The impact of vitamin D pathway genetic variation and circulating 25-hydroxyvitamin D on cancer outcome: systematic review and meta-analysis

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Background: Vitamin D has been linked with improved cancer outcome. This systematic review and meta-analysis investigates the relationship between cancer outcomes and both vitamin D-related genetic variation and circulating 25-hydroxyvitamin D (25OHD) concentration.

Methods: A systematic review and meta-analysis of papers until November 2016 on PubMed, EMBASE and Web of Science pertaining to association between circulating vitamin D level, functionally relevant vitamin D receptor genetic variants and variants within vitamin D pathway genes and cancer survival or disease progression was performed.

Results: A total of 44 165 cases from 64 studies were included in meta-analyses. Higher 25OHD was associated with better overall survival (hazard ratio (HR) = 0.74, 95% CI: 0.66–0.82) and progression-free survival (HR = 0.84, 95% CI: 0.77–0.91). The rs1544410 (BsmI) variant was associated with overall survival (HR = 1.40, 95% CI: 1.05–1.75) and rs7975232 (ApaI) with progression-free survival (HR = 1.29, 95% CI: 1.02–1.56). The rs2228570 (FokI) variant was associated with overall survival in lung cancer patients (HR = 1.29, 95% CI: 1.0–1.57), with a suggestive association across all cancers (HR = 1.26, 95% CI: 0.96–1.56).

Conclusions: Higher 25OHD concentration is associated with better cancer outcome, and the observed association of functional variants in vitamin D pathway genes with outcome supports a causal link. This analysis provides powerful background rationale to instigate clinical trials to investigate the potential beneficial effect of vitamin D in the context of stratification by genotype.

The importance of vitamin D for bone health is well established, but the role of vitamin D beyond the skeletal system has been under debate for decades (Theodoratou et al, 2014). In recent years, it has become apparent that the vitamin D receptor (VDR) is expressed in most cells, and that multiple tissues have the ability to convert the primary circulating form of vitamin D into the active form (Bouillon et al, 2013), implying that extra-skeletal effects of vitamin D are likely.

While typically thought of as ‘vitamin’, it may be more appropriate to regard the primary circulating form, 25-hydroxyvitamin D (25OHD), as a pre-hormone and the primary active form, 1,25-dihydroxyvitamin D (1,25(OH)₂D), as a hormone. It has been previously recognised that mutations in genes involved in response to hormones, their metabolism or actions may affect the prognosis of disease and thus act as modifiers. Correspondingly, 1,25(OH)₂D binds to the VDR (a ligand-dependent
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transcription factor) and polymorphisms in the VDR gene have been shown to modify the activity of this VitD–VDR complex (Anderson et al, 2003): for example, rs11568820 is situated in the VDR promoter region and can influence transcriptional activity (Yamamoto et al, 1999), while rs2228570 affects the translational start site (Uitterlinden et al, 2004). Therefore, it is hypothesised that not only vitamin D status but also expression and structure of VDR determine molecular actions, and can potentially modify cancer risk and survival (Flugge et al, 2007; Li et al, 2007; Zgaga et al, 2014). The VitD–VDR complex has the ability to exert downstream biological effects; amongst others, it can regulate the expression of multiple target genes, including several with antimetabolism (Ramagopalan et al, 2010). Moreover, polymorphisms in the VDR gene have been linked to cancer risk, including prostate (Taylor et al, 1996), breast (Lowe et al, 2005), skin and bowel (Ingles et al, 2001; Xu et al, 2014; Serrano et al, 2016), and VDR expression has been linked to survival in prostate and breast cancer (Berger et al, 1991; Hendrickson et al, 2011; Ditsch et al, 2012). Unlike highly variable environmental exposures (sunlight, diet and supplements) or seasonally varying 25OHD levels (Kelly et al, 2015), genetic variants are constant, present since conception and cannot be modified by the disease; thereby removing reverse causation concerns.

Three aspects further strengthen the case for understanding the associations between vitamin D and cancer outcomes: first, cancer incidence and mortality are increasing (CRUK, 2015); second, vitamin D deficiency is common worldwide among otherwise healthy individuals (Holick, 2007; Zgaga et al, 2011), and particularly among cancer patients (Crew et al, 2009; Fakih et al, 2009; Shanafelt et al, 2011; Vrieling et al, 2011; Zgaga et al, 2014), and third, vitamin D deficiency is a modifiable risk factor; based on the studies that report an association between vitamin D deficiency and poorer cancers outcomes. Unsurprisingly, it has been proposed that vitamin D may have potential value as an adjuvant chemotherapeutic agent, particularly since vitamin D supplements are cheap, safe and readily available (Newton-Bishop et al, 2009, 2015; Drake et al, 2010; Hatse et al, 2012; Zgaga et al, 2014).

Here we present a systematic review and meta-analysis examining the role of vitamin D on cancer progression and survival. We conducted a comprehensive evaluation of the literature that examines the associations between cancer outcomes and genetic factors involved in the vitamin D pathway, in addition to circulating 25OHD concentration. Focus on vitamin D-related genetic variation allowed us to partially mitigate against potential confounding or reverse causation, biases that typically limit implications of findings from observational vitamin D studies.

MATERIALS AND METHODS

Literature search. We performed a systematic literature review and meta-analysis following PRISMA guidelines (Moher et al, 2009). The electronic databases PubMed (NCBI, 2015), EMBASE (EMBASE, 2015), and Web of Science (JISC, 2015) were searched up to week 3, November 2015. We searched for studies that examined the association between cancer outcomes and (i) measured vitamin D levels and (ii) genetic factors known to affect vitamin D metabolism or pathways. A list of search terms was compiled using a number of core papers in the field. For cancer outcomes, we included a combination of terms: cancer, neoplasm, malignant, malignancy with survival, outcome, prognosis, mortality, death, recurrence. For vitamin D levels, we included terms: 25-hydroxyvitamin D, calcidiol and 25OHD; for vitamin D receptor, and for commonly studied variants, we searched for: vitamin D receptor, VDR, rs1544410, BsmI, rs10735810, rs2228570, FokI, rs7975232, ApaI, rs11568820, Cdx-

2, rs2282679, rs12785878, rs10741657 and rs6013897. Finally, we also included variation in genes related to vitamin D synthesis, transport or metabolism: 1-α-hydroxylase, CYP27B1, 25-hydroxy-, CYP2R1, 24-hydroxylase, CYP24A1, vitamin D binding protein, 27-hydroxylase and CYP27A1. Genetic variants beyond those explicitly searched for were not included if previously shown to affect vitamin D metabolism. We considered all human research full text articles, with no restriction on language or article type. Bibliographies of retrieved papers and previous reviews were hand-searched to identify other relevant studies.

Selection criteria and selection of relevant studies. Study inclusion ‘PICO’ criteria were as follows: (i) participants: individuals of any age who received a diagnosis of cancer; (ii) intervention/exposures: assessment of vitamin D status or genetic factors known to affect vitamin D concentration, metabolism or pathways; (iii) comparators: study reports a quantitative association between cancer outcome and either vitamin D status (e.g., concentration, quartiles, low/high levels) sampled at most 1 year prior to the diagnosis, or any germline genetic variation or gene expression in normal tissue; and (iv) outcome: cancer-specific or all-cause mortality, or disease progression (e.g., disease-free survival, local recurrence or metastasis). Observational retrospective and prospective cohorts were included.

In relation to patients, exclusion criteria were: (i) pre-cancerous lesions, and (ii) mixed-cancer cohort without site-specific reporting; in relation to exposures: (iii) vitamin D intake and supplementation, (iv) acquired non-germline mutations or tumour gene expression, and (v) predicted vitamin D status; in relation to outcomes: (vi) prognostic markers such as Prostate Specific Antigen or Breslow thickness, (vii) population cancer mortality rates; in relation to study/publication type: (viii) ecological studies, and (ix) reviews, editorials, case reports, conference abstracts and nonclinical publications. If the same patient cohort was reported on more than once, we used the highest quality, largest sample size or most recent publication. Article titles and abstracts were screened for eligibility, independently by two authors (PVS and LZ or FOS). Disagreements were resolved by discussion and review of full text.

Data extraction. The data extraction was performed by a single investigator (PVS or FOS) using the predefined data fields and extraction was cross-checked by a second investigator in its

| Box 1. Conversion of continuous HR and 95% CI estimate to per 10 ng ml⁻¹ HR estimates. |
|---------------------------------|
| To achieve this, we raised the continuous HR (or HR per 1ng ml⁻¹) to the power of 10 to get [A], per 10 ng ml⁻¹ HR (e.g., continuous HR: 0.89, hence per 10 ng ml⁻¹ HR: 0.89¹⁰ = 0.3188). In order to calculate the confidence intervals we first found the exp(s.e.(beta)) = [B]; the standard errors (s.e.) were calculated using the formula below. We then calculated 1.96*(x-bar) = [C], where x was fixed at 10 ng ml⁻¹ and xbar was the median of all cohort means from the rest of the studies included in the meta-analysis. We found this to be 23 ng ml⁻¹, therefore 1.96*(10–23) = –25.872 = [C]. [B] was then raised to the power of [C], to get [D]. Finally, the HR per 10 ng ml⁻¹ [A] was multiplied or divided by [D] in order to derive the upper and lower 95% confidence intervals. Therefore, the resulting HR was A and 95% CI: (A*D) to (A/D).
| Continuous HR 10⁻¹ [A] – per 10 ng/ml HR |
|---------------------------------|
| exp(s.e.(beta)) = [B] |
| 1.96*(x–xbar) = [C] |
| B*C = [D] |
| A*D = lower 95% CI |
| A/D = upper 95% CI. |
entirety (FOS or PVS). The data from eligible studies were extracted using a tailored data extraction form that included the following information: first author, publication year, location or ethnicity of patients, sample size, mean age, gender, cancer site (subtype/histology where relevant), cancer stage, any interventions (e.g., chemotherapy), vitamin D exposure studied and important meta-data (time of sampling, mean/median 25OHD values or range for categories being compared; SNP position, name and rs ID, genotypes compared and model: additive, recessive or dominant), covariates considered, details of outcomes studied,

**Figure 1. PRISMA Flowchart of the study selection process.** Two studies used the same prostate cancer cohort but one reported on circulating 25OHD and the other on genetic variants, and so both were retained. (Holt et al, 2010, 2013) Three publications used the same initial cohort of lung cancer patients but two reported on different subpopulations of patients (according to disease stage) and so were retained, (Zhou et al, 2007; Heist et al, 2008), while a third reported on different exposures to the first two and so was also retained (Zhou et al, 2006). Finally, four studies reported on the same melanoma patient cohort (Newton-Bishop et al, 2009, 2015; Field et al, 2013; Davies et al, 2014) (one paper scored lower in NOS scoring was excluded (Field et al, 2013), while the remaining three, which reported different exposure or outcomes were retained. *Only a single study reported impact of circulating vitamin D-binding protein levels on outcome and so could not be included in the meta-analysis.

*Includes only exposures and outcomes included in MA. Articles may report on multiple exposure-outcome pairs hence the sum of the pairs is greater than the number of articles included. For example, several papers studied the effect of more than one SNP for example, Zgaga et al, (Zgaga et al, 2014), while many papers studied the impact on both overall survival or progression-free survival for example, Lohman et al (Lohmann et al, 2015). However, where multiple estimates were extracted, no patient was included more than once for a certain exposure or outcome. †Study authors were contacted to provide HR, RR or OR when not reported; 13 did not respond. ‡One study (Vrieling et al, 2011) used the same breast cancer cohort as a later, larger study (Vrieling et al, 2014) and as both had the same NOS score, the newer study was included. 25OHD: 25-hydroxyvitamin D; DBP: vitamin D binding protein; HR: hazard ratio; PSA: prostate specific antigen; WOK: Web of Knowledge.
Table 1. Characteristics of studies (N = 64) included in the meta-analysis

| First author, year | Cancer (subtype) | HR/OR | Sample Size | Site | Follow-up (m) | Events | NOS | 25OHD | Genetic | Progression | Survival |
|-------------------|------------------|-------|-------------|------|---------------|--------|-----|-------|---------|-------------|----------|
| Anic et al (2012) | Brain (glioma)   | HR    | 320         | USA  | 28            | 248 cancer deaths | 5   |       | BT     |             | CS       |
| Lim et al (2015)  | Breast           | HR    | 491         | Korea| 86            | 32 recurrences, 22 cancer deaths | 8   |       | BT     | DFS        | CS       |
| Lohmann et al (2015) | Breast     | HR    | 934         | Canada| 112         | Not given          | 4   |       | BT     | RFS        | OS       |
| Clark et al (2014) | Breast          | HR    | 82          | USA  | >36           | 23 relapses or deaths | 5   |       | BT     | RFS        | OS       |
| Vrieling et al (2014) | Breast   | HR    | 2177        | Germany| 64          | 206 cancer deaths, 241 recurrences or deaths | 7   |       | 66% BT | DFS        | CS       |
| Mishra et al (2013) | Breast        | OR    | 232         | USA  | NA            | Not given          | 5   |       | DF     |             | OS       |
| Pande et al (2013) | Breast         | HR    | 1029        | USA  | 114           | 266 recurrences or deaths | 6   |       | DF     |             | OS       |
| Perna et al (2013a) | Breast        | HR    | 498         | Germany| 60          | 48 cancer deaths    | 7   |       | CS     |             |          |
| Villasenor et al (2013) | Breast    | HR    | 585         | USA  | 110           | 48 cancer deaths    | 7   |       | AT     | DFI        | CS       |
| Hatte et al (2012) | Breast         | HR    | 1800        | Belgium| 56          | 118 relapses, 64 cancer deaths | 5   |       | BT     |            | OS       |
| Jacobs et al (2011) | Breast        | OR    | 512         | USA  | 88            | Not given          | 5   |       | AT     | R          | OS       |
| Kim et al (2012)  | Breast         | HR    | 310         | Korea| 23            | 33 metastases or deaths | 7   |       | BT     | DFS        | OS       |
| Goodwin et al (2009) | Breast      | HR    | 512         | Canada| 139         | 116 recurrences, 106 deaths | 7   |       | BT     | R          | OS       |
| Goode et al (2002) | Breast         | HR    | 721         | UK    | NA            | 200 deaths          | 6   |       |       | OS         |          |
| Lundin et al (1999) | Breast        | RR    | 111         | Sweden| 67          | 44 deaths           | 4   |       |       | OS         |          |
| Tretti et al (2012) | Breast, colon, lung, and lymphoma | HR | 658 | Norway | >60 | 343 cancer deaths | 7   |       | BT     | CS         |          |
| Wesa et al (2015)  | Colorectal     | HR    | 250         | USA  | NA            | 153 deaths          | 5   |       | BT     | OS         |          |
| Zgaga et al (2014) | Colorectal     | HR    | 1598        | UK    | 107           | 363 cancer deaths    | 8   |       | AT     | CS         |          |
| Perna et al (2013b) | Colorectal     | HR    | 1397        | Germany| 60          | 336 cancer deaths    | 6   |       | CS     |             |          |
| Szkandera et al (2013) | Colorectal | HR    | 264         | Austria| 53          | 45 recurrences      | 5   |       | R      |             |          |
| Fedirko et al (2012) | Colorectal     | HR    | 1202        | Europe| 73          | 444 cancer deaths    | 8   |       | BD     | CS         |          |
| Ng et al (2011)   | Colorectal     | HR    | 515         | USA  | 61            | 440 progression, 475 deaths | 5   |       | BT     | TTP        | OS       |
| Mezawa et al (2010) | Colorectal     | HR    | 257         | Japan | 32          | 30 cancer deaths, recurrences not given | 5   |       | NS     | DFS        | CS       |
| Ng et al (2008)   | Colorectal     | HR    | 304         | USA  | 78            | 96 cancer deaths     | 7   |       | BD     | CS         |          |
| Ren et al (2012)  | Gastric        | HR    | 197         | China | >60         | 106 deaths          | 5   |       | BT     | OS         |          |
| Lee et al (2014)  | Haematological (AML) | HR | 97  | China | >60 | 55 relapses, 51 deaths | 4   |       | BT     | R          | OS       |
| Shanafelt et al (2011) | Haematological (CLL) | HR | 543  | USA  | 118         | 201 progression, 96 deaths | 8   |       | NS     | TTT        | OS       |
| Aref et al (2013) | Haematological (CLL, NHL) | HR | 195 | Egypt | 60 | 118 deaths | 5   |       | BT     |             |          |
| Drake et al (2010) | Haematological (DLBCL) | HR | 983  | USA  | 35 | 404 events, 168 cancer deaths | 6   |       | 66% BT | EFF        | CS       |
| Pardanani et al (2011) | Haematological (PMF, MDS) | HR | 321 | USA | 34 | 36 progression, 171 deaths | 4   |       | BT     | LFS        | OS       |
| Bittenbring et al (2014) | Haematological (BCL) | HR | 359 | Germany | 49 | Not given | 4   |       | AT     | EFF        | OS       |
| Kelly et al (2015) | Haematological (FL) | HR | 423 | USA | 65 | 193 progression, 58 deaths | 5   |       | BT     | PFS        | OS       |
| Azad et al (2013) | Head and neck | HR | 522 | Canada | >53 | 214 deaths | 8   |       |       | OS         |          |
| Zeljic et al (2012) | Head and neck | OR | 110 | Serbia | 28–100 | Not given | 5   |       |       | CS         |          |
| Meyer et al (2011) | Head and neck | HR | 540 | Canada | 96 | 119 recurrences, 223 deaths | 8   |       | BT     | R          | OS       |
| Gugatschka et al (2011) | Head and neck (SCC) | RR | 88 | Austria | NA | 31 progression, 29 deaths | 4   |       | BT     | DFS        | OS       |
| Hama et al (2011) | Head and neck (SCC) | HR | 204 | Japan | 34 | 103 progression or deaths | 6   |       |       | DFS        |          |
| Finklemeier et al (2014) | Liver (HCC) | HR | 200 | Germany | 11 | 60 deaths | 6   |       | BT     | OS         |          |
| Zhou et al (2007) | Lung           | HR    | 447         | USA  | 72            | 126 cancer deaths    | 7   |       | BT     | CS         | OS       |
| Liu et al (2011)  | Lung (AC, SCC) | HR    | 568         | China | 19          | 311 deaths          | 6   |       | NS     | OS         |          |
| Heist et al (2008) | Lung (AC, SCC) | HR    | 294         | USA  | 42            | 233 deaths          | 6   |       | NS     | OS         |          |
The association between circulating 25OHD and outcomes was summarised in meta-analyses by comparing the risk in the highest to the lowest reported category. The majority of studies used vitamin D categories such as quartiles or tertiles. To enable inclusion of studies that used 25OHD as a continuous variable, we sought to transform the 'continuous HR' into a 'HR per 10 ng ml⁻¹' (Box 1).

**Genetic factors.** For SNPs, the rs number naming convention was typically used in the paper and some recoding was needed to ensure that uniform reference system was followed. For example, where a restriction fragment length polymorphism was referenced, the mutation and risk allele were recoded (e.g., FokI, rs10735810 and rs2228570 are the same variant).

| First author, year | Cancer (subtype) | HR/OR | Sample Size | Site | Follow-up (m) | Events | NOS | 25OHD | Genetic | Progression | Survival |
|--------------------|------------------|-------|-------------|------|--------------|--------|-----|-------|---------|-------------|----------|
| Zhou et al (2004)  | Lung (AC, SCC)   | HR    | 373         | USA  | 71           | 186    | 7   | ✔     |         | ✔           | OS       |
| Xiong et al (2013) | Lung (NSCC)      | HR    | 755         | China| NA           | Not given | 4   | ✔     |         | ✔           | OS       |
| Newton-Bishop et al (2015) | Melanoma | HR | 2182       | UK   | NA           | Not given | 6   | ✔     |         | NS          | CS       |
| Davies et al (2014) | Melanoma        | HR    | 3137        | Various | 96      | 653     | 7   | ✔     |         | ✔           | OS       |
| Orlov et al (2014) | Melanoma        | HR    | 3566        | Worldwide | 91     | 254 cancer deaths | 7   | ✔     |         |             | CS       |
| Newton-Bishop et al (2009) | Melanoma | HR | 872        | UK   | 56          | 173 relapses | 5   | ✔     | NS          |             | DFS      |
| Halsall et al (2004) | Melanoma       | HR    | 171         | UK   | 75          | 18 metastases | 4   | ✔     |         | M           |          |
| Webb et al (2015)  | Ovarian         | HR    | 670         | Australia | >60   | 491 progression; 435 deaths | 7   | ✔     |         | BT          | PFS      |
| Tamez et al (2009) | Ovarian         | HR    | 101         | Japan | 85          | 28 cancer deaths; total deaths not given | 7   | ✔     | ✔           |             | OS       |
| Van Loon et al (2014) | Pancreatic | HR | 256       | Europe | 35     | progression not given; 254 deaths | 4   | ✔     | ✔           |             | PFS      |
| Cho et al (2013)   | Pancreatic      | HR    | 178         | USA  | 33          | 82 deaths | 5   | ✔     | ✔           |             | OS       |
| Gupta et al (2015) | Prostate        | HR    | 125         | USA  | 31          | 49 deaths | 7   | ✔     | ✔           |             | PFS      |
| Trummer et al (2015) | Prostate | HR | 702       | Austria | 73-91  | 93 metastases; 123 deaths | 6   | ✔     | ✔           |             | M CS     |
| Holt et al (2013)  | Prostate        | HR    | 1476        | USA  | 130         | 325 progression; 95 cancer deaths | 7   | ✔     |             | NS          | P CS     |
| Pao et al (2013)   | Prostate        | HR    | 601         | Taiwan | 60-120 | 415 progression; 101 cancer deaths | 8   | ✔     |             | P CS        |          |
| Fang et al (2011)  | Prostate        | HR    | 1822        | USA  | 120         | 166 cancer deaths | 8   | ✔     | ✔           |             | BD CS     |
| Holt et al (2010)  | Prostate        | HR    | 1294        | USA  | 102         | 139 recurrences; 57 cancer deaths | 8   | ✔     | ✔           | R CS        |          |
| Penney et al (2010) | Prostate     | OR    | 1292        | USA  | >60         | Not given | 5   | ✔     | ✔           |             | OS CS     |
| Trettli et al (2009) | Prostate | HR | 160       | Norway | 44     | 52 cancer deaths | 6   | ✔     | 77% BT       |             | CS       |
| Williams et al (2004) | Prostate   | HR    | 728         | USA  | 60-120      | Not given | 7   | ✔     | ✔           |      DFS     |          |
| Renal              | HR              | 630   | Europe       | 30   | 152 cancer deaths | 8   | ✔     | ✔           |             | CS CS     |
| Obara et al (2007) | Renal (RCC)    | RR    | 135         | Japan | >60         | Not given | 5   | ✔     | ✔           |             | CS CS     |
| Samimi et al (2014) | Skin (Merkel cell) | HR | 89       | France | NA      | 33 metastases; 19 deaths | 6   | ✔     | ✔           | NS CS        | M CS     |

**Abbreviations:** AC—adenocarcinoma; ALL—acute lymphocytic leukaemia; AML—acute myeloid leukaemia; AT—25OHD—assayed after cancer treatment; BCL—B-cell lymphoma; BD—25OHD—assayed before diagnosis; BT 25OHD—assayed before treatment; CML—chronic myeloid leukaemia; CS—cancer-specific survival; DFI—disease-free interval; DFS—disease-free survival; DLBCL—diffuse large B-cell lymphoma; EFF—event-free survival; FL—follicular lymphoma; HCC—hepatocellular carcinoma; LFS—locoregional failure; M—metastasis; MDS—myelodysplastic syndrome; NA—not available; NHL—Non-Hodgkins lymphoma; NOS—Newcastle-Ottawa score; NS—non-Hodgkins lymphoma; NSCC—non-small-cell lung carcinoma; OS—overall survival; P—progression not otherwise specified; PFS—progression-free survival; PMF—primary myelofibrosis; R—recurrence or relapse not otherwise specified; RCC—renal cell carcinoma; RFS—relapse/recurrence-free survival; SCC—squamous cell carcinoma; TTP—time to progression; TTT—time to treatment.
subpopulations, outcomes, and/or exposures. The extracted HRs and 95% CIs were used to calculate the pooled HR estimates. The standard errors (s.e.) were used to calculate weighting for each study. The DerSimonian and Laird random-effects model was used to calculate pooled HR because of the a priori expected heterogeneity between studies, due to differences among populations and methodological dissimilarities between studies; most notably, different definition of 25OHD categories. All analyses were performed in R (R Core Team, 2013), and the R-package ‘metafor’ was used for meta-analyses (Viechtbauer and Cheung, 2010). P-value <0.05 was considered statistically significant.

Table 2. Characteristics of studies (*N* = 17) included in the qualitative synthesis

| First author, year | Cancer (subtype) | Size | Follow-up (m) | Events | NOS | 25OHD | Genetic | Progression | Survival | Author conclusion | Reason excluded |
|-------------------|------------------|------|---------------|--------|-----|-------|---------|-------------|----------|------------------|-----------------|
| Obermannova et al (2015) | Colorectal | 84 | 24 | Not given | 4 | ⬤ | PFS | OS | Consistently low 25OHD (always <16 ng/ml) associated with worse PFS and OS | Serial 25OHD |
| Turner et al (2013) | Lung (NSCC) | 142 | 52 | Not given | 7 | ⬤ | CS | OS | Low serum DBP levels predicted lung cancer-specific death (P = 0.04) | Only paper reporting DBP |
| Tuma et al (2012) | Lung(NSCC) | 62 | NA | Not given | 5 | ⬤ | OS | OS | Haplotype analysis revealed rs731236 (Tagl) = c2228570 (FokI) TTF1/TTF1 haplotype associated with reduced OS (P = 0.04) | No individual SNP HR |
| Bade et al (2014) | Melanoma | 324 | NA | Not given | 6 | ⬤ | OS | OS | Increased 25OHD (Q4 v Q1) associated with increased OS 195 months v 80 months (P = 0.049) | No HR |
| Der et al (2014) | Prostate | 16535 | 60 | 4613 deaths | 5 | ⬤ | OS | Vitamin D deficiency significantly associated with reduced survival (<0.001) | No HR |
| Dickinson et al (2010) | Haematological (AML, CML) | 228 | NA | 55 relapses, 84 deaths | 5 | | R | OS | No data provided on impact of VDR variants | No HR |
| Furuya et al (1999) | Prostate | 66 | NA | Not given | 3 | | PFS | | Taql TT genotype associated with shorter PFS (P = 0.07) | No HR |
| Hansson et al (2014) | Haematological (AML, ALL, CML, MDS) | 123 | 96 | 29 relapses, 31 deaths | 6 | | R | OS | 25OHD < 20 ng/ml associated with reduced OS (P = 0.01) and increased relapse (P = 0.03) | No HR |
| Kim et al (2012) | Haematological | 100 | 105 | 12 relapses, 4 deaths | 4 | | EFS | OS | VDR rs2228570 FokI genotype did not impact survival in paediatric ALL | No HR |
| Nurnberg et al (2009) | Melanoma | 205 | NA | 118 metastases | 4 | | M | OS | 25OHD > 20 ng/ml associated with increased time to distant metastatic disease (P = 0.64) | No HR |
| Peiris et al (2013) | Bladder | 4126 | NA | 2025 deaths | 6 | | OS | OS | 25OHD < 20 ng/ml associated with reduced OS (Q2 = 10.44; P = 0.001) | No HR |
| Silvagno et al (2010) | Ovarian (Epithelial) | 26 | NA | Not given | 2 | | OS | OS | Increased platelet VDR expression (>50 fMol) associated with increased OS (P = 0.12) | No HR |
| Walentowicz-Sadlejka et al (2012) | Ovarian | 72 | 60 | 45 deaths | 6 | | OS | OS | 25OHD < 10 ng/ml associated with reduced OS (P = 0.04) | No HR |
| Yagmurdkur et al (2009) | Breast | 56 | 60 | 5 recurrences | 3 | | R | | rs1544410 (BsmI) genotype not associated with local recurrence or metastasis (P > 0.55) | No HR |
| Yalikavak et al (2014) | Breast | 87 | 60 | Not given | 3 | | PFS | OS | rs2228570 FokI ff genotype not associated with reduced DFS 35 months vs > 54 months (P = 0.08) | No HR |
| Field et al (2013) | Melanoma | 795 | 56 | 137 cancer deaths | 4 | | CS | OS | 8 ng/ml incremental increase in 25OHD associated with improved DFS (P = 0.02) and MSS (P = 0.05) | Duplicate patient cohort |
| Vrieling et al (2011) | Breast | 1295 | 70 | 182 recurrence or metastases, 183 deaths | 7 | | DFS | OS | Low 25OHD significantly associated with worse DFS and OS | Duplicate patient cohort |

Abbreviations: AML = acute myeloid leukaemia; ALL = acute lymphocytic leukaemia; CML = chronic myeloid leukaemia; CS = cancer-specific survival; DFS = disease-free survival; DBP = vitamin D binding protein; EFF = event-free survival; Fmol = femtomol; HR = hazard ratio; m = months; M = metastasis; MDS = myelodysplastic syndrome; MSS = melanoma specific survival; NOS = Newcastle-Ottawa score; NSCC = non-small-cell lung carcinoma; OS = overall survival; PFS = progression-free survival; R = recurrence or relapse not otherwise specified; SNP = single nucleotide polymorphism.
'high' and 'low' categories compared (below or $\geq 20$ ng ml$^{-1}$), and (vii) the degree of deficiency in 'low' category (mean/median 25OHD concentration below or $\geq 12.5$ ng ml$^{-1}$). Publication and selection bias was investigated by checking for asymmetry in the funnel plots and running the Egger's regression test (Sterne and Egger, 2001).

RESULTS
A flowchart illustrating study selection is shown in Figure 1. After removal of duplicates, the search yielded 3070 potential articles. Irrelevant articles were eliminated after screening titles ($N = 2708$).
or abstracts (N = 262). One hundred full-texts were considered for inclusion and assessed for eligibility and 19 were excluded. Finally, 81 articles were kept for the systematic review and 64 of these were included in the meta-analysis. The main characteristics of included studies are summarised in Table 1 and Table 2.

Assessment of included studies. The risk of bias assessment revealed that 35 studies (43%) had a low risk of bias, 35 (43%) had an uncertain, and 11 (14%) had a high risk of bias. The risk of bias assessment summary per each domain is shown in Supplementary Figure S1 and individual study scores in Supplementary Figure S2. Sixty-four studies were included in the meta-analysis, with a total of 44 165 patients. Most studies were conducted in the USA (N = 24) and Europe; breast cancer was most commonly studied (N = 15), followed by nine studies (each) on prostate cancer and colorectal cancer. In total, 157 HR estimates for a range of exposure-outcome pairs were included in meta-analyses: 77 estimates (from 41 studies) for association with 25OHD, and 80 estimates (from 27 studies) relating to genetic factors. Separate estimates were extracted for different patient subgroups (e.g., different type of haematological malignancy (Drake et al., 2010)), different exposures (e.g., multiple polymorphisms (Zgaga et al., 2014)), or different outcome (i.e., survival or disease progression (Lohmann et al., 2015)). No patients were included more than once in meta-analysis, as separate meta-analyses have been conducted for each exposure-outcome pair. Very large differences were observed in definition of vitamin D categories being compared. For example, the median 25OHD concentration was 18.26 ng ml⁻¹ in the ‘high’ category in one study, (Zgaga et al., 2014) yet this was actually lower than the median (19.7 ng ml⁻¹) in the ‘low’ category in another study (Hatse et al., 2012). The variety of vitamin D categories, cutoffs and means/medians used are presented in Figure 2 and Supplementary Figure S3.

Meta-analysis of 25OHD studies

Circulating vitamin D and survival. Forty-eight estimates from 38 studies were included in the meta-analysis of 25OHD and survival (17 studies (45%) examined cancer-specific mortality), comprising in total 24 013 cancer patients. Twelve cancer types were represented: breast, haematological, head and neck, colorectal, lung, prostate, skin, pancreatic liver, gastric, kidney and ovarian cancers. Overall, a significantly reduced risk of death was observed in definition of vitamin D categories or approximation of median. No category (Q1(median) = 9.86 – ((24.4 – 9.86)/2))/2 = 6.225 and Q4(median) = 24.4 + ((24.4 – 9.86)/2)/2 = 28. Insufficient data were reported in three studies to allow graphical illustration of categories or approximation of median. NA = data not reported; For Tretti et al., study: B = breast; C = colon; L = lung; Ly = lymphoma.
Figure 3. Cancer survival and 25-hydroxyvitamin D concentration: meta-analysis of adjusted hazard ratios. HR are sorted by cancer site and the difference in median between ‘high’ and ‘low’ vitamin D categories compared. Acute myeloid leukaemia (AML), Chronic Lymphoid Leukaemia (CLL), and subtypes of non- Hodgkin’s lymphoma (NHL) (large B-cell lymphoma (DLBCL), T-cell lymphoma (TCL), Follicular Lymphoma (FL) and mantle cell lymphoma (MCL)). Myelodysplastic syndrome (MDS) and primary myelofibrosis (PMF). F = breast; 0, haematological: 0, colorectal: 0.91, prostate: 0.68, head and neck: 0, pancreatic: 0.66, lung: 0.93, skin: 0, overall cancer: 0.18. Approximated Median in studies using quartiles/tertiles (ng ml−1): Treti breast (lower: 12.9, upper: 33.9), Treti Haematological: (lower: 14.3, upper: 34.1), Treti colorectal: (lower: 16.4, upper: 38), Treti lung: (lower: 14.3, upper: 34.1), Vnieling: (lower: 10.6, upper: NA), Kelly (NA), Fedirko: (lower: 11.8, upper: 33.4), Ng et al (2011): (lower: 9.6, upper: 30.7), Zgaga: (lower: 4.4, upper: 18.3), Ng et al (2008): (lower: 21, upper: 30.6), Liu: (lower: 7, upper: 25.4), Zhou: (lower: 7.4, upper: 24.5), Heist: (lower: 10.4, upper: 23.9), Meyer: (lower: 16.2, upper: 34.2), Fang: (NA), Muller: (NA).

| Author and Year | Population | N.O. score | N | Exposure (ng ml−1) | Weight | HR (95% CI) |
|-----------------|------------|------------|---|-------------------|--------|-------------|
| **Breast**      |            |            |   |                   |        |             |
| Hatse, 2012     | Belgium    | 5          | 1800 | >30 vs <30        | 1.6%   | 0.49 (0.27, 0.89) |
| Vitassena, 2013 | USA        | 7          | 580 | >30 vs <30        | 0.9%   | 1.21 (0.52, 2.80) |
| Goodwin, 2009   | Canada     | 5          | 712 | >29 vs <38        | 2.2%   | 0.63 (0.38, 1.04) |
| **Treti, 2012** | Norway     | 8          | 251 | Quartile 4 vs Quartile 1 | 1.3%   | 0.42 (0.21, 0.82) |
| Jacobs, 2011    | USA        | 5          | 712 | >30 vs <30        | 2.5%   | 0.88 (0.56, 1.39) |
| Lohmann, 2015   | Canada     | 5          | 934 | >20 vs >16        | 1.4%   | 1.07 (0.57, 2.02) |
| Lin, 2015       | Korea      | 8          | 491 | >20 vs >20        | 0.6%   | 0.46 (0.17, 1.22) |
| Vnieling, 2014  | Germany    | 7          | 2177 | Tertile 3 vs tertile 1 | 3.3%   | 0.79 (0.53, 1.16) |
| **Subtotal (breast)** |          |            |   |                   | 0.75 (0.56, 0.95) |
mortality. By far, the most commonly studied were polymorphisms in VDR gene, particularly rs228570 (FokI), rs1544410 (BsmI), rs731236 (TaqI), rs11568820 (Cdx2), and rs7975232 (Apal). In meta-analysis, rs1544410 TT/TC genotypes were associated with worse survival compared to CC genotype (HR = 1.40, 95% CI = 1.05–1.75; Figure 5). The same direction of the effect was observed in the sensitivity analyses after exclusion of studies with NOS < 7 (Supplementary Figure S4) and those reporting on cancer-specific mortality, but the association was no longer significant (Supplementary Figure S5). In lung cancer patients, a poorer outcome was observed to be associated with rs228570 TT/TC carriers (HR = 1.29, 95% CI = 1.00–1.57) and a consistent albeit non-significant association was found across all cancers (HR = 1.26, 95% CI = 0.96–1.56). A significant association was observed with rs731236 (TaqI) variant when limited to studies at low risk of bias (NOS score ≥ 7; HR = 0.79, 95% CI = 0.62–0.95, Supplementary Figure S4). Other genetic factors were investigated in at most three original studies and no other statistically significant results were observed.

VDR and vitamin D pathway SNPs and disease progression. Ten studies examined the effect of genetic variation on disease progression (Figure 6; for sensitivity analysis see Supplementary Figure S6). In meta-analysis of three studies with a total of 1588 patients, it was observed that rs7975232 AA carriers had significantly worse survival than CC carriers (HR = 1.29, 95% CI = 1.02–1.56). Additionally, a suggestive association was observed for vitamin D binding protein variant rs2282679 (HR = 1.22, 95% CI = 0.99–1.46) in meta-analysis of two studies.

Testing for publication bias and study heterogeneity. There was some evidence of heterogeneity between studies in meta-analysis of 25OHD and some evidence of publication bias (Supplementary Figures S7 and S8). A non-significant degree of heterogeneity and evidence of publication bias were observed in some subgroup analysis. Heterogeneity was observed for subgroup analysis of rs1544410, rs7975232, rs22825870 and rs731236, as well as for some individual cancer types while publication bias was observed for rs1544410, rs22825870 and rs731236 (Supplementary Figures S7 and S8).

Studies not included in meta-analysis. Seventeen papers were excluded from the meta-analysis, but their findings were nonetheless considered (Table 2). Eight studies report improved overall and/or progression-free survival among those with higher 25OHD concentration (Vrieling et al, 2011; Walentowicz-Sadlecka et al, 2012; Peiris et al, 2013; Field et al, 2013; Bade et al, 2014; Der et al, 2014).
2014; Hansson et al, 2014; Obermannova et al, 2015) and one study found no association between 25OHD and incidence of metastases (Nurnberg et al, 2009). Seven studies investigated genetic variants and outcome (median sample size: 66). One study reported that the rs731236/rs2228570 (Tafl-FokI, TTFf/TTFf) haplotype was significantly associated with reduced overall survival (HR = 1.81, 95% CI: 1.23–3.48, P = 0.04) (Turna et al, 2012): suggestive associations were reported between progression-free survival and rs731236 (AA) genotype in prostate cancer (Furuya et al, 1999) and rs2228570 TT genotype in breast cancer (Yiallourou et al, 2014), while there was no association found between rs2228570 and paediatric ALL (Kim et al, 2012). No association was observed between rs1544410 and breast cancer outcome (Yagmurdu et al, 2009). There was a suggestive association between platelet VDR expression and survival in ovarian cancer (Silvagno et al, 2010). Finally, low vitamin D binding protein (DBP) levels were found to be predictive of lung cancer death (Turner et al, 2013).

![Adjusted Meta-analysis: Survival for Vitamin D receptor and pathway polymorphisms](image)

Figure 5. Cancer survival and vitamin D receptor polymorphisms and other vitamin D-related genetic factors: adjusted meta-analysis. \( \chi^2 \) for Apal: 0.95, BsmI prostate: 0.93, BsmI Lung: 0.93, BsmI colorectal: 0, BsmI All: 0.85, Cdx2 prostate: 0, Cdx2 lung: 0, Cdx2 colorectal: 0, Cdx2 All: 0, FokI Prostate: 0, FokI lung: 0, FokI colorectal: 0, FokI All: 0.83, TaqI breast: 0.88, TaqI skin: 0.46, TaqI all: 0.86, Cyp24a1(1) all: 0.75, Cyp24a1(2) all: 0.67, GC; all: 0, Rs2107301 all: 0, Rs4516035: 0, Rs2238135: 0.
25OHD being a marker of healthier lifestyle (i.e., healthier diet improved survival observed in the original studies might be due to deficiency. For example, the association between 25OHD and cancer outcome are often also associated with vitamin D cancer progression is challenging because risk factors associated with reduction in disease progression was also found in those breast, haematological and skin cancer. With this in mind, we conducted a systematic review and meta-analysis to examine the relationship between vitamin D status and cancer outcome.

Our review suggests that higher circulating vitamin D pathway genes, and also by far the largest review on vitamin D status the relationship between cancer outcomes and variation in vitamin D 25OHD being a marker of healthier lifestyle (i.e., healthier diet improved survival observed in the original studies might be due to 25OHD being a marker of healthier lifestyle (i.e., healthier diet containing more fish; physical activity and spending time outdoors). However, evidence that genetic factors linked to vitamin D metabolism and pathways impact upon cancer survival may be used to counter such concerns and support a causal link. In our meta-analysis, we found evidence of an association between the VDR gene variants with functionally characterised effects and cancer outcome. Forty percent higher rate of death was observed in TT carriers at rs1544410 locus and 26% higher rate in TT carriers at rs2228570, while 29% increased risk of disease progression was observed in carriers at rs7975232 have been associated with changes in VDR messenger RNA expression (Staal et al, 1996; Uitterlinden et al, 2004). We hypothesise that interactions between mutations in the vitamin D pathway and vitamin D status exist,

| rs2228570 (Fokl)  | Breast  |  |
|-------------------|---------|-----------------------------|
| Perna,2013        | Germany | 6 498 TT vs CC 4.4% 0.70 (0.20, 2.10) |
| Prostate          |         |                            |
| Pao,2013          | Taiwan  | 8 601 TT vs CC 8% 0.81 (0.46, 1.44) |
| Holt,2010         | USA     | 8 1292 TT vs CC 6.1% 0.80 (0.30, 1.80) |
| Subtotal (Prostate)|        |                            |
| Lung              |         |                            |
| Heist,2008        | USA     | 7 294 TT vs CC 9.5% 1.41 (0.96, 2.07) |
| Zhou,2006         | USA     | 7 373 TT vs CC 9.1% 1.13 (0.74, 1.74) |
| Subtotal (Lung)   |         |                            |
| Colorectal        |         |                            |
| Perna,2013        | Germany | 7 1379 TT vs CC 10% 0.96 (0.68, 1.32) |
| Zgaga,2014        | Scotland | 8 1598 TT vs CC 10% 0.94 (0.59, 1.29) |
| Fedirko,2012      | Europe  | 8 1202 TT vs CC 10% 0.94 (0.70, 1.28) |
| Subtotal (Colorectal) |     |                            |
| Head and neck     |         |                            |
| Zeljic,2012       | Serbia  | 6 110 TT/TC vs CC 9% 1.72 (1.08, 2.70) |
| Skin              |         |                            |
| Orlow, 2014       | Worldwide | 7 3566 TT vs CC 10.7% 0.96 (0.77, 1.19) |
| Gloma 2014        | USA     | 5 320 TT vs CC 9.3% 1.42 (0.96, 2.13) |
| Ovarian           |         |                            |
| Tamez,2009        | Japan   | 7 101 TT/CT vs CC 3.6% 5.56 (1.64, 20.00) |
| SUBTOTAL          |         |                            |
| Rs731236 (TaqI)   | Breast  | 11,334                      |
| Lundin, 1999      | Sweden  | 4 111 GG vs AA 12.3% 0.90 (0.59, 1.54) |
| Goode, 2002       | UK      | 8 721 GG vs AA 11.2% 0.85 (0.54, 1.32) |
| Perna, 2013       | Germany | 6 498 GG vs AA 8.6% 3.00 (1.10, 8.10) |
| Subtotal (Breast) |         |                            |
| Prostate          |         |                            |
| Holt, 2010        | USA     | 8 1292 GG vs AA 8.9% 0.50 (0.20, 1.30) |
| Colorectal        |         |                            |
| Perna, 2013       | Germany | 7 1379 GG vs AA 13.8% 0.79 (0.57, 1.12) |
| Lung              |         |                            |
| Liu, 2011         | China   | 6 586 GG vs AA 7.4% 4.26 (1.32, 13.80) |
| Head and neck     |         |                            |
| Zeljic, 2011      | Serbia  | 6 110 AG/GG 9.7% 1.37 (0.90, 2.10) |
| Skin              |         |                            |
| Orlow, 2014       | Worldwide | 7 3566 GG vs AA 14.5% 0.81 (0.67, 0.99) |
| Gloma 2012        | USA     | 5 320 GG vs AA 13.4% 1.32 (0.88, 1.96) |
| Subtotal          |         |                            |
| Rs2296241 (CYP24A1(1)) |    |                            |
| Head and neck     |         |                            |
| Zeljic, 2012      | Serbia  | 6 110 AG/AA vs GG 40.5% 0.65 (0.38, 1.11) |
| Azad, 2013        | Canada  | 8 522 AA vs GG 59.5% 1.23 (1.00, 1.51) |
| Subtotal          |         |                            |

Figure 5. (Continued)

**DISCUSSION**

This is the first systematic review with meta-analysis that examines the relationship between cancer outcomes and variation in vitamin D pathway genes, and also by far the largest review on vitamin D status and cancer outcome. Our review suggests that higher circulating vitamin D in cancer patients is associated with a 26% lower rate of death and a 16% lower rate of disease progression. The clear association with survival was also observed in site-specific analyses of breast, haematological and colorectal cancers, while an association with reduction in disease progression was also found in those diagnosed with breast, haematological and skin cancer.

Establishing a causal relationship between vitamin D status and cancer progression is challenging because risk factors associated with cancer outcome are often also associated with vitamin D deficiency. For example, the association between 25OHD and improved survival observed in the original studies might be due to 25OHD being a marker of healthier lifestyle (i.e., healthier diet containing more fish; physical activity and spending time outdoors). However, evidence that genetic factors linked to vitamin D metabolism and pathways impact upon cancer survival may be used to counter such concerns and support a causal link. In our meta-analysis, we found evidence of an association between the VDR gene variants with functionally characterised effects and cancer outcome. Forty percent higher rate of death was observed in TT carriers at rs1544410 locus and 26% higher rate in TT carriers at rs2228570, while 29% increased risk of disease progression was observed in AA carriers at rs7975232 and 22% in GG carriers at GC locus.

Evidence from biological studies support a role for these polymorphisms in modulating vitamin D biology. For example, rs2296241 has been shown to affect the translational start site of 1,25(OH)2D and hence its downstream effects (Staal et al, 1996; Uitterlinden et al, 2004). We hypothesise that interactions between mutations in the vitamin D pathway and vitamin D status exist,
and that this interaction could have a critical role in cancer prognosis. Indeed, Han et al (Han et al, 2007) have shown an interaction between vitamin D intake and rs1544410 polymorphism on cancer risk, and we and others have previously shown a modification of the relationship between vitamin D intake or status and cancer outcome by other VDR variants, thus suggesting an interaction of genetic and environmental factors (Li et al, 2007; Theodoratou et al, 2008; Anderson et al, 2011; Zgaga et al, 2014).

In conjunction with the strong associations observed for vitamin D status, evidence from genetic studies further supports an important role of vitamin D in cancer progression. Few studies to date have analysed the associations between VDR or vitamin D pathway genetic variants and cancer outcomes, and no meta-analyses have been published to date. A review by Kostner et al (Kostner et al, 2009) concluded that associations between VDR polymorphisms and cancer prognosis are strongest for prostate cancer (rs2228570), breast cancer (rs1544410, rs731236) malignant melanoma (rs1544410), and renal cell carcinoma (rs731236) but did not perform meta-analysis on these data.

Interestingly, Afzal et al (Afzal et al, 2014) have employed principles of Mendelian randomization in a study comprising 95 766 participants and found that variation in genes involved in vitamin D and 25OHD synthesis (DHCR7 and CYP2R1) were associated with both all-cause and cancer mortality, supporting a causal role of vitamin D. To date, there are no published findings from randomised controlled trials (RCT) assessing the effect of vitamin D supplementation on survival in cancer patients, although several ongoing trials (unfortunately only some of which have disease progression as an outcome) were identified (ClinicalTrials.gov, 2016). Meanwhile, the data on cancer mortality from RCTs conducted in the general population can offer some insight; most notably, a Cochrane review of randomised studies comparing vitamin D supplements to placebo identified a significant reduction in cancer mortality in those taking vitamin D supplements (HR = 0.88, 95% CI = 0.78–0.98; Bjelakovic et al, 2014).

A major issue that is typically taken poor notice of in vitamin D meta-analyses—namely, a very large variability in vitamin D category definition amongst studies, is for the first time being highlighted and transparently shown in our review. Vitamin D categories differed in level as well as range—as a result, large heterogeneity in exposure definition occurred and study point-estimates are difficult to compare: it is, for example, unsurprising that the reported effect per 20 ng ml⁻¹ is greater than effect per 5 ng ml⁻¹ increase. Therefore, there is a need for a consensus in category definition and reporting of effect sizes: future original studies should report effect sizes using internationally agreed cutoffs, such as those given by the Institute of Medicine, solely or in addition to study-specific cutoff values chosen. Generally, variability in exposure categories results in a more heterogeneous estimates and is likely to increase statistical uncertainty and hence bias results towards the null. Nonetheless, our summary findings remain largely unchanged when the analysis was limited according to the difference in 25OHD between the compared groups.

There are some additional limitations of the present work. First, a number of relevant studies were published after the time limits stipulated in our search strategy and so are not included in our...
meta-analysis. Some such papers support the conclusions presented here (Brandstedt et al., 2016; Fang et al., 2016; Fanidi et al., 2016; Mondul et al., 2016; Orlow et al., 2016; Yao et al., 2016; Yuan et al., 2016), while others reported no association between circulating vitamin D and cancer outcome (Vashi et al., 2015; Ahn et al., 2016; Danilovic et al., 2016; McGovern et al., 2016).

**Figure 6.** Cancer progression and vitamin D receptor polymorphisms and other vitamin D-related genetic variants: adjusted meta-analysis. $I^2$ for Apal: 0, BsmI prostate: 0.52, BsmI breast: 0.1, BsmI All: 0.61, FokI Prostate: 0, FokI All: 0.90, TaqI all: 0, Rs4516035: 0.94, Rs22382679: 0.

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| Author and Year | Population | N.o. score | N | Exposure | Weight | HR (95% CI) |
|-----------------|------------|------------|---|----------|--------|-------------|
| Rs7975232 Apal  | Breast     | USA        | 6 | AA vs AC/CC | 16.5%  | 1.08 (0.59, 2.00) |
| Prostate        | Taiwan     | 8          | 601| AA vs CC   | 64.2%  | 1.31 (1.94, 1.84) |
| Lung            | China      | 4          | 755| AA vs CC   | 19.2%  | 1.43 (0.99, 2.78) |
| SUBTOTAL        |            |            |    |           |        | 1.29 (1.02, 1.56) |
| Rs1544410 BsmI  | Breast     | USA        | 6 | AA vs AC/CC | 10.4%  | 1.49 (0.53, 3.33) |
| Prostate        | Taiwan     | 8          | 601| TT vs TC/CC| 2.3%   | 1.00 (0.14, 7.21) |
| Williams, 2004  | USA        | 7          | 728| TT vs CC   | 18.4%  | 1.10 (0.60, 1.80) |
| Holt, 2010      | USA        | 8          | 1294| TT vs CC  | 18.4%  | 1.10 (0.60, 1.80) |
| Subtotal (Prostate) |         |        |    |           | 0.93 (0.54, 1.32) |
| Lung            | China      | 6          | 568| TT vs CC   | 24.1%  | 1.64 (1.16, 2.31) |
| SUBTOTAL        |            |            |    |           | 1.19 (0.88, 1.50) |
| Rs2228570 FokI  | Breast     | USA        | 6 | TT vs TC/CC| 21.7%  | 0.57 (0.30, 0.90) |
| Prostate        | Taiwan     | 8          | 601| TT vs CC   | 22.6%  | 0.99 (0.74, 1.33) |
| Holt, 2010      | USA        | 8          | 1292| TT vs CC  | 19.9%  | 1.01 (0.60, 1.33) |
| Subtotal (Prostate) |         |        |    |           | 0.99 (0.72, 1.33) |
| Head and Neck   | Japan      | 6          | 204| TT vs TC/CC| 15.5%  | 3.03 (1.62, 5.67) |
| Skin            | UK         | 5          | 171| TT vs TC/CC| 20.4%  | 0.77 (0.24, 2.50) |
| SUBTOTAL        |            |            |    |           | 1.28 (0.49, 2.08) |
| Rs731236 TaqI   | Breast     | USA        | 6 | GG/AG vs AA | 40.7%  | 1.30 (0.70, 2.40) |
| Prostate        | USA        | 8          | 1292| GG vs AA  | 48.8%  | 0.80 (0.50, 1.40) |
| Skin            | UK         | 5          | 171| GG vs AA/AG| 10.5%  | 0.60 (0.22, 2.00) |
| SUBTOTAL        |            |            |    |           | 0.98 (0.59, 1.37) |
| Rs4516035 EcoRV | Breast     | USA        | 6 | GG/AG vs AA | 40.7%  | 1.30 (0.70, 2.40) |
| Prostate        | USA        | 8          | 1292| GG vs AA  | 48.8%  | 0.80 (0.50, 1.40) |
| Skin            | UK         | 5          | 171| GG vs AA/AG| 10.5%  | 0.60 (0.22, 2.00) |
| SUBTOTAL        |            |            |    |           | 0.98 (0.59, 1.37) |
| Rs2282679 GC    | Prostate   | Austria    | 6 | TG vs TT   | 60.1%  | 1.26 (0.93, 1.70) |
| Colorectal      | Austria    | 5          | 264| GG vs TT/TG| 39.9%  | 1.17 (0.81, 1.69) |
| SUBTOTAL        |            |            |    |           | 1.22 (0.99, 1.46) |
Second, various assays were used for 25OHD measurement in the different studies, while 25OHD was also sampled at variable timepoints, including pre-diagnosis, before treatment and after treatment, which may impact the results. Also, in disease progression studies, different outcome definitions were used for example, disease-free survival, local or distant recurrence.

In the present study, results for all cancers combined are given, in addition to site-specific findings, we yet fully acknowledge that cancer is a heterogeneous disease. However, numerous studies have shown involvement of vitamin D on key hallmarks of cancer, many of which are common to all cancers; preclinical studies demonstrate effects on cell cycle arrest, cell adhesion, differentiation, proliferation, tumour angiogenesis, and apoptosis in human cancer cell lines (Simboli-Campbell et al., 1997; Chen et al., 2000; Krishnan et al., 2003; Deeb et al., 2007; Kizilag et al., 2010; Hsu et al., 2011; Ting et al., 2012), while reduction in cancer proliferation has been shown in carcinogen-exposed rats (Mokady et al., 2000) and cancer phenotypes are more commonly observed in vitamin D receptor (VDR) knockout mice (Zheng et al., 2012). Nevertheless, the heterogeneity in pooled results between different cancer types and the small number of studies for certain cancers limits the strength of the current study in demonstrating an association between circulating 25-hydroxyvitamin D and total cancer survival.

Next, in reporting the impact of genetic variation on outcome, we acknowledge that ethnic differences in VDR variation exist, which might interfere with the findings from genetic studies, as ethnicity is directly linked to the skin type and vitamin D synthesis. Meanwhile, VDR variants may interact with circulating 25OHD to impact outcome, yet only a small number of studies examined these putative gene–environment interactions. Finally, we observed some evidence of heterogeneity and publication bias overall; however, findings from sensitivity analysis were highly consistent and supportive of main findings.

Despite these limitations, the present work includes a novel meta-analysis, investigating the association between vitamin D-related genetic variation and cancer outcome, in addition to a 50% larger meta-analysis of circulating 25OHD and cancer outcome compared to a previous review (Li et al., 2014). Moreover, stringent quality assessment of original studies and corresponding sensitivity analysis were conducted and strikingly inconsistent 25OHD category definitions were addressed in stratified analysis.

In conclusion, the consistent evidence across the studies inform prognosis.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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