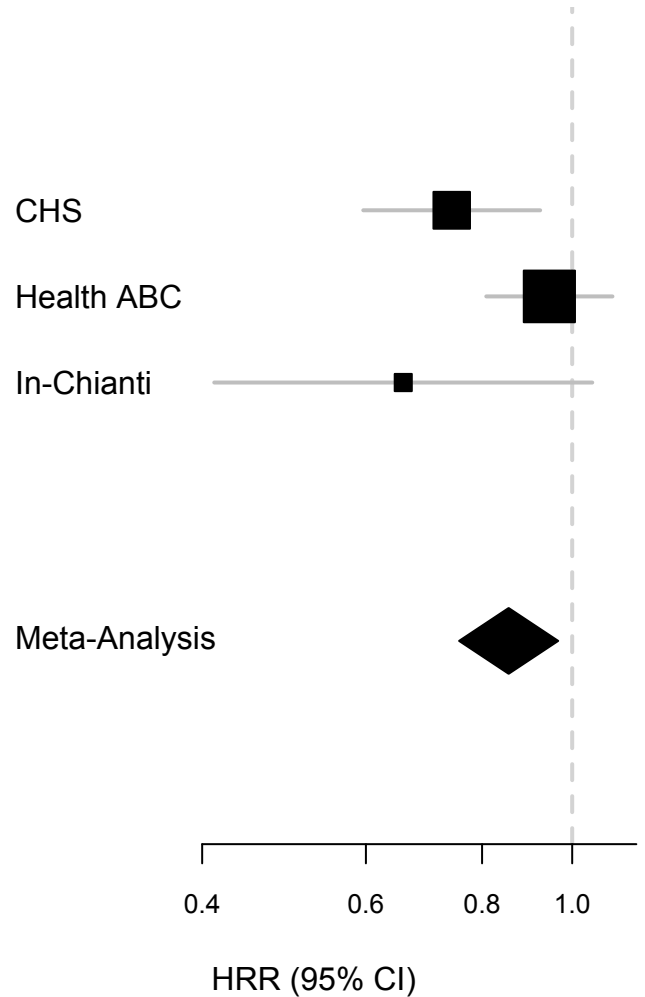
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**eFigure 3.** Estimates of interaction between low vitamin D and the number of minor alleles on risk of the composite outcome, by study and overall, for the two VDR variants: rs7968585 and rs2239179

a) rs7968585



b) rs2239179



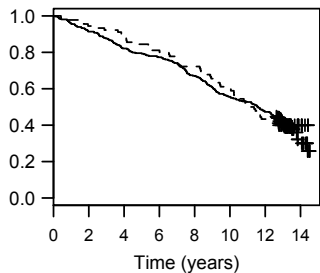
The diamond displays the meta-analysis hazard ratio (HRR). The size of the squares is inversely proportional to the estimated variance.

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**eFigure 4.** Kaplan-Meier plots describing the association of low vitamin D with risk of the composite outcome in the Cardiovascular Health Study, stratified according to the number of copies of the minor allele for the 2 *VDR* variants: **rs7968585 and rs2239179**

## a) rs7968585

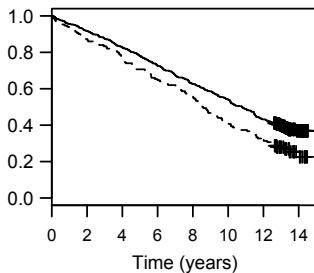
0 Minor Alleles



# At Risk

Normal	313	257	210	148
Low	90	79	65	39

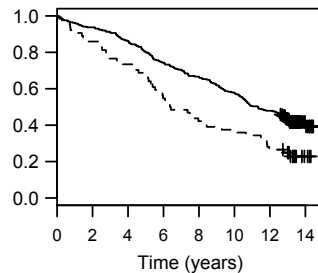
1 Minor Allele



# At Risk

Normal	609	504	381	263
Low	150	113	84	48

2 Minor Alleles

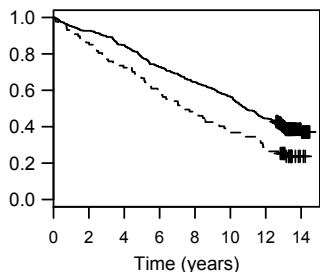


# At Risk

Normal	288	248	192	138
Low	64	47	27	17

## b) rs2239179

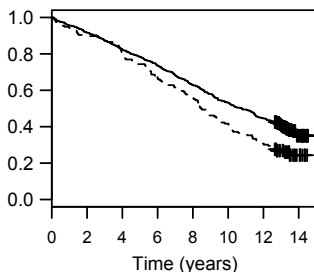
0 Minor Alleles



# At Risk

Normal	409	347	264	182
Low	87	63	41	23

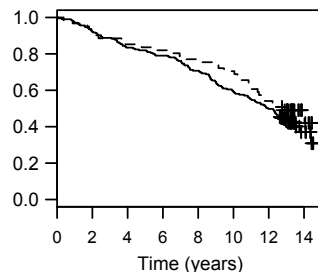
1 Minor Allele



# At Risk

Normal	595	490	374	265
Low	156	124	88	48

2 Minor Alleles



# At Risk

Normal	205	171	145	102
Low	61	52	47	33

Proportion of participants free of hip fracture, myocardial infarction, cancer, and death is presented on the y-axis. The numbers at risk at baseline, and at 4, 8, and 12 years of follow-up are given below each plot. Vertical lines indicate censoring. Normal/Low 25(OH)D defined by above/below the season-specific 20th percentile of 25(OH)D concentrations. Solid and dotted lines indicate participants with normal and low 25(OH)D levels respectively.

## eReferences

1. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol.* Feb 1991;1(3):263-276.
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4. Melhus H, Snellman G, Gedeberg R, et al. Plasma 25-hydroxyvitamin D levels and fracture risk in a community-based cohort of elderly men in Sweden. *J Clin Endocrinol Metab.* Jun;95(6):2637-2645.