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, Apal, 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, Apal, 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 Apal 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, Apal, TaqI, Cdx2, GATA, and FokI polymorphisms. Previous studies have shown that the BsmI, Apal, 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...
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 LSA 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-hydroxyvitamin 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 χ² test (categorical variables). The Hardy–Weinberg equilibrium was tested for every polymorphism by comparing expected and observed genotype frequencies using a χ² 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
and GATA demonstrated a linkage disequilibrium defining three haplotypes (7). Data are only presented for haplotypes 1, 2, and 3 of the BsmI–ApaI–TaqI haplotype. The number of individuals with BsmI–ApaI–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–ApaI–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 ± 6.5, 75.1 ± 6.5, and 75.8 ± 6.7 years respectively, P = 0.03).

Participants without copies of haplotype 3 (AA) had higher MAP (0, 1, and 2 copies: 107 ± 16, 107 ± 16, and 100 ± 15 mmHg respectively, P = 0.01) and higher concentrations of 25(OH)D (52.0 ± 23.4, 49.7 ± 23.2, and 44.0 ± 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 ± 6.5, 75.3 ± 6.5, and 75.1 ± 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 (inter-quartile 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–ApaI–TaqI haplotype 1 allele (bAt) were associated with lower all-cause mortality (Table 3). In individuals, homozygous for the BsmI–ApaI–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–ApaI–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 1: Baseline characteristics of the study population. Data are n or mean ± s.d.

<table>
<thead>
<tr>
<th>Total population</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>935</td>
<td>459</td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.7 ± 6.6</td>
<td>75.5 ± 6.5</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>65 (7)</td>
<td>27 (6)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>311 (33)</td>
<td>45 (10)</td>
</tr>
<tr>
<td>Current</td>
<td>188 (20)</td>
<td>123 (27)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 ± 4.2</td>
<td>26.0 ± 3.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>106 ± 16</td>
<td>107 ± 16</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69 ± 12</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.7 ± 1.1</td>
<td>5.5 ± 1.0</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>97 ± 40</td>
<td>107 ± 40</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>23.4, 49.7</td>
<td>23.3, 45.8</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>3.8 ± 2.7</td>
<td>3.9 ± 2.7</td>
</tr>
</tbody>
</table>

### Table 2: Frequency of genotypes in the study population.

<table>
<thead>
<tr>
<th>Frequency of VDR genotype (n)</th>
<th>Total population</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI polymorphism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/CT/TT</td>
<td>361/429/136</td>
<td>37.9</td>
<td>37.3</td>
</tr>
<tr>
<td>Cdx2–GATA haplotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haplotype 1: GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>271/466/187</td>
<td>45.5</td>
<td>47.1</td>
</tr>
<tr>
<td>Haplotype 2: GA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>386/422/116</td>
<td>35.4</td>
<td>37.3</td>
</tr>
<tr>
<td>Haplotype 3: AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>612/270/42</td>
<td>19.2</td>
<td>16.3</td>
</tr>
<tr>
<td>BsmI–ApaI–TaqI haplotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haplotype 1: bAt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>307/429/187</td>
<td>43.5</td>
<td>42.5</td>
</tr>
<tr>
<td>Haplotype 2: BAt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>313/435/175</td>
<td>42.5</td>
<td>41.9</td>
</tr>
<tr>
<td>Haplotype 3: bAt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/1/2 copies</td>
<td>742/169/12</td>
<td>10.5</td>
<td>11.7</td>
</tr>
</tbody>
</table>
VDR genotypes and mortality was not significant (P > 0.2).

Analysis of the separate single nucleotide polymorphisms (BsmI, ApaI, 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: 1.09, 0.81–1.47). 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–ApaI–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–ApaI–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–ApaI–TaqI haplotype 1 showed a tendency to be associated with differences in all-cause mortality risk. Statistical adjustment for common cardiovascular risk factors was performed to assess the effect of VDR polymorphisms on all-cause mortality.
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 naïve 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.

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

<table>
<thead>
<tr>
<th></th>
<th>CV mortality</th>
<th>Non-CV mortality</th>
<th>Relative contribution</th>
<th>Cancer mortality</th>
<th>Non-cancer mortality</th>
<th>Relative contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>HR (95% CI)</td>
<td>n</td>
<td>HR (95% CI)</td>
<td>n</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Cdx2–GATA haplotype 1 (GG)</td>
<td>44</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>31</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>0 copy</td>
<td>87</td>
<td>1.09 (0.74–1.62)</td>
<td>1.20 (0.93–1.57)</td>
<td>1.33 (0.97–1.84)</td>
<td>0.91 (0.50–1.55)</td>
<td>0.71 (0.45–1.13)</td>
</tr>
<tr>
<td>1 copy</td>
<td>165</td>
<td>0.90 (0.51–1.56)</td>
<td>0.89 (0.65–1.16)</td>
<td>0.63 (0.78–1.86)</td>
<td>1.49 (1.05–2.65)</td>
<td>0.71 (0.52–0.95)</td>
</tr>
<tr>
<td>2 copies</td>
<td>22</td>
<td>1.13 (0.90–1.48)</td>
<td>1.14 (0.60–1.11)</td>
<td>1.09 (0.67–1.77)</td>
<td>1.09 (0.67–1.77)</td>
<td>1.49 (0.84–2.65)</td>
</tr>
<tr>
<td>BsmI–ApaI–TaqI haplotype 1 (baT)</td>
<td>54</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>38</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
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<tr>
<td>0 copy</td>
<td>112</td>
<td>0.80 (0.56–1.15)</td>
<td>0.88 (0.69–1.13)</td>
<td>0.91 (0.58–1.40)</td>
<td>0.91 (0.59–1.39)</td>
<td>0.84 (0.67–1.06)</td>
</tr>
<tr>
<td>1 copy</td>
<td>148</td>
<td>0.91 (0.58–1.40)</td>
<td>0.91 (0.58–1.40)</td>
<td>0.87 (0.50–1.51)</td>
<td>0.87 (0.50–1.51)</td>
<td>0.87 (0.50–1.51)</td>
</tr>
<tr>
<td>2 copies</td>
<td>28</td>
<td>0.63 (0.45–1.13)</td>
<td>0.63 (0.45–1.13)</td>
<td>0.63 (0.45–1.13)</td>
<td>0.63 (0.45–1.13)</td>
<td>0.63 (0.45–1.13)</td>
</tr>
</tbody>
</table>
The BsmI–ApaI–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 the data. The HR was not influenced by correction for several cardiovascular risk factors, suggesting that the effect is not mediated by these factors.

The BsmI, ApaI, 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 ApaI 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–ApaI–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–ApaI–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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