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Review

Association of Vitamin D Receptor Gene Polymorphisms With Melanoma Risk: A Meta-analysis and Systematic Review

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Abstract. *Background/Aim: Increasing evidence indicates a relevance of the vitamin D endocrine system for pathogenesis of malignant melanoma. We performed a systematic review and meta-analysis to update previous reports that investigated the association between vitamin D receptor (VDR) gene polymorphisms and melanoma risk. Materials and Methods: A comprehensive literature search (PubMed, ISI Web of Science) identified a total of 14 studies that were eligible for inclusion in our meta-analysis. In the statistical analysis, the ORs and the 95% CIs were calculated for the dominant and recessive models for seven VDR gene polymorphisms, namely rs2228570 (FokI), rs731236 (TaqI), rs1544410 (BsmI), rs4516035 (A-1012G), rs11568820 (Cdx2), rs7975232 (ApaI) and rs739837 (BglI). Results were illustrated in Forest Plots. Publication bias was tested using Funnel Plots and the Egger's test. Results: Our meta-analysis showed in the dominant model (Bb + BB vs. bb) a significant association of a 15% risk reduction in malignant melanoma incidence for carriers of the rarer allele B of rs1544410 (Bsml). Notably, the dominant model (Ff + ff vs. FF) of rs2228570 (FokI) demonstrates that carriers of the rarer allele f are 22% more likely to develop malignant melanoma. For rs7975232 (ApaI), there is a 20% higher risk of melanoma for carriers of the rarer a allele (Aa + aa vs. AA). The results of the meta-analysis revealed no significant association between melanoma risk and the other investigated VDR polymorphisms. Conclusion: The VDR variants FokI, ApaI and BsmI may influence the susceptibility to developing melanoma. These findings support the concept, that the vitamin D endocrine system is of importance for pathogenesis of malignant melanoma.*

Increasing evidence indicates a relevance of the vitamin D endocrine system for pathogenesis and progression of various malignancies, including malignant melanoma. Under most living conditions, humans have to produce the major part of the vitamin D needed in the skin upon exposure to ultraviolet B (UV-B) radiation from 7-dehydrocholesterol (7-DHC) (1). After being transferred to the circulation, vitamin D is hydroxylated in the liver by CYP2R1 (and CYP27A1) to generate 25-hydroxyvitamin D, (25(OH)D), the major vitamin D metabolite in the blood, where its concentration defines a person's individual vitamin D status (1). Interestingly, 25(OH)D serum concentration has been reported to be inversely associated with incidence, disease-free survival and mortality of various malignancies, including colorectal, lung, breast and prostate cancer (2-15). (1). After being transferred from the liver to the circulation, 25(OH)D is hydroxylated in the kidneys and in many extrarenal cells by CYP27B1 to generate 1,25-dihydroxyvitamin D, $1,25(OH)_2D$, the classical biologically active vitamin D metabolite (1). Biologically active vitamin D metabolites, including $1,25\ (OH)_2\ D$, exert their effects at least in part *via* binding to the nuclear vitamin D receptor (VDR), which induces conformational changes that enable hetero-dimerization of VDR with retinoid-x receptor (RXR) (1). The VDR/RXR heterodimer regulates the transcription of many target genes *via* binding to specific DNA sequences in their promotor region, named vitamin D response elements (1). Inhibitory effects on pathogenesis and progression of malignancies that are mediated by biologically active vitamin D metabolites may at least, in part, be exerted *via* VDR-mediated suppression of cell

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growth, adhesion and migration (16, 17), and *via* stimulation of apoptosis (18). Interestingly, $1,25(OH)_2D$ suppresses growth of cultured malignant melanoma cells *in vitro* and in various animal models that include xenografts (17, 19-21). Notably, the hypothesis that a person's vitamin D status and its putative surrogates, including season, geographic latitude and continuous moderate sun exposure, are associated with a more favorable outcome and prognosis in melanoma patients is also supported by observational studies (22-24).

VDR expression and function, that can be regulated by many factors including epigenetic and genetic modifications (25, 26), is a hallmark that determines responsiveness of target cells for biological effects of vitamin D metabolites. Notably, it has been reported that VDR expression is reduced in many advanced solid tumor entities as compared with corresponding unaffected tissue, and that elevated VDR expression is associated with improved prognosis/survival in various types of cancer, including lung (27, 28) and breast cancer (29, 30). Interestingly, it has been demonstrated immunohistologically that VDR expression is reduced in situ in malignant melanomas as compared with benign acquired melanocytic nevi or normal human skin, with a marked reduction of VDR expression in vertical versus radial melanoma growth phases (31). Moreover, VDR expression has been reported to be inversely associated with progression of malignant melanoma (32), indicating that VDR-mediated cellular signaling may be of importance for preventing the multi-step progression of benign acquired melanocytic nevi to malignant melanoma.

During the last decades, numerous single nucleotide polymorphisms (SNPs) of the *VDR* gene have been identified. However, despite extensive research on this topic, the functional significance of many of these variants still remains to be elucidated. It has been speculated that some VDR variants may modulate or affect the expression and/or function of VDR, including stability or downstream transactivation by the translated VDR protein. Several reports have indicated that some common SNPs in the *VDR* gene might alter disease-specific outcomes including disease-free and overall-survival in patients with breast cancer (33), lung cancer (34, 35), renal cell carcinoma (36), ovarian cancer (37), prostate cancer (38, 39), head and neck cancers (40, 41), colorectal cancer (13) and glioma (42); however, other investigators reported no effect on these outcomes (43-45). In melanoma patients, Newton-Bishop *et al*. (23) investigated the *VDR* variants Cdx-2, GATA, FokI, BsmI, ApaI and TaqI in a cohort of 872 cases and found no main effect on overall survival. However, the authors of this study (23) concluded that BsmI and VDR variants in high linkage disequilibrium (LD) with this SNP modified the risk for relapse in individuals with lower levels (≤ 50.4 nmol/l) of serum 25(OH)D.

To gain further insight into the relevance of VDR variants for pathogenesis of malignant melanoma, it was the aim of this study to perform a systematic review and meta-analysis to investigate the association between VDR gene polymorphisms and melanoma risk.

Materials and Methods

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (46).

Search strategy and inclusion criteria. A comprehensive literature search (PUBMED and ISI Web of Science, including cross-referenced studies) was conducted to identify relevant publications. Combinations of the following search terms were used: "Vitamin D Receptor", "VDR", "Polymorphism", "Polymorphisms", "VDR polymorphism", "Vitamin D receptor polymorphism", "Vitamin D receptor polymorphisms", "malignant melanoma" and "skin cancer". Inclusion criteria: The studies were selected according to study type (observational studies), language (German, English, Spanish, French), date (published up until February 25, 2018) and content. In terms of content, only studies were included that examined the association of vitamin D receptor (VDR) gene polymorphisms with melanoma risk. In cases where more than one study covering the same study population was identified, the most recent or most relevant study was selected for inclusion.

Assessing the quality of studies and level of evidence. The quality of the studies was assessed using a modification of the Newcastle Ottawa scale (47). Level of evidence was determined according to the Oxford Centre for Evidence-based Medicine (48).

Statistical analysis. For statistical analysis, StatsDirect Statistical Software Version 3.0.150 was used. For every single polymorphism the odds ratios (OR) and the 95% confidence intervals (CI) were calculated for the dominant and recessive models from the allele frequencies reported in the studies or requested from the authors (49, 50). The dominant and recessive models were formed following the genetic association studies (51). The results including the summary risk estimates were illustrated in Forest Plots. Heterogeneity was considered using I^2 statistics and Cochran-Q test. The Cochran-Q test was used to determine whether the random or the fixed effects model was to be used (p-Value<0.05: random effects model; p-Value≥0.05: fixed effects model). Potential publication bias was assessed using Funnel Plots (asymmetry could indicate publication bias) and the Egger's test (p-Values<0.05: potential publication bias). If only a small number of studies (<10 publications) was eligible for inclusion in the meta-analysis of a certain polymorphism, only an uncertain conclusion could be made (52).

Sensitivity analysis/meta-regression. Sensitivity analysis and meta-regression were conducted to detect possible causes of heterogeneity. To identify potential population bias in control selection or genotyping errors, the Hardy-Weinberg equilibrium (HWE) was used. Chi-squared test was used to determine the HWE for the genotype distribution in the control groups (p-Value<0.05: HWE was not met) (51, 53). If the genotype distribution was deviated from the HWE, the corresponding study was excluded as part of the sensitivity analysis. Meta-regression was used to investigate how natural UV exposure influences the odds ratio of malignant melanoma in relation to the models used.

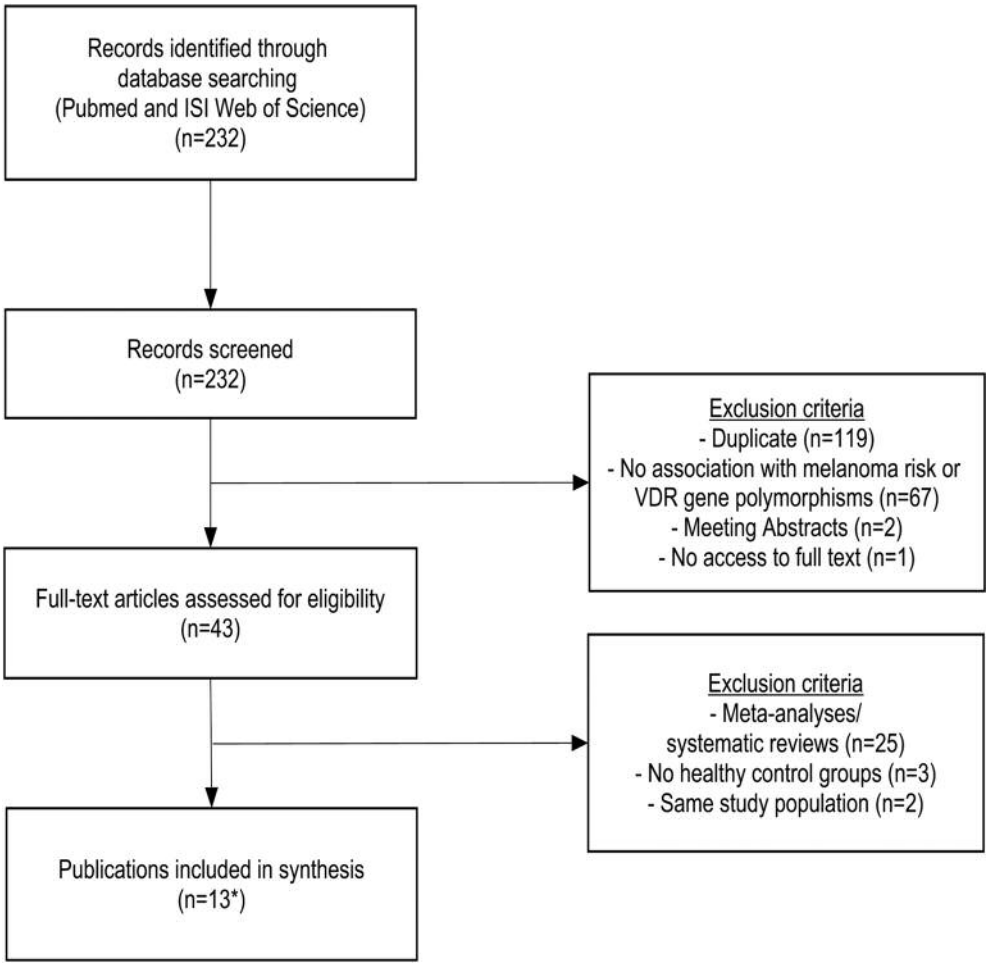

Figure 1. *Flowchart illustrating the literature search process. *One of the studies (64) includes two separate studies.*

Results

Literature search. A flowchart of the literature search process is presented in Figure 1. Two hundred thirty-two articles were identified by literature search. Of these studies, 119 were excluded because of being duplicates, 67 did not investigate the association between VDR gene polymorphisms with melanoma risk, two articles were Meeting Abstracts and for one article there was no access to full text. Thus, 43 articles were assessed for eligibility. After excluding meta-analyses/systematic reviews, articles with no healthy control groups or the same study population, 13 publications (one paper includes two separate studies and therefore the included publications are counted as 14 studies in the following) were eligible for the meta-analysis.

Study characteristics. Selected characteristics of the included studies (n=14) with respect to the association between VDR gene polymorphisms and melanoma risk are shown in Table I (only the relevant polymorphisms for the current meta-analysis are listed). All participants were Caucasians. Twelve of the 14 selected studies were conducted in Europe and two in the USA. Overall, included studies comprised 5,658 malignant melanoma cases and 5,340 healthy controls regarding the association between VDR gene polymorphisms and melanoma risk. Every single of the seven VDR gene polymorphisms rs2228570 (FokI), rs731236 (TaqI), rs1544410 (Bsml), rs4516035 (A-1012G), rs11568820 (Cdx2), rs7975232 (ApaI) and rs739837 (BglI) appeared in at least two studies and were included in the current meta-analysis.

Meta-analysis: FokI polymorphism and melanoma risk. The *VDR* gene polymorphism rs2228570 FokI was examined in 11 eligible studies encompassing 4,506 melanoma cases und 4,409 healthy controls. Calculation of the summary risk estimate revealed a significant association between

Table I. *Characteristics of studies included in the meta-analysis.*

Study (reference number)	Year	Country	Study design	Source of cases	Source of controls	Number cases/ controls	VDR gene polymorphism
Hutchinson *et al.* (70)	2000	UK	CC	Hospitals	Hospitals	316/108	rs2228570 (FokI), rs731236 (TaqI)
Halsall *et al.* (71)	2004	UK	CC	Hospitals	No information	176/80	rs4516035 (A-1012G)
Han *et al.* (58)	2007	USA	CC	Nurses' Health Study (women only)	Nurses' Health Study (women only)	219/873	rs2228570 (Fokl), rs1544410 (Bsml), rs11568820 (Cdx2)
Santonocito *et al.* (61)	2007	Italy	CC	Hospitals	Population (blood donors)	101/101	rs1544410 (BsmI), rs4516035 (A-1012G), rs2228570 (FokI)
Povey *et al.* (60)	2007	UK (Scotland)	CC	Hospitals	Population	596/441	rs4516035 (A-1012G)
Li *et al.* (62)	2008	USA (Texas)	CC	Hospitals	Population	805/841	rs731236 (TaqI), rs1544410 (BsmI), rs2228570 (FokI)
Barroso *et al.* (49)	2008	Spain	CC	Hospitals	Hospitals and Madrid College of Lawyers	283/245	rs2228570 (FokI), rs731236 (TaqI), rs739837 (BgIl), rs4516035 (A-1012G)
Gapska *et al.* (63)	2009	Poland	CC	Hospitals	Population	763/763	rs1544410 (BsmI), rs731236 (TaqI), rs2228570 (FokI), rs4516035 (A-1012G)
Randerson-Moor *et al.* (Leeds CCS1) (64)	2009	UK	CC	Hospitals, Krebsregister	Population	1028/402	rs11568820 (Cdx2), rs4516035 (A-1012G), rs2228570 (FokI), rs1544410 (Bsml), rs7975232 (ApaI), rs731236 (TaqI)
Randerson-Moor *et al.* (Leeds CCS2) (64)	2009	UK	CC	No information	Population (women only)	299/560	rs11568820 (Cdx2), rs4516035 (A-1012G), rs2228570 (FokI), rs1544410 (Bsml), rs7975232 (ApaI), rs731236 (TaqI)
Schäfer *et al.* (50)	2012	Germany	CC	Hospitals	Hospital and population	305/370	rs731236 (TaqI), rs7975232 (ApaI)
Gapska *et al.* (63)	2009	Poland	CC	Hospitals	Population	763/763	rs1544410 (BsmI), rs731236 (TaqI), rs2228570 (FokI), rs4516035 (A-1012G)
Peña-Chilet *et al.* (72)	2013	Spain	CC	Hospitals	Hospital and Madrid College of Lawysers	530/314	rs2228570 (FokI), rs731236 (TaqI), rs739837 (BgIl), rs34516035 (A-1012G)
Zeljic *et al.* (54)	2014	Serbia	CC	Hospitals	Population (blood donors)	117/122	rs731236 (TaqI), rs2228570 (FokI), rs4516035 (A-1012G), rs7975232 (ApaI)
Cauci *et al.* (73)	2017	Italy	CC	Hospitals	No information	120/120	rs2228570 (Fokl), rs1544410 (Bsml)

CC: Case-control, VDR: vitamin D receptor, OR: odds ratio, CI: confidence interval.

melanoma risk and FokI polymorphism in the dominant model (Ff + ff *vs.* FF) (Figure 2A). The meta-analysis of FokI polymorphism with an OR of 1.22 (95% CI=1.06-1.40) shows that carriers of the rarer f allele, compared to FF homozygotes, are 22% more likely to develop a malignant melanoma. The recessive model (ff *vs.* FF + Ff) did not reveal an association between FokI polymorphism and melanoma risk (OR=1.14; 95% CI=0.91-1.42) (Figure 2B). According to the funnel plots (Figures 2C and 2D) and the Egger's test, no significant publication bias was found.

Meta-analysis: TaqI polymorphism and melanoma risk. TaqI polymorphism was examined by nine eligible studies encompassing 4,319 melanoma cases und 3,630 healthy controls. The summary risk estimate revealed no association between the TaqI polymorphism and melanoma risk neither in the dominant model (Tt + tt *vs.* TT) with an OR=1.03 (95% CI=0.86-1.23) (Figure 3A) nor in the recessive model (tt *vs.* TT + Tt) with an OR=0.92 (95% CI=0.81-1.05) (Figure 3B).

Meta-analysis: Bsml polymorphism and melanoma risk. Seven of the eligible studies examined the association between the VDR gene polymorphism Bsml and melanoma risk with a total of 3,324 melanoma cases and 3,622 healthy controls. The pooled OR revealed a significant association between melanoma risk and BsmI polymorphism in the dominant model (Bb + BB *vs.* bb) (Figure 4A). The results of the meta-analysis showed a significant risk reduction of 15% in malignant melanoma incidence for the rarer allele B with an OR=0.85 (95% CI=0.77-0.94). There was no significant association using the recessive model (BB *vs.* bb + Bb) (OR=0.91; 95% CI=0.79-1.04) (Figure 4B).

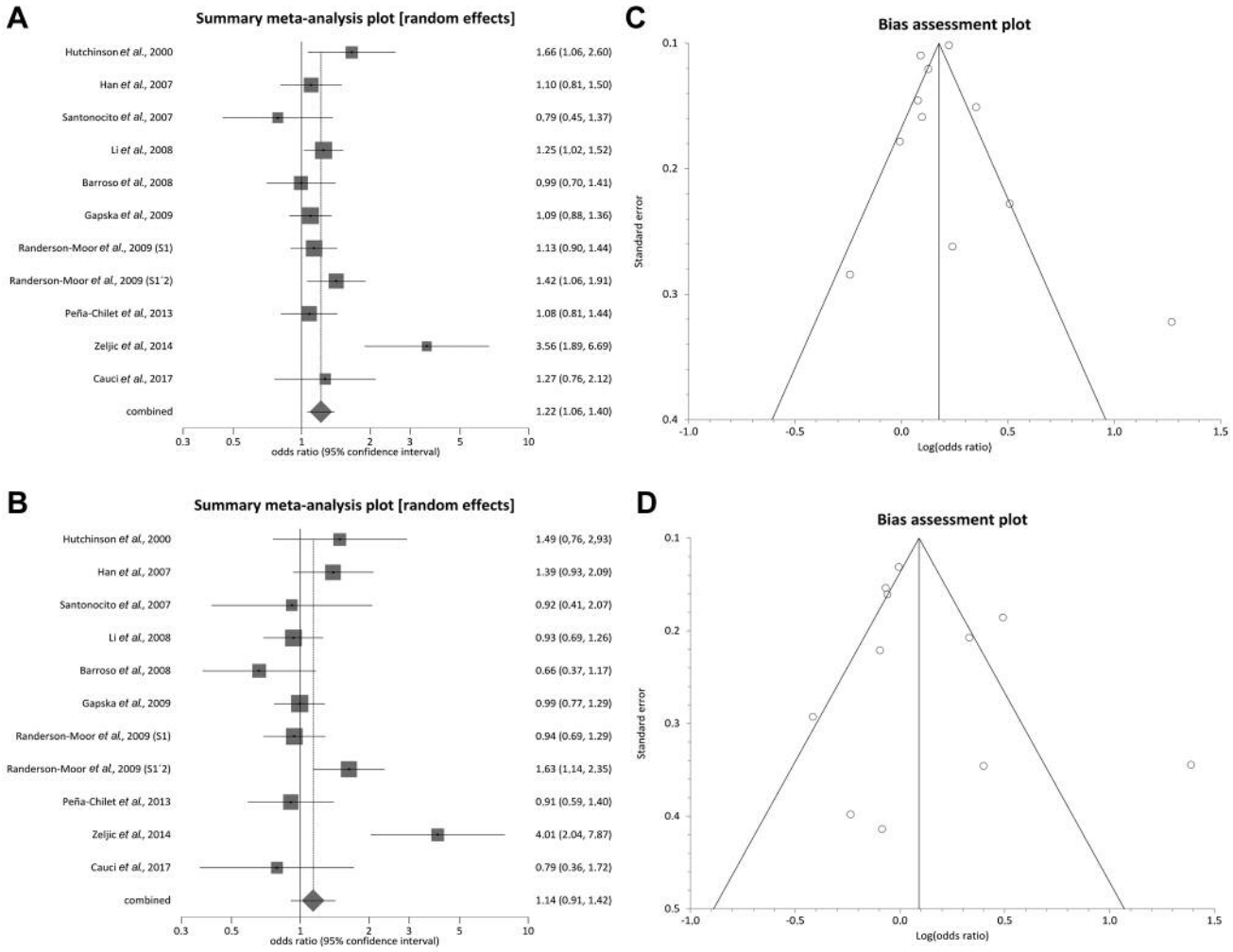

Figure 2. *Forest (A, B) and Funnel (C, D) plots demonstrating the association between melanoma risk and VDR variant rs2228570 (FokI) in the dominant (A, C; Ff + ff vs. FF), and recessive (B, D; ff vs. FF + Ff) models of our meta-analysis. Note that in the dominant model, carriers of the rarer allele f are 22% more likely to develop a malignant melanoma. To investigate potential publication bias, Funnel plot asymmetry was tested using Egger's-test: p=0.30 (C) and p=0.48 (D).*

Meta-analysis: A-1012G polymorphism and melanoma risk. The VDR gene polymorphism A-1012G was examined by nine studies with a total of 3,855 melanoma cases and 2,962 controls. The summary risk estimate revealed no association between A-1012G polymorphism and melanoma risk neither in the dominant model (AG + GG *vs.* AA) with an OR=1.03 (95% CI=0.93-1.15) (Figure 5A) nor in the recessive model (GG *vs.* AA + AG) with an OR=1.00 (95% CI=0.88-1.13) (Figure 5B).

Meta-analysis: Cdx2 polymorphism and melanoma risk. Three of the eligible studies examined the association between Cdx2 polymorphism and melanoma risk in 1,532 melanoma cases and 1,815 controls. The summary risk estimate showed no association in the dominant model (GA + AA *vs.* GG) with an OR=0.96 (95% CI=0.82-1.12) (Figure 6A). The pooled OR in the recessive model (GG *vs.* AA + AG) also did not reveal an association with an OR=1.02 (95% CI=0.70-1.48) (Figure 6B).

Meta-analysis: ApaI polymorphism and melanoma risk. The *VDR* gene polymorphism ApaI was examined in four studies with a total of 1,724 melanoma cases and 1,421 controls. The summary risk estimate did neither result in a significant association in the dominant model (Aa + aa *vs.* AA) with an OR=1.15 (95% CI=0.98-1.36) (Figure 7A) nor in the recessive model (aa *vs.* AA + Aa) with an OR=1.03 (95% CI=0.86-1.23) (Figure 7B).

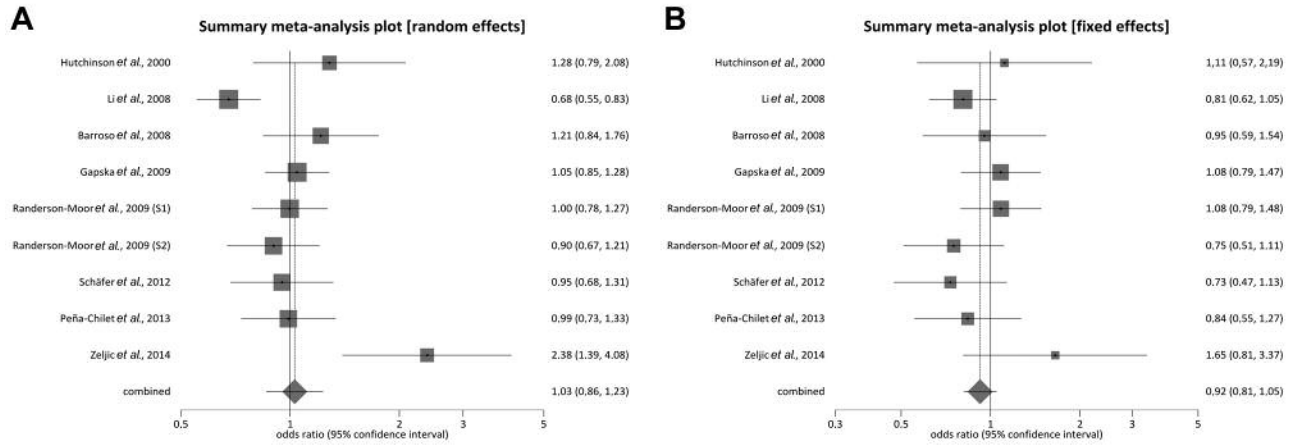

Figure 3. *Forest plots demonstrating no significant association between melanoma risk and VDR variant rs731236 (TaqI) in the dominant (A; Tt + tt vs. TT) and recessive (B; tt vs. TT + Tt) models of our meta-analysis.*

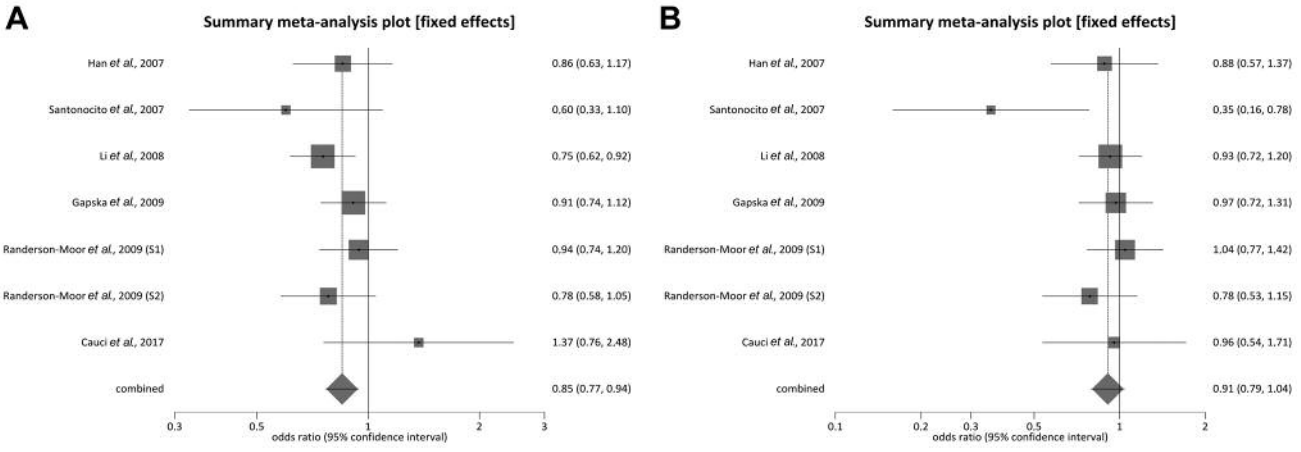

Figure 4. *Forest plots demonstrating the association between melanoma risk and VDR variant rs1544410 (Bsml) in the dominant (A; Bb + BB vs. bb) and recessive (B; BB vs. bb + Bb) models of our meta-analysis. Note that in the dominant model (Bb + BB vs. bb), a 15% risk reduction in malignant melanoma incidence for carriers of the rarer allele B is shown.*

In the publication performed by Zeljic *et al.* (54) the distribution of genotypes was not consistent with the HWE (p=0.001) in the control group for ApaI polymorphism. The corresponding study was excluded in the sensitivity analysis. The excluding study affected the result of meta-analysis of ApaI polymorphism (Figure 7C). For the ApaI polymorphism, there is a 20% higher risk of melanoma with an OR=1.20 (95% CI=1.01-1.42) for carriers of the rarer a allele in the dominant model (Aa + aa *vs.* AA) compared to AA homozygotes.

Meta-analysis: BglI polymorphism and melanoma risk. Two of the included studies examined the association between BglI polymorphism and melanoma risk with a total of 761 melanoma cases and 535 healthy controls. The summary risk estimate revealed no significant association in the dominant model (TG + GG *vs.* TT) with an OR=0.90 (95% CI=0.70-1.17) (Figure 8A). The pooled OR in the meta-analysis of the recessive model neither revealed a significant association (GG *vs.* TT + TG) (OR=0.95; 95% CI=0.73-1.23) (Figure 8B).

Sensitivity analysis and publication bias. Only one of the included studies, that was performed by Zeljic *et al.* (54), was deviated from the HWE for genotype distribution in the control group for one polymorphism (ApaI). Excluding the study affected the result: ApaI-meta-analysis turned into a significant result as described above. Excluding the study performed by Zeljic *et al.* (54) did not affect the meta-analyses of the other polymorphisms in the sensitivity

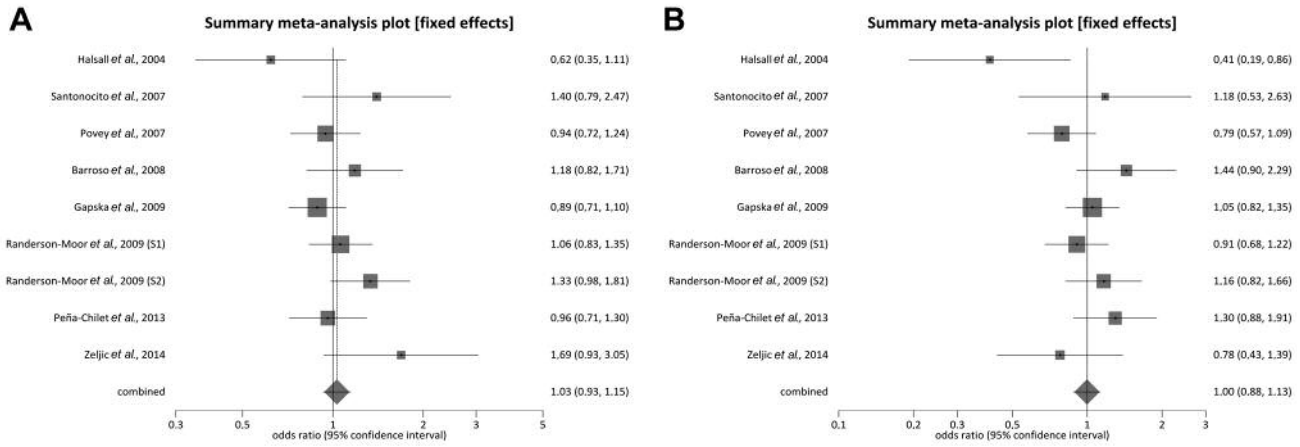

Figure 5. *Forest plots demonstrating no significant association between melanoma risk and VDR variant rs4516035 (A-1012G) in the dominant (A; AG + GG vs. AA), and recessive (B; GG vs. AA + AG) models of our meta-analysis.*

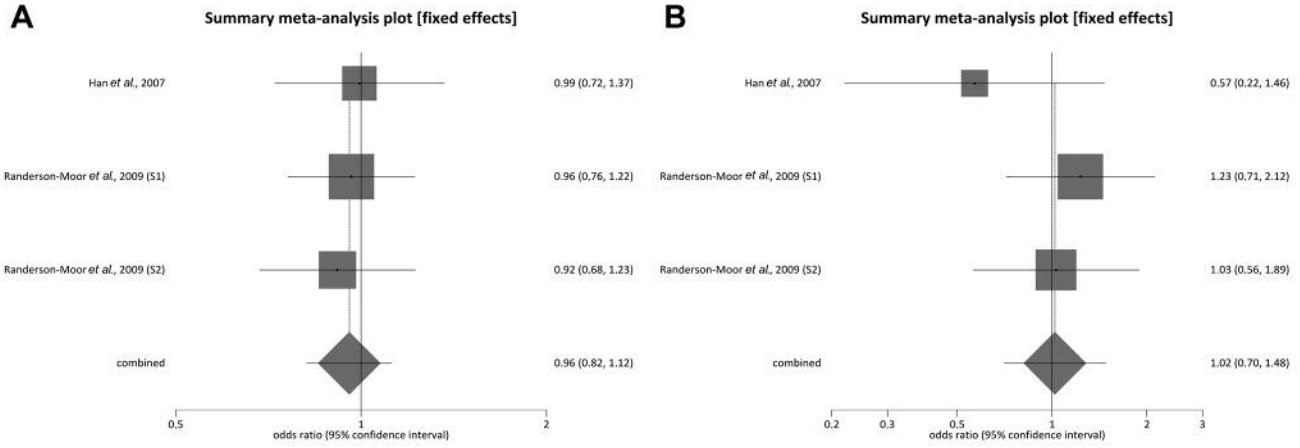

Figure 6. *Forest plots demonstrating no significant association between melanoma risk and VDR variant rs11568820 (Cdx2) in the dominant (GA + AA vs. GG), and recessive (B; GG vs. AA + AG) models of our meta-analysis.*

analysis. Because of the relatively small number of studies in most meta-analyses (assessment with less than 10 studies is unreliable), it was difficult to interpret the funnel plots, which are used to detect publication bias (52).

Meta-regression. The results of the meta-regression revealed no significant association with the influence of UV exposure.

Discussion

In this meta-analysis, we investigated the association of seven *VDR* gene polymorphisms (for which risk estimates could be retrieved in at least two studies of our literature search), namely rs2228570 (FokI), rs731236 (TaqI), rs1544410 (BsmI), rs4516035 (A-1012G), rs11568820 (Cdx2), rs7975232 (ApaI) and rs739837 (BglI), and melanoma risk. The most relevant results were obtained when SNPs for FokI (rs2228570), Bsml (rs1544410), and ApaI (rs7975232) were analysed. Our meta-analysis shows for carriers of the rarer allele B of the SNP rs1544410 (Bsml) in the dominant model (Bb + BB *vs*. bb) a significant association with a 15% reduction in malignant melanoma incidence. For carriers of the rarer allele f of the polymorphism rs2228570 (FokI), the dominant model (Ff + ff *vs*. FF) demonstrates an association with a 22% increased risk to develop a malignant melanoma. For the rs7975232 (ApaI) polymorphism, the presence of the

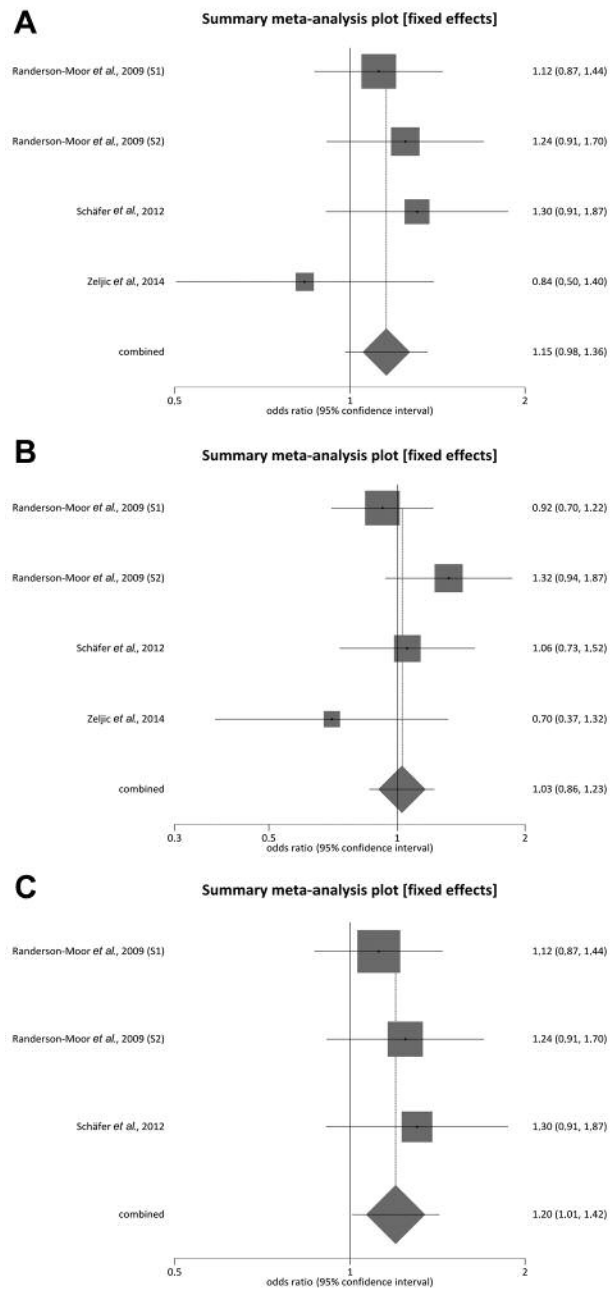

Figure 7. *Forest plots demonstrating the association between melanoma risk and VDR variant rs7975232 (ApaI) in the dominant (A; sensitivity analysis in C; Aa + aa vs. AA) and recessive (B; aa vs. AA + Aa) models of our meta-analysis. Note a 20% increased risk of melanoma for carriers of the rarer a allele in the dominant model.*

rarer a allele (Aa + aa *vs.* AA) was associated with a 20% higher melanoma risk.

Two of these three SNPs (BsmI/rs1544410 and ApaI/rs7975232) belong to a group of adjacent restriction fragment length polymorphism (RFLP) at the 3' end of the VDR gene, that represent some of the most frequently previously investigated VDR variants in malignancies including malignant melanoma (Table II). Notably, the functional significance of the majority of *VDR* SNPs identified is so far unknown. However, some progress has been made and *in vitro* laboratory investigations suggest that at least some VDR SNPs modulate and affect the VDR mRNA and/or protein levels (55-57). The functional characterization of the three relevant SNPs (rs1544410/BsmI; rs2228570/FokI; rs7975232/ApaI) identified in this meta-analysis has produced inconsistent results concerning their biological effects across previous investigations. It has to be noted that, even if these SNPs are nonfunctional, the effects observed in this meta-analysis and in some other investigations may be caused by other, truly relevant SNPs in strong LD located elsewhere in the VDR gene. Some investigations aiming to characterize the differences in VDR expression for SNPs in the 3' end of the *VDR* gene have shown higher levels of VDR mRNA expression for the BsmI-ApaI-TaqI haplotype BAt (rs1544410-A/rs7975232-A/rs731236-C) as compared with the haplotype baT (rs1544410-G/rs7975232-C/rs731236-T). However, conflicting, opposite results have been found in human fibroblasts, as well as in leukemia and prostate cancer cell lines regarding *VDR* mRNA expression VDR mRNA stability and transactivation (26). Findings of a previous study for rs1544410 suggest that the A(B) might be the adverse allele in relation to melanoma risk, which is in conflict with results from other studies (49, 58-64). A population-based observational study that investigated 763 cases and 763 controls in a Polish population found that carriers of haplotypes containing the B allele had an increased risk for development of a malignant melanoma (63). Results from our meta-analysis are in line with previous meta-analyses (64-66), suggesting a protective role for the B allele. However, considering the results after adjusting for covariates, it has been argued that the findings from these meta-analyses should be interpreted with caution and that the BsmI variant of the VDR gene needs to be further characterized in large population-ascertained cohorts and in functional *in vitro* assays preferentially using melanoma and/or skin models.

Our meta-analysis revealed no significant association between melanoma risk and the four other investigated polymorphisms [rs4516035 (A-1012G), rs11568820 (Cdx2), rs7975232 (ApaI) and rs739837 (BglI)]. These findings are partly in line with previous investigations, *e.g.* with a large population-based case-control study comprising 3,676 people with incident single and multiple primary malignant melanomas that evaluated 38 different *VDR* gene variants for their association with the development of cutaneous melanomas and that found in addition to the BsmI (rs1544410), seven SNPs (rs10875712, rs4760674, rs7139166, rs4516035, rs11168287, rs7305032 and

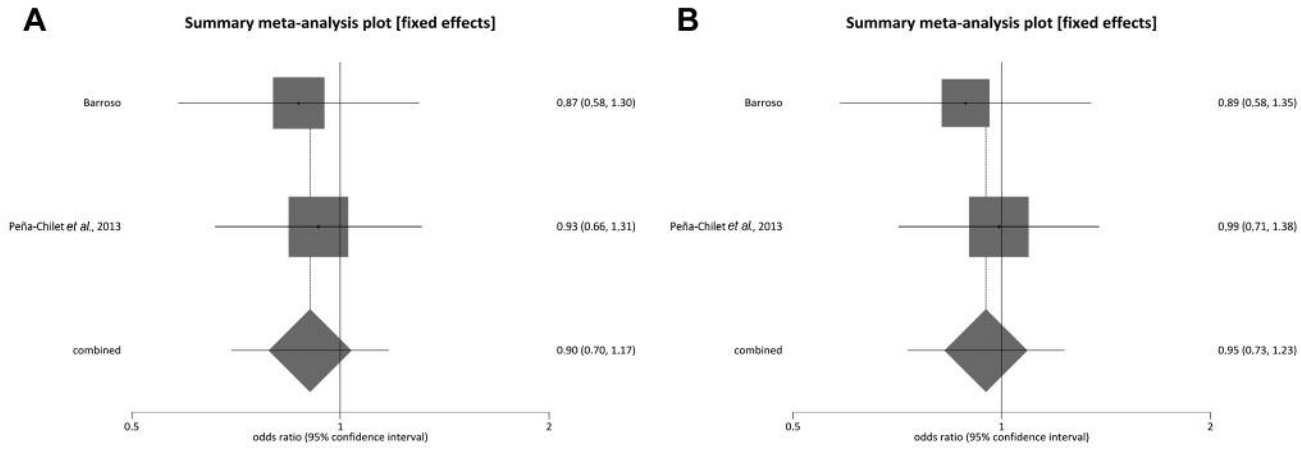

Figure 8. *Forest plots demonstrating no significant association between melanoma risk and VDR variant rs739837 (BglI) in the dominant (A; TG + GG vs. TT) and recessive (B; GG vs. TT + TG) models of our meta-analysis.*

Table II. *Summary risk estimates from meta-analyses about the association of VDR gene polymorphisms with melanoma risk.*

SNPs	Number of studies	Number of cases	Number of controls	OR (95% CI)	Model	I^2 (%)
rs2228570 (FokI)	11	4506	4409			
Ff + ff *vs*. FF (dominant)				1.22 (1.06-1.40)	R	50
ff *vs*. FF + Ff (recessive)				1.14 (0.91-1.42)	R	64.3
rs731236 (TaqI)	9	4319	3630			
Tt + tt *vs*. TT (dominant)				1.03 (0.86-1.23)	R	69.8
tt *vs*. TT + Tt (recessive)				0.92 (0.81-1.05)	F	2.9
rs1544410 (Bsml)	7	3324	3622			
Bb + BB *vs*. bb (dominant)				0.85 (0.77-0.94)	F	9.4
BB *vs*. bb + Bb (recessive)				0.91 (0.79-1.04)	F	14.2
rs4516035 (A-1012G)	9	3855	4953			
AG + GG *vs*. AA (dominant)				1.03 (0.93-1.15)	F	35.9
GG *vs*. AA + AG (recessive)				1.00 (0.88-1.13)	F	42.4
rs11568820 (Cdx2)	3	1532	1815			
GA + AA *vs*. GG (dominant)				0.96 (0.82-1.12)	F	0
AA *vs*. GG + GA (recessive)				1.02 (0.70-1.48)	F	0
rs7975232 (ApaI)	4	1724	1421			
Aa + aa *vs*. AA (dominant)				1.15 (0.98-1.36)	F	0
aa *vs*. AA + Aa (recessive)				1.03 (0.86-1.23)	F	26.1
Sensitivity analysis:	3	1607	1299			
Aa + aa *vs*. AA (dominant)				1.20 (1.01-1.42)	F	0
aa *vs*. AA + Aa (recessive)				1.06 (0.88-1.28)	F	21.2
rs739837 (BglI)	2	761	535			
TG + GG *vs*. TT (dominant)				0.90 (0.70-1.17)	F	0
GG *vs*. TT + TG (recessive)				0.95 (0.73-1.23)	F	0

CI: Confidence interval, F: Fixed effects model, OR: odds ratio, R: Random effects model, SNP: single nucleotide polymorphism, VDR: vitamin D receptor.

rs7965281) that were significantly associated with melanoma risk (67). In that study (67), 38 individual statistical tests were performed and consequently, the increased risk of false positive findings due to these "multiple comparisons" was recognized. The authors expected about two of the tests to be significant simply due to chance and concluded that the fact that they observed eight significant SNPs, reflected by the skewness in the Q–Q plot, suggested that the evidence resulting from that study broadly favors the hypothesis that the gene harbors at least some causal variants.

Previously, it has been speculated that the A allele in position 1A-1012 (rs4516035) of the *VDR* gene may indirectly stimulate a GATA-3 driven T-cell switch of naïve T cells to T-helper 2 cells and that this allele may play differential, opposing roles in susceptibility and in metastasis possibly as a function of the transcription factors secreted by various different backgrounds of the cellular micromilieu (55, 68, 69). In our meta-analysis of nine studies with a total of 3,855 melanoma cases and 2,962 controls the summary risk estimate revealed no association between the A-1012G VDR gene polymorphism and melanoma risk neither in the dominant model (AG + GG *vs*. AA) with an OR=1.03 (95% CI=0.93-1.15) (Figure 5A) nor in the recessive model (GG *vs*. AA + AG) with an OR=1.00 (95% CI=0.88-1.13) (Figure 5B). In summary, the results of our meta-analysis are in agreement with a previous meta-analysis (64) of a total of six published reports on this SNP and melanoma that found no evidence overall that this SNP may be associated with melanoma risk, although the results of this carefully conducted investigation were inconsistent, with significant heterogeneity in the observed relative risks across individual studies.

In conclusion, our study indicates that the *VDR* variants FokI, ApaI and Bsml may influence the susceptibility to developing cutaneous malignant melanoma, with an increased risk of developing a malignant melanoma for carriers of the rarer allele f of rs2228570 (FokI) and the rarer allele a of rs7975232 (ApaI) polymorphism. In contrast, the less common allele B of the rs1544410 (Bsml) polymorphism, may be associated with a protective effect and thus a lower disease risk. In summary, results of our study provide some evidence in support of the hypothesis that the vitamin D signaling pathway may be of relevance for pathogenesis of malignant melanoma.

Key Findings

This meta-analysis demonstrated an association between the presence of malignant melanoma and three out of seven analysed VDR variants. For melanoma development, dominant models indicate a 15% reduced and a 22% increased risk for carriers of the rarer allele B of rs1544410 (Bsml; Bb + BB *vs*. bb) and for carriers of the rarer allele f of rs2228570 (FokI and Ff + ff *vs*. FF), respectively. For rs7975232 (ApaI), a 20% higher risk of melanoma for carriers of the rarer a allele (Aa + aa *vs*. AA) was found. These findings support the concept, that VDR variants influence the susceptibility to developing melanoma and that the vitamin D endocrine system is of importance for pathogenesis of malignant melanoma.

Future Directions

Future studies are needed to confirm that VDR variants influence the susceptibility to developing melanoma and to identify the underlying functional mechanisms that cause this association, *e.g*. whether individual VDR variants modulate or affect the expression and/or function of VDR, including stability or downstream transactivation by the translated VDR protein. Additionally, it needs to be elucidated whether SNPs in the *VDR* gene alter disease-specific outcomes including disease-free and overall-survival in melanoma patients.

Conflicts of Interest

Saarland University with Jörg Reichrath as principal investigator received funding from the Jörg Wolff Foundation.

Authors' Contributions

Michelle Birke: Study design, literature search, data analysis, manuscript preparation; Jakob Schöpe: Study design, literature search, data analysis, manuscript preparation; Stefan Wagenpfeil: Study design, data analysis; Thomas Vogt: Study design, manuscript preparation; Jörg Reichrath: Study design, literature search, data analysis, manuscript preparation.

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