CLINICAL STUDY

Associations between vitamin D receptor genotypes and mortality in a cohort of older Dutch individuals

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Abstract

Context: Vitamin D receptor (VDR) polymorphisms are associated with a variety of diseases, which may translate into an effect on mortality.

Objective: To investigate the associations between VDR gene variants and mortality among older people.

Design: The analyses were conducted in a population-based, prospective cohort of the Longitudinal Aging Study Amsterdam. Adequate DNA analysis was performed in 923 men and women (≥ 65 years). We aimed to assess the associations between mortality and the VDR polymorphism *FokI*, three haplotypes of the *Cdx2* and *GATA* polymorphisms, and three haplotypes of the *BsmI*, *ApaI*, and *TaqI* polymorphisms.

Results: During the median follow-up of 10.7 years, 480 participants deceased (51%). Homozygosity for the *Cdx2*–*GATA* haplotype 1 allele was associated with a 30% higher mortality risk compared to the absence of alleles (hazard ratios (HR) 1.30, 95% confidence intervals (CI) 1.01–1.68). Adjustment for cardiovascular risk factors and 25-hydroxyvitamin D levels did not affect this HR. The number of copies of the *Cdx2*–*GATA* haplotype 1 allele was associated, although not significantly, with an increased risk of osteoporotic fractures (0 copies = reference, HR, 95% CI: 1 copy 2.01, 0.99–4.07 and 2 copies 1.81, 0.87–4.18). After adjustment for osteoporotic fractures, homozygosity for the *Cdx2*–*GATA* haplotype 1 allele was no longer associated with higher mortality risk (HR 1.08, 95% CI 0.83–1.41).

Conclusions: The *Cdx2*–*GATA* haplotype 1 allele was related to increased mortality risk, which may be partly explained by osteoporotic fractures. As the biological mechanism is uncertain and this study size is limited, our results should be interpreted as hypothesis generating.

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Introduction

It is increasingly acknowledged that vitamin D plays a central role in a large variety of metabolic pathways. Vitamin D exerts its action at a cellular level through binding of the active metabolite 1,25-dihydroxyvitamin D to the vitamin D receptor (VDR). The VDR is expressed in many different cell types such as pancreatic β cells (1), vascular smooth muscle cells (2), and osteoblasts and chondrocytes (3). Several polymorphisms in the gene encoding the VDR, such as *Cdx2*, *FokI*, *BsmI*, *ApaI*, and *TaqI*, have been demonstrated to be related to bone characteristics and risk of fractures, although results remain equivocal (4–6). Moreover, specific combinations of these polymorphisms, so-called haplotypes, have been identified as a risk factor for fractures (7). Beside effects on bone metabolism, VDR polymorphisms, in particular *BsmI*, are associated with cardiovascular

risk factors such as increased blood pressure, an unfavorable lipid profile (8, 9), increased left ventricular mass in hemodialysis patients (10), and intimal medial thickening of the carotid artery (11). In addition, VDR polymorphisms have been related to an increased risk of myocardial infarction (12) and diabetes mellitus type 2 (13–15). Moreover, the *FokI*, *GATA*, *BsmI*, and *ApaI* polymorphisms are related to an increased risk and a worse prognosis of cancer, in particular prostate cancer, breast cancer, and malignant melanoma (16).

Although many studies have been published on associations between VDR genotypes and risk factors or disease states, the relationship with total mortality remains unknown. To our knowledge, only one population-based study reported on the relationship between VDR genotyping and mortality, which was absent in a population of 85 years and older with a mean follow-up of 4.2 years (17). The main aim of this

study is to assess whether VDR genotypes, which have been associated with several diseases, i.e. the *FokI* polymorphism, *Cdx2*–*GATA* haplotypes and *BsmI*–*ApaI*–*TaqI* haplotypes, are associated with increased or decreased mortality risk in a large cohort of men and women aged 65 years and older followed for more than 10 years.

Subjects and methods

Subjects

The analyses in this report were conducted within the Longitudinal Aging Study Amsterdam (LASA). The LASA is an ongoing cohort study that focuses on physical, cognitive, emotional, and social functioning in the aging population of the Netherlands. The sampling, data collection, and non-response have been described in detail previously (18). Briefly, a random sample of older men and women (aged 55–85 years) was drawn from the population registers of 11 municipalities in three different areas of the Netherlands. Initially, a total of 3107 individuals participated in the study. More than 98% of the participants were of Caucasian descent. At baseline (1992–1993) and every 3 years subsequently, a cycle of measurements was carried out, consisting of a general and a medical interview and blood sampling. Written informed consent was obtained from all participants. The study was approved by the medical ethics committee of the VU University Medical Center.

The study sample consisted of 1509 persons who participated in both the main and medical interview in 1995–1996 and were 65 years and older as of January 1, 1996. Those, who refused to provide a blood sample ($n=157$) or whose sample was insufficient or inappropriate for DNA isolation ($n=399$), genotyping was not performed. This resulted in 953 participants with samples for DNA analyses. After additionally excluding 18 participants (inconsistency in gender determination between DNA and self-report ($n=8$) (see below), not enough DNA for analyses ($n=3$), technical failure ($n=6$), and a sample with inconsistent results ($n=1$), 935 participants (459 men and 476 women) were included in the present analyses.

On 1 June 2007, it was determined if any deaths had occurred among the study participants by collecting data from the registers of the municipalities where participants were living. Primary causes of death were obtained from the Dutch Central Bureau of Statistics. The cause of death was coded according to the International Classification of Diseases, 10th Revision (ICD-10). Mortality from cardiovascular diseases (CVDs) included deaths due to hypertensive disease (codes I10–I13), ischemic heart disease (codes I20–I25), arrhythmia (codes I44–I49), heart failure (code I50), cerebrovascular disease (codes I60–I69), or atherosclerosis or other diseases of the arteries

(codes I70–I78). Cancer mortality included deaths coded as malignant neoplasm deaths (codes C00–C95). For two participants who moved abroad, data on mortality was missing and for two participants data on causes of death was missing due to unknown death certificate numbers.

DNA isolation and genotyping

Determination of VDR polymorphisms was performed during the second cycle in 1995–1996. The initial aim of genotyping of the *VDR* gene was to examine the associations of polymorphisms with bone mineral density and fractures. These data have been published as part of a participant-level meta-analysis (6). In this study, we examined the associations between VDR genotypes and mortality. We restricted our analysis to VDR polymorphisms, which have been shown to be associated with relevant clinical conditions, i.e. the *BsmI*, *ApaI*, *TaqI*, *Cdx2*, *GATA*, and *FokI* polymorphisms. Previous studies have shown that the *BsmI*, *ApaI*, and *TaqI* polymorphisms may demonstrate strong linkage disequilibrium, defining five haplotypes in total (7). Moreover, the *Cdx2* and *GATA* polymorphisms have been described to show a linkage disequilibrium describing three haplotypes (7). In case of linkage disequilibrium it is more appropriate to study the associations for haplotypes instead of separate polymorphisms (7, 19, 20). In Fig. 1, the genomic organization of the *VDR* locus at gene 12q13 and the polymorphisms and haplotypes examined are depicted.

Of the 935 participants with sufficient and appropriate blood samples for DNA analyses, genotyping for polymorphisms of the *VDR* gene *FokI* was complete in 926 samples, *Cdx2*–*GATA* haplotyping in 924 samples, and *BsmI*–*ApaI*–*TaqI* haplotyping was complete in

Figure 1 The genomic structure of the *VDR* gene on chromosome 12q13. The *VDR* gene spans a genomic region of 100 kb. The black boxes indicate the 14 exons of the *VDR* gene. The approximate location of the six polymorphisms analyzed in this study has been indicated with arrows. The *BsmI*–*TaqI*–*ApaI* and *Cdx2*–*GATA* haplotypes included in this study are studied and indicated.

923 samples. The failure of genotyping in a small number of samples was because of the insufficient quality of DNA.

Genomic DNA was isolated from peripheral venous blood specimens according to standard protocols. The rs1544410 (*BsmI* G/A), rs7975232 (*ApaI* A/C), rs1788009 (*TaqI* A/G), rs11568820 (*Cdx2* G/A), rs4516035 (*GATA* A/G), and rs2228570 (*FokI* G/A) were genotyped by Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA). The *Cdx2*–*GATA* and *BsmI*–*ApaI*–*TaqI* haplotypes were constructed by use of the PHASE program as previously described (7, 21). The PHASE program implements the Bayesian statistical method for reconstruction of haplotypes from population genotype data (22). To check the genotyping procedures, gender as documented in the LASA was compared with DNA gender (*SRY* gene) and testosterone level. The number of persons with inconsistent gender determination was 8 of 953 samples (0.8%).

Covariates

The following covariates were determined in 1995–1996: body mass index (BMI), mean arterial pressure (MAP), heart rate, total cholesterol, high-density lipoprotein (HDL) cholesterol, creatinine, 25-hydroxy-vitamin D (25(OH)D), and parathyroid hormone (PTH). Self-reported variables included smoking (never, former, or current) and diabetes mellitus. Blood pressure (mmHg) and heart rate were measured using an oscillometric blood pressure monitor (model HEM-706; Omron Corporation, Tokyo, Japan). Blood samples were obtained in the morning (semi-fasting state), immediately centrifuged, and stored at -20°C . PTH was measured by IRMA (Instar Corp., Stillwater, MN, USA) and serum 25(OH)D was determined by a competitive protein binding assay (Nichols Diagnostics, San Juan Capistrano, CA, USA). Serum total cholesterol was measured by enzymatic colorimetric test with a Hitachi model 747 analyzer. HDL cholesterol was determined by an enzymatic colorimetric test (Roche Diagnostics). Intra- and interassay coefficients of variation of these measurements were $<12\%$. Data on covariates were missing in some participants: presence of diabetes mellitus, $n=1$; BMI, $n=8$; blood pressure and heart rate, $n=12$; total cholesterol, $n=16$; HDL cholesterol, $n=17$; and creatinine, $n=14$.

Data on fractures were collected and checked from 1995/1996 to September 1, 2002. Data on fractures were obtained from self-recorded calendars (1995–1999) and medical interviews (1999–2002). Only the first fractures during the study period were included. If a participant died, their general practitioner or caregiver was contacted to supply information on whether a fracture had occurred since the last interview contact. The fracture was considered osteoporotic if it had occurred from a standing or sitting position; all accidental fractures, fractures of hands, fingers, feet,

toes, and skull were excluded. Of all reported osteoporotic fractures, 90% were confirmed following verification with the general practitioner or the hospital. Data of fractures was missing in five participants.

Statistical analysis

Differences in baseline characteristics between different genotypes were tested with one-way ANOVA (continuous variables) and χ^2 test (categorical variables). The Hardy–Weinberg equilibrium was tested for every polymorphism by comparing expected and observed genotype frequencies using a χ^2 test. Differences in age of death between different genotypes were tested by ANOVA. Hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause mortality and cause-specific mortality from CVD and cancer for the different VDR polymorphisms and haplotypes were estimated with Cox proportional hazards analyses without and with adjustment for potential imbalances in baseline variables. Gender, age, presence of diabetes mellitus, smoking status, blood pressure, total cholesterol, and HDL cholesterol were included in the model because they were identified as classical cardiovascular risk factors (23). In addition, blood pressure, BMI, and 25(OH)D were included in the model because these variables might mediate the relationship between VDR genotypes and mortality whereas these variables are associated with both VDR genotypes (8, 9, 24–26) and mortality (27, 28).

We examined the possible role of osteoporotic fractures by adding this variable as covariate to the regression model. The possible causal role for CVD was studied as follows: for a specific allele associated with an increased mortality risk we divided the HR for cardiovascular mortality by the HR for non-cardiovascular mortality. A ratio higher than 1 indicates that cardiovascular mortality risk exceeds non-cardiovascular mortality risk for a specific VDR genotype or haplotype. Similarly, relative excess cancer mortality was calculated. All statistical analyses were performed with SPSS for Windows, release 16.0 (SPSS, Inc., Chicago, IL, USA). A P value <0.05 was considered statistically significant for all the analyses.

Results

Baseline characteristics

Mean age of the 935 included participants was 75.7 years, with an almost equal gender distribution (49% male). Details on the cardiovascular risk profile and other baseline characteristics are described in Table 1. The distribution of genotypes for all VDR gene polymorphisms was in the Hardy–Weinberg equilibrium. In this study population, the *BsmI*, *ApaI*, and *TaqI* polymorphism demonstrated a strong linkage disequilibrium defining five haplotypes, and the *Cdx2*

Table 1 Baseline characteristics of the study population. Data are *n* or mean $\pm$ S.D.

	Total population	Men	Women
Number	935	459	476
Age (years)	75.7 $\pm$ 6.6	75.5 $\pm$ 6.5	75.8 $\pm$ 6.6
Diabetes (%)	65 (7)	27 (6)	38 (8)
Smoking (%)			
Never	311 (33)	45 (10)	266 (56)
Former	436 (47)	291 (63)	145 (30)
Current	188 (20)	123 (27)	65 (14)
BMI (kg/m ²)	26.9 $\pm$ 4.2	26.0 $\pm$ 3.4	27.8 $\pm$ 4.7
Mean arterial pressure (mmHg)	106 $\pm$ 16	107 $\pm$ 16	106 $\pm$ 16
Heart rate (beats/min)	69 $\pm$ 12	69 $\pm$ 12	70 $\pm$ 11
Total cholesterol (mmol/l)	5.7 $\pm$ 1.1	5.5 $\pm$ 1.0	5.9 $\pm$ 1.0
HDL-cholesterol (mmol/l)	1.4 $\pm$ 0.4	1.3 $\pm$ 0.4	1.5 $\pm$ 0.4
Creatinine (μ mol/l)	97 $\pm$ 40	107 $\pm$ 40	87 $\pm$ 37
25(OH)D (nmol/l)	51.2 $\pm$ 23.3	55.8 $\pm$ 24.2	46.8 $\pm$ 21.5
PTH (pmol/l)	3.8 $\pm$ 2.7	3.9 $\pm$ 2.7	3.7 $\pm$ 2.7

and *GATA* demonstrated a linkage disequilibrium defining three haplotypes (7). Data are only presented for haplotypes 1, 2, and 3 of the *BsmI*–*Apal*–*TaqI* haplotype. The number of individuals with *BsmI*–*Apal*–*TaqI* haplotypes 4 and 5 was too low to perform statistical analysis (<5%). The first three haplotypes have previously been described as haplotypes 1, 2, and 3 or *baT*, *BAT*, and *bAT* respectively (7, 29). The *FokI* polymorphism was not in linkage disequilibrium with any of the other examined VDR polymorphisms. The distribution of VDR genotypes and minor allele and haplotype frequencies are reported in Table 2.

Baseline characteristics demonstrated the differences between the *Cdx2*–*GATA* haplotypes and *BsmI*–*Apal*–*TaqI* haplotypes. Participants without copies of the *Cdx2*–*GATA* haplotype 2 (GA) were older than participants with 2 copies (0, 1, and 2 copies: 76.6 $\pm$ 6.5, 75.1 $\pm$ 6.5, and 75.8 $\pm$ 6.7 years respectively, $P=0.03$). Participants without copies of haplotype 3 (AA) had higher MAP (0, 1, and 2 copies: 107 $\pm$ 16, 107 $\pm$ 16, and 100 $\pm$ 15 mmHg respectively, $P=0.01$) and higher concentrations of 25(OH)D (52.0 $\pm$ 23.4, 49.7 $\pm$ 23.2, and 44.0 $\pm$ 21.7 nmol/l respectively, $P=0.04$) than participants with 2 copies. Participants without copies of haplotype 2 (*BAT*) were older than participants with 2 copies (76.5 $\pm$ 6.5, 75.3 $\pm$ 6.5, and 75.1 $\pm$ 6.7 respectively, $P=0.02$). For the *FokI* polymorphism and other haplotypes, no clear differences in baseline characteristics were observed (data not shown).

VDR genotyping and mortality

During the median 10.7 years of follow-up (interquartile range 5.7–11.3 years), 480 participants deceased (52%). In 152 persons (32% of deceased participants), mortality was ascribed to CVD and in

115 persons (24% of deceased participants) to cancer. HRs of all-cause mortality according to VDR polymorphisms and haplotypes are presented in Table 3.

Persons with 2 copies of *Cdx2*–*GATA* haplotype 1 allele had a 30% higher risk of all-cause mortality compared with 0 copies of the haplotype allele (Table 3; Model 1). Gender-specific analyses demonstrated a 25% higher risk in men (HR 1.25, 95% CI 0.90–1.73, $P=0.18$) and a 32% higher risk in women (HR 1.32, 95% CI 0.87–2.00, $P=0.19$). The increased mortality risk associated with 2 copies of *Cdx2*–*GATA* haplotype 1 allele in the total population did not change materially after the adjustment for baseline imbalances in cardiovascular risk factors and 25(OH)D (Table 3; Model 2). In a regression analysis, the number of *Cdx2*–*GATA* haplotype 1 alleles was related to mortality risk (HR 1.14, 95% CI 1.01–1.30, $P=0.045$). More copies of the *BsmI*–*Apal*–*TaqI* haplotype 1 allele (*baT*) were associated with lower all-cause mortality (Table 3). In individuals, homozygous for the *BsmI*–*Apal*–*TaqI* haplotype 1 allele (*baT*) a 22% reduction of all-cause mortality was shown, which did not reach statistical significance (Table 3; Model 1). Gender-specific analyses demonstrated a 26% reduction in men (HR 0.74, 95% CI 0.52–1.04, $P=0.08$) and an 18% reduction risk in women (HR 0.82, 95% CI 0.56–1.19, $P=0.29$). In the total population, the number of *BsmI*–*Apal*–*TaqI* haplotype 1 allele was borderline significantly related to a larger mortality reduction (HR 0.88, 95% CI 0.78–1.00, $P=0.05$). Adjustment for baseline imbalances in cardiovascular risk factors and 25(OH)D did not influence these HRs (Table 3; Model 2). None of the other polymorphisms or haplotypes were associated with an increased or decreased risk of all-cause mortality. Interaction analysis demonstrated that the interaction of gender on associations between

Table 2 Frequency of genotypes in the study population.

	Frequency of VDR genotype (n)	Minor or haplotype allele frequency (%)		
		Total population	Men	Women
<i>FokI</i> polymorphism				
CC/CT/TT	361/429/136	37.9	37.3	38.4
<i>Cdx2</i> – <i>GATA</i> haplotypes				
Haplotype 1: GG				
0/1/2 copies	271/466/187	45.5	47.1	43.9
Haplotype 2: GA				
0/1/2 copies	386/422/116	35.4	37.3	34.6
Haplotype 3: AA				
0/1/2 copies	612/270/42	19.2	16.3	21.6
<i>BsmI</i> – <i>Apal</i> – <i>TaqI</i> haplotypes				
Haplotype 1: <i>baT</i>				
0/1/2 copies	307/429/187	43.5	42.5	44.3
Haplotype 2: <i>BAT</i>				
0/1/2 copies	313/435/175	42.5	41.9	43.1
Haplotype 3: <i>bAT</i>				
0/1/2 copies	742/169/12	10.5	11.7	9.2

Table 3 Associations between VDR genotypes and haplotypes and the hazard ratios (HR) of all-cause mortality according to different regression models. Model 1 is adjusted for age and gender. Model 2 is adjusted for age, gender, mean arterial pressure (MAP), BMI, smoking, diabetes mellitus, total cholesterol, high-density lipoprotein (HDL) cholesterol, and 25-hydroxyvitamin D. Model 3 is adjusted for age, gender, MAP, BMI, smoking, diabetes mellitus, total cholesterol, HDL cholesterol, 25-hydroxyvitamin D, and osteoporotic fractures.

	No. of copies	No. at risk	No. deceased	Mean age of death (years)	Model 1, HR (95% CI)	Model 2, HR (95% CI)	Model 3, HR (95% CI)
<i>FokI</i> polymorphism							
CC		358	186	84.4 ± 7.0	1 (Reference)	1 (Reference)	1 (Reference)
CT		428	221	84.5 ± 6.7	0.96 (0.79–1.16)	0.96 (0.79–1.17)	1.05 (0.86–1.29)
TT		136	68	85.0 ± 6.3	0.97 (0.74–1.29)	0.96 (0.72–1.28)	1.08 (0.80–1.47)
<i>Cdx2</i> – <i>GATA</i> haplotypes							
Haplotype 1 (GG)	0	271	130	85.1 ± 6.0	1 (Reference)	1 (Reference)	1 (Reference)
	1	464	239	85.1 ± 6.6	1.16 (0.93–1.43)	1.14 (0.92–1.32)	0.93 (0.75–1.17)
	2	187	106	84.0 ± 7.2	1.30 (1.01–1.68)*	1.29 (0.99–1.67) [‡]	1.08 (0.83–1.41)
Haplotype 2 (GA)	0	386	210	84.6 ± 6.5	1 (Reference)	1 (Reference)	1 (Reference)
	1	420	211	84.2 ± 7.1	1.00 (0.82–1.21)	1.00 (0.82–1.21)	1.21 (0.98–1.48)
	2	116	54	85.1 ± 6.7	0.83 (0.61–1.13)	0.83 (0.61–1.13)	1.08 (0.79–1.48)
Haplotype 3 (AA)	0	610	315	84.1 ± 6.8	1 (Reference)	1 (Reference)	1 (Reference)
	1	270	140	84.6 ± 6.9	0.90 (0.73–1.10)	0.89 (0.72–1.10)	0.81 (0.66–1.09)
	2	42	20	84.5 ± 6.8	0.82 (0.53–1.31)	0.84 (0.53–1.35)	0.79 (0.50–1.27)
<i>BsmI</i> – <i>Apal</i> – <i>TaqI</i> haplotypes							
Haplotype 1 (<i>baT</i>)	0	306	166	83.6 ± 6.8	1 (Reference)	1 (Reference)	1 (Reference)
	1	428	214	84.6 ± 6.9 [†]	0.86 (0.70–1.05)	0.85 (0.69–1.04)	0.82 (0.66–1.01) [‡]
	2	185	94	85.7 ± 6.3 [†]	0.78 (0.60–1.01) [‡]	0.79 (0.61–1.03) [§]	0.77 (0.59–1.01) [‡]
Haplotype 2 (<i>BA</i> †)	0	311	159	85.3 ± 6.6	1 (Reference)	1 (Reference)	1 (Reference)
	1	433	225	84.1 ± 6.9	1.15 (0.94–1.41)	1.22 (0.98–1.51)	1.14 (0.92–1.42)
	2	175	90	84.0 ± 6.9	1.14 (0.88–1.48)	1.14 (0.87–1.50)	1.17 (0.89–1.53)
Haplotype 3 (<i>bAT</i>)	0	738	376	85.7 ± 6.8	1 (Reference)	1 (Reference)	1 (Reference)
	1	169	93	83.7 ± 6.7	1.15 (0.92–1.45)	1.11 (0.87–1.41)	1.08 (0.85–1.37)
	2	12	5		–	–	–

* $P=0.043$, $^{\dagger}P=0.046$, $^{\ddagger}P=0.06$ and $^{\S}P=0.08$.

VDR genotypes and mortality was not significant ($P>0.2$).

Analysis of the separate single nucleotide polymorphisms (*BsmI*, *Apal*, *TaqI*, *Cdx2*, and *GATA*) instead of haplotypes did demonstrate associations that were in line with the results found with the haplotypes, although the CIs were wider (data not shown).

To examine the potential role for osteoporotic fractures in the relationships of VDR genotypes with mortality, we assessed the risk of an osteoporotic fracture for the different VDR genotypes. During the median 5.9 years of follow-up (interquartile range 4.4–6.1 years), 79 first fractures (8%) have occurred of which 55 were classified as osteoporotic (6%). Of the osteoporotic fractures, 24 (44%) fractures have occurred in men and 31 (56%) in women and 22 (40%) were hip fractures. The presence of *Cdx2*–*GATA* haplotype 1 allele was associated, although not significantly, with an increased risk for osteoporotic fractures (HR, 95% CI: 1 copy 2.01, 0.99–4.07, $P=0.054$ and 2 copies 1.81, 0.87–4.18, $P=0.17$). Adjustment for osteoporotic fractures reduced the risk of all-cause mortality associated with *Cdx2*–*GATA* haplotype 1 homozygosity with 22% in the total population (Table 3; Model 3). Gender-specific analyses demonstrated a 17% reduction of total mortality in men (HR 1.13, 95% CI 0.69–1.86, $P=0.63$) and a 27% reduction risk in women (HR 1.03, 95% CI 0.74–1.36, $P=0.78$). This showed that the

mortality excess associated with *Cdx2*–*GATA* haplotype 1 may be partly mediated by an increased osteoporotic fracture risk. All other VDR genotypes were not associated with an increased risk of an osteoporotic fracture ($P>0.5$).

The hazard rate ratios of cardiovascular to non-cardiovascular mortality, i.e. the relative contribution of cardiovascular mortality to all-cause mortality for the *Cdx2*–*GATA* haplotype 1 and *BsmI*–*Apal*–*TaqI* haplotype 1, are depicted in Table 4. Similarly, excess mortality due to cancer is depicted. Excess mortality related to the *Cdx2*–*GATA* haplotype 1 allele was not determined only by excess mortality due to CVD or cancer. Similarly, the data in Table 4 demonstrate that the number of copies of *BsmI*–*Apal*–*TaqI* haplotype 1 was not associated with a relative decrease in mortality specifically due to CVD or cancer.

Discussion

This study was performed to assess the effect of VDR polymorphisms and haplotypes on all-cause mortality in a large population-based cohort of older individuals. The main findings were that *Cdx2*–*GATA* haplotype 1 and *BsmI*–*Apal*–*TaqI* haplotype 1 showed a tendency to be associated with differences in all-cause mortality risk. Statistical adjustment for common cardiovascular risk

Table 4 Associations between *VDR* genotypes and risk of mortality due to cardiovascular disease or cancer. All models are adjusted for age and gender.

	CV mortality		Non-CV mortality		Relative contribution CV mortality	Cancer mortality		Non-cancer mortality		Relative contribution cancer mortality
	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	HR (95% CI)	<i>n</i>	HR (95% CI)	<i>n</i>	HR (95% CI)	HR (95% CI)
<i>Cdx2</i> – <i>GATA</i> haplotype 1 (GG)										
0 copy	44	1 (Reference)	87	1 (Reference)	1 (Reference)	31	1 (Reference)	100	1 (Reference)	1 (Reference)
1 copy	75	1.09 (0.74–1.62)	165	1.20 (0.93–1.57)	0.90 (0.51–1.56)	60	1.13 (0.65–1.96)	180	1.14 (0.90–1.48)	0.81 (0.53–1.33)
2 copies	33	1.19 (0.75–1.91)	73	1.33 (0.97–1.84)	0.91 (0.50–1.55)	22	1.20 (0.78–1.86)	84	1.36 (1.05–1.90)	0.88 (0.48–1.62)
<i>BsmI</i> – <i>ApaI</i> – <i>TaqI</i> haplotype 1 (baT)										
0 copy	54	1 (Reference)	112	1 (Reference)	1 (Reference)	38	1 (Reference)	128	1 (Reference)	1 (Reference)
1 copy	66	0.80 (0.56–1.15)	148	0.88 (0.69–1.13)	0.91 (0.58–1.40)	49	0.91 (0.59–1.39)	165	0.84 (0.67–1.06)	1.09 (0.67–1.77)
2 copies	28	0.71 (0.45–1.13)	66	0.82 (0.60–1.11)	0.87 (0.50–1.51)	27	1.04 (0.63–1.70)	67	0.71 (0.52–0.95)	1.49 (0.84–2.65)

factors and 25(OH)D did not materially influence the results. These differences could not be explained by a relative increase or decrease in cardiovascular or cancer mortality. Statistical adjustment for osteoporotic fractures, however, diminished the association of *Cdx2*–*GATA* haplotype 1 with all-cause mortality, indicating a possible causal role.

The strengths of this study are the population-based sample and the long follow-up period of more than 10 years. Moreover, risk factors (genotypes) and main outcome (date of death) were determined reliably and are almost immune to misclassification. Nevertheless, several considerations have been taken into account when interpreting this study results. First, the fact that most of the polymorphisms studied have functional effects that are not known in full detail hinders the interpretation. Second, statistical power of the findings in this study is limited due to study size and the fact that certain polymorphisms occur in only a low proportion of the population. Finally, *VDR* polymorphisms and haplotypes were not determined in all participants. Because this selection was mostly due to absence of sufficient material, the missing genotype determinations can be assumed to be missing completely at random. The results are therefore likely to be unbiased by missing genotype determination (30). Despite these limitations, we observed an association of *Cdx2*–*GATA* haplotype 1 and a tendency toward an association of *BsmI*–*ApaI*–*TaqI* haplotype 1 with all-cause mortality. The presence of a gradual change in hazard of all-cause mortality depending on the number of copies of both haplotypes is in support of a true effect. However, given the hypothesis generating character of the study, the results have to be reproduced in other population-based studies and further research on possible underlying mechanisms is warranted.

To our knowledge, only one previous population-based study on associations between *VDR* polymorphisms and mortality has been performed. In this population of 85 years and older the authors did not find an association between several *VDR* haplotypes (among which *BsmI*–*ApaI*–*TaqI* haplotype 1) and

all-cause mortality (17). The authors did not study the *GATA* polymorphism. The inconsistency in the results of *BsmI*–*ApaI*–*TaqI* haplotype 1 (baT) may be explained by a shorter follow-up period and the smaller study population, both leading to a lower power for detecting an association in that particular study.

The *GATA* polymorphism is situated within the promoter region of the *VDR* gene. Functional studies have shown that the G allele is associated with a decrease in transcription rate compared to the A allele which may result in lower *VDR* expression and consequent decreased vitamin D signaling efficiency (7). In addition, a possible negative role for the G allele in activation of the immune system has been hypothesized (31, 32). The A allele forms a potential binding site in the promoter region for *GATA3*, which is a transcription factor leading to activation of naive T cells to T helper 2 cells (31). Interestingly, the *GATA* G allele has been shown to be associated with less bone mineral accrual (33). In addition, a previous study demonstrated an association between *Cdx2*–*GATA* haplotype 1, which contains the *GATA* G allele, and an increased incidence of fractures in another older, Caucasian population (7). In accordance with these findings, we demonstrated a tendency toward an association between the *Cdx2*–*GATA* haplotype 1 and osteoporotic fractures. The occurrence of an osteoporotic fracture in this study, at least statistically, partly explained the increased mortality associated with *Cdx2*–*GATA* haplotype 1, although the data on osteoporotic fractures were limited to the first 6 years of follow-up. The partial explanation by osteoporotic fractures seems plausible since occurrence of fractures is a well-known risk factor of mortality (34–36). However, it is not excluded that underlying common denominators influenced both the risk of osteoporotic fractures and mortality. For example, *VDR* receptor genotypes may influence calcium homeostasis, which may have effects on both fracture risk and mortality. This and other possible mechanisms by which the *Cdx2*–*GATA* haplotype may influence physiological and clinical endpoints require further study.

The *BsmI*–*Apal*–*TaqI* haplotype 1 (*baT*) was associated with a positive effect on cognitive function and depressive symptoms (17). In hemodialysis patients, the *BsmI* b allele, which is part of haplotype 1, was related to increased survival (37). In addition, the b allele is associated with a lower prevalence of coronary artery disease and diabetes mellitus type 2 (12, 13). These studies support the notion that haplotype 1 may be associated with survival benefit, as was shown by a tendency in this study. Studies examining the associations between the *BsmI*, *Apal*, and *TaqI* polymorphisms and cardiovascular risk factors, such as blood pressure and lipid profile, have shown conflicting results (8, 15, 38). In this study, the HR was not influenced by correction for several cardiovascular risk factors, suggesting that the effect is not mediated by these factors.

The *BsmI*, *Apal*, and *Taq* polymorphisms are located near the 3' UTR of the gene, which is known to play a role in the regulation of mRNA stability (39). The *BsmI* and *Apal* polymorphisms, however, are not affecting any transcription factor binding or splicing site. In addition, the *TaqI* polymorphism is not changing the amino acid sequence of the encoded protein (4). Therefore, it is believed that the *BsmI*–*Apal*–*TaqI* haplotype is not functional itself but that associations with clinical conditions are caused through linkage with certain functional alleles on the same chromosome (4, 5, 39).

In conclusion, in this study, we have demonstrated the associations of *Cdx2*–*GATA* haplotype 1 with increased all-cause mortality, which may be partly explained by the occurrence of osteoporotic fractures. This may suggest, but does not prove, a causal role in this association. In addition, the *BsmI*–*Apal*–*TaqI* haplotype 1 tends to be related to decreased all-cause mortality. Given the fact that a clear biological mechanism is lacking that can account for these findings and that the power of this study is limited, the results from this study should be interpreted as hypothesis generating. Further studies after the associations between VDR genotypes and mortality in large population-based cohorts, and after the potential underlying biological mechanisms are warranted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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